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Rules for Identifying Potentially Reactive or Promiscuous Compounds

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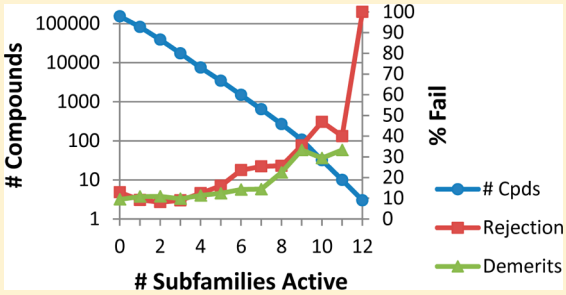
Supporting Information

ABSTRACT: This article describes a set of 275 rules, developed over an 18-year period, used to identify compounds that may interfere with biological assays, allowing their removal from screening sets. Reasons for rejection include reactivity (e.g., acyl halides), interference with assay measurements (fluorescence, absorbance, quenching), activities that damage proteins (oxidizers, detergents), instability (e.g., latent aldehydes), and lack of druggability (e.g., compounds lacking both oxygen and nitrogen). The structural queries were profiled for frequency of occurrence in druglike and nondruglike compound sets and were extensively reviewed by a panel of experienced medicinal chemists. As a means of profiling the rules and as a filter in its own right, an index of biological promiscuity was developed. The 584 gene targets with screening data at Lilly were assigned to 17 subfamilies, and the number of subfamilies at which a compound was active was used as a promiscuity index. For certain compounds, promiscuous activity disappeared after sample repurification, indicating interference from occult contaminants. Because this type of interference is not amenable to substructure search, a "nuisance list" was developed to flag interfering compounds that passed the substructure rules.



INTRODUCTION

Screening of large compound collections is perhaps the most common method for identifying novel leads toward new drugs. However, several decades of experience have shown that many screening hits derive their activity from undesirable mechanisms. In addition to simple chemical reactivity, more subtle mechanisms such as aggregation[1] and redox cycling[2] can produce misleading screening results. Interference with assay readouts via light absorption, fluorescence, etc. can also cause false hits.[2] The first clue that a compound acts by an undesirable mechanism is often promiscuity, defined as activity of the same compound at several unrelated biological targets.[3−5] Since it is unlikely that a molecule would occupy multiple, diverse binding sites by the types of simple, noncovalent interactions usually seen in specific screening hits, a promiscuous activity profile usually implies a nonspecific mode of action.

The problems mentioned above have led to the development of structural queries to identify compounds that are unstable, reactive, promiscuous, or otherwise unsuitable to be used as input for drug discovery programs.[4−9] At Lilly, such queries have been developed and refined since the mid-1990s, resulting in a set of 275 rules used to remove undesirable compounds. These queries are used both to prefilter screening sets and to pare down lists of compounds offered by outside suppliers. The current publication provides a detailed account of the Lilly rejection rules and the computational engine used to implement them. In addition, a novel index of promiscuity is applied to the Lilly activity database and the structural motifs identified are used to refine the rejection rules.

An important impetus for publishing our internal rejection rules is Lilly's Open Innovation Drug Discovery initiative (OIDD[10]). OIDD allows academic and biotechnology collaborators to submit novel molecules for no-charge in vitro testing against a variety of Lilly assays. Current tests include assays related to strategic areas of interest such as oncology, endocrine, cardiovascular, and neuroscience. Because of the volume of screening requests, it has been necessary to filter the compounds computationally before testing. The first step of the filtration process, resulting in the largest number of rejections, is the application of the Lilly structural filters. Early rejection of molecules unlikely to lead to a new drug helps both Lilly and the OIDD submitters, focusing resources on those molecules most likely to succeed. Questions of course arise as to why certain compounds are rejected. The current article is intended to provide guidance to medicinal chemists about structural features that can detract from the value of their compounds in the process of screening for new drugs.

METHODS

Software and queries are available at https://github.com/IanAWatson/Lilly-Medchem-Rules.git or from watson_ian_a@lilly.com.

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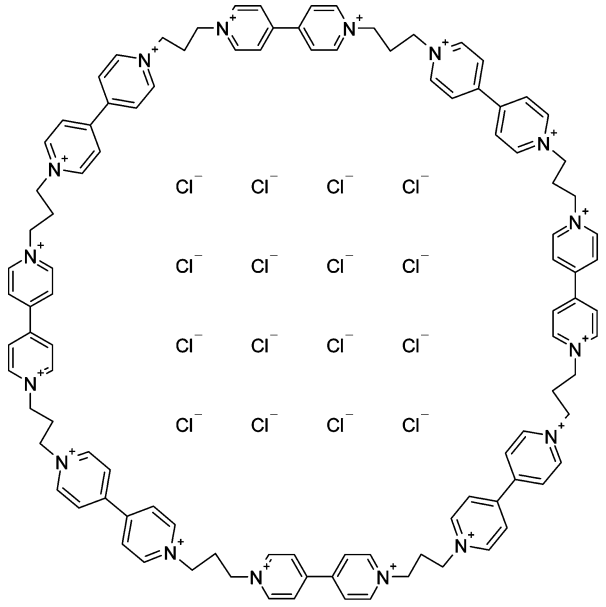

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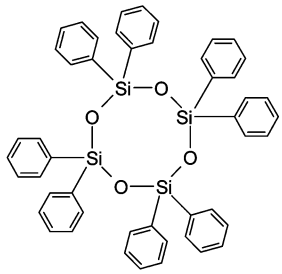

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Figure 1. Examples of nondruggable hits from an early diversity screenset.

Substructure Searching Tools. Many concepts voiced by the medicinal chemists who helped shape the rules were not readily amenable to traditional atom-matching substructure search specifications: an atom that is part of a fused ring system, no attached heteroatoms, no match if there is an electron withdrawing group on the ring, only reject if more than three occurrences, two heteroatoms in an aromatic ring, etc. To facilitate implementation of such concepts, a traditional atom matching specification was augmented with the ability to create a variety of chemically meaningful specifications about the search conditions. The search engine consists of three separate executables. A first pass, written in C++ for speed, quickly eliminates molecules with obvious flaws: too many atoms, too few atoms, too many rings, excessively large rings or ring systems, nonallowed elements, isotopes, valence errors, lack of pharmacophoric features, etc. Another executable performs substructure searches and rejects all matched molecules. A third executable applies demerits and rejects molecules that accumulate excessive demerits. The query file format for the second and third executables is an extension of the MSI Cerius2 file format.[11] A detailed description of the search engine, along with text of the substructure queries, is included in Supporting Information.

Data Sets. The Available Chemicals Directory (ACD) database, version 2010.3, and MDL Drug Data Report (MDDR), version 2009.1, purchased from Symyx Corp, were profiled versus the structural filters. Because of recent changes in the size and content of ACD, a version from early 2005 was also profiled. The database of internal Lilly compounds consisted of those with at least 5 mg inventory as of July 2010. Third-party compounds were from a compendium of offerings from 11 vendors assembled in April 2009; exact matches to Lilly compounds with at least 20 mg inventory were excluded from the list. The number of compounds in each data set is listed in Table 2 (see Results and Discussion).

Activity Data. Whenever possible, Lilly screening assays are annotated with links to Entrez gene names. Activity data for Lilly compounds (with or without current inventory) were extracted along with the gene names. An active result was defined as a concentration−response curve with an IC_{50} of 9.9 μM or less or a single-point result of at least 90% inhibition, regardless of concentration. By these criteria, 2.1 million out of 120 million data points were defined as active.

RESULTS AND DISCUSSION

History of the Lilly Rules. The need for a set of rejection rules became apparent when one of us (R.F.B.) designed the first general diversity screenset at Lilly in 1991. Although the screenset was successful in producing usable hits, including a series that progressed to phase III clinical trials,[12] scrutiny of the hits revealed many compounds with poor druggability (for two examples, see Figure 1). This experience was taken into account in the design of a druglike diversity screenset in 1994. Each compound was required to have at least moderate similarity to a known drug candidate (at least 60% MACCS similarity to a compound in the Comprehensive Medicinal Chemistry or MDDR database) and in addition was required to pass 23 structural filters. The filters were written as MACCS queries by Stephen W. Kaldor and included queries for well-known functional groups such as Michael acceptors, acid halides, etc.

For the next 4 years, the druglike screenset described above was the core set used in all HTS campaigns. In the course of screening, additional undesirable compounds were identified, resulting in the creation of additional rules. Reasons for rejecting these compounds fell into one or more of three categories: reactivity or instability, nuisance activity, and manual rejection by medicinal chemists. Although chemist acceptance is subjective,[13] it is often an insurmountable obstacle to expansion of a hit. The new queries were aggregated with the

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previous Kaldor queries in a script in MACCS sequence language.

In 1995, one of us (I.A.W.) created a substructure query engine on the UNIX platform (see Supporting Information). The new engine was more than 2 orders of magnitude faster than MACCS and had features that facilitated the creation of complex and highly specific queries. The new engine also allowed a more nuanced approach: features that were considered undesirable but not crippling could be assigned demerits, which would not result in rejection unless total cumulative demerits passed a predetermined threshold (100 by default). For instance, each occurrence of an ester in a molecule was assigned 35 demerits because of the group's vulnerability to hydrolysis in storage or in biological assays. The previous queries were ported to the new engine. Over the next 6 years, additional rules were created on an ad hoc basis, resulting in over 200 rules by the beginning of 2004.

With the proliferation of rules and their widespread application to both primary and follow-up screensets, it became necessary to systematize and standardize the rules. A working group was created for the purpose of enumerating and classifying the rules, reviewing the existing rules, and when necessary creating new ones. The group consisted of four high-ranking medicinal chemists, responsible for decisions on reactivity and chemist acceptance, and five computational chemists, responsible for statistical analysis and conversion of medicinal chemists' guidance into substructure queries.

Profiling of various databases revealed 246 rejection reasons, which were broken into classes and subclasses. For instance, the acylated_enol rule (Table S1) was assigned to the class "acylating" and the subclass "ester". By sorting on class and then subclass, it was possible to identify redundancies. For example, there were 11 Michael acceptor rules that had sprung up to deal with individual examples of compounds deemed undesirable. After an analysis of the reaction mechanism, it was possible to reduce the 11 rules to 2: michael_rejected for strongly reactive compounds without electron-donating groups, and michael_demerited for more weakly reactive compounds with electron-donating groups.

The rules were divided into three categories: rejections, demerits, and innocuous. Rules falling into the last category were deleted. The full set of rules was reviewed by one team member, and only the ∼60 rules deemed potentially controversial were carried on to the full group. For a more thorough review, the group was split into two, and each remaining rule was rated independently by both subgroups. The ∼20 rules where the two subgroups differed were discussed by the full team. Criteria included reactivity, stability, nuisance activity, and chemist acceptance. In addition, the full group reviewed the numeric penalty value assigned to each demerit rule.

After rating the existing rules, the group looked for gaps in the rules. The main mechanism was the clustering and hand-inspection of 2340 promiscuous screening actives that passed existing rules. The list of promiscuous compounds was generated by a preliminary version of the method described later in this paper. An additional ∼15 rules were created as a result of this exercise. For example, a rule was created to reject biotin analogues that interfere in assays that use avidin−biotin coupling. The final set consisted of 222 rules, which subsequently grew to 275 at the time of the writing of this manuscript.

An important use of the Lilly rules has been to filter compound offerings from outside vendors ("third-party offerings"). Because the number of compounds available is typically ∼100× the number that can be purchased in a given year, a fairly stringent threshold of 100 demerits was used to ensure that money was not wasted on mediocre compounds. However, a rejection threshold of 100 demerits is not necessarily suitable for other applications. For instance, the cost of screening a compound already in the collection is typically less than 10% of the cost of purchasing a compound. For the purpose of hit follow-up, it makes sense to ease the stringency of the filters to include somewhat less desirable compounds that nevertheless may provide useful information at modest cost. Therefore, an option to run a "relaxed" version of the rules with a demerit cutoff of 160 was made available. The relaxed version of the rules is used by the OIDD collaboration. It is also possible to run the rejection rules alone, without demerits or heavy-atom cutoffs ("outright rejection" version). Because the demerit information is sometimes used computationally to prioritize compounds, all rejection rules are assigned demerits equal to the threshold for rejection on demerits alone: 100 demerits when running in regular mode and 160 in relaxed mode.

Open Innovation Drug Discovery. When Lilly launched OIDD in 2009, there was a strong desire to ensure that the molecules accepted for testing would have the highest possible chance of resulting in viable chemical starting points for drug discovery programs. As part of the OIDD agreement, Lilly personnel are not permitted to see submitted structures, so only automated computational methodologies can be applied in the selection process. One of the checks OIDD performs is application of the Lilly structural filters. As of May 2012, 72 000 structures had been submitted to OIDD. Of these, 56 000 passed the Lilly structural filters. This pass rate is slightly better than those we have historically seen from commercial compound offerings. Reasons for failure are reported back to the submitter. An alternative channel is available for compounds that fail the rules but are of specialized interest, such as natural products, emerging areas of chemical diversity, etc.[14]

Description of the Individual Rules. The Supporting Information contains a table listing the individual rules and their match rates against various compound databases, accompanied by an example structure for each rule (Tables S1 and S2). The rules fall into 17 classes (Table 1), which will be described below.

Acylating Agents. This is by far the largest class, with 10 subclasses and 51 rules. A few of the rules in this class simply flag structures that may be unstable under assay conditions (e.g., "ester"), but most guard against more troublesome behavior. Many acylating agents inhibit serine or cysteine hydrolases by reacting with the active serine or cysteine, respectively.[15] The acylated enzyme may recover slowly or not at all. Since the main driver of potency is a simple chemical reaction, it is difficult to obtain specificity starting with this type of mechanism. As a result, this mechanism has fallen out of favor in the pharmaceutical industry, creating a need to identify and reject potential acylating agents. In addition, some acylating agents produce hydrolysis products that interfere with assays (e.g., thioester) or cause toxicity (e.g., acylhydrazides). Fused β-lactams such as penicillins and cephalosporins are very successful drugs, but the compounds are not stable on long-term storage at room temperature and the degradation

Table 1. Summary of Rule Classes

rule class	number of rules
acylating	51
aldehyde	29
alkylating	13
chelator	7
color	3
halogen	11
misc	14
nitrogen	40
nuisance	12
phosphorus	10
protecting group	4
quat	2
redox	7
ring	23
sulfur	19
vinyl	30
total	275

products often are active across a wide variety of targets (R.F.B., unpublished observation). The parent compounds also acylate active-site serine or cysteine of many hydrolase enzymes, a mechanism of action that is considered undesirable for most current projects that target these enzymes.

Aldehydes and Ketones. This rule class contains five subclasses and 29 individual members. Like the acylating agents, aldehydes and ketones can react with active-site serine or cysteine, in this case forming hemiacetals or hemiketals. The reaction is usually reversible, but the time scale can vary greatly.[16] Although this mechanism is not considered to be as undesirable as the acylation mechanism described above, specificity is still a problem, and most Lilly projects choose not to screen the most reactive examples of this class, aldehydes and electron-deficient ketones. Several rules identify latent aldehydes and ketones, such as acetals and hemiacetals. These structures are more stable when at least one heteroatom is confined to a ring, as is seen naturally with sugars such as glucose and ribose; structures in which one or both oxygens are in a ring accrue 30 demerits, whereas those with neither oxygen in a ring are rejected.

Alkylating Agents. There are 5 subclasses and 13 rules. The reactivity of most of these is well-understood. An interesting example is the alkylthio_N_aromatic rule. This group has a reputation for lability under typical chemical reaction conditions and may have toxicity liabilities as well, but it seems to be stable under biological assay conditions and is not a predictor of promiscuity (Table S1); hence, it is demerited and not rejected. The prototypical compound of this class, 2-(methylthio)pyridine, was not toxic to mammalian cells at concentrations up to 1 mM.[17]

Chelators. This is a small class of seven rules. 8-Hydroxyquinolines are rejected because they are common nuisance hits, and crown-ether-like compounds are rejected because of potential to act as ionophores in addition to their poor druggability.

Color. The compounds matching these three rules (Table S1) interfere with assays that have light emission or absorption as readout. Additional rules might be profitably added to this category. However, this problem is often controlled by creating "no-screen" lists of compounds known to interfere in these assays. Interference is often due to colored impurities, which are usually related more closely to the origin and storage history of the sample than to the nominal structure.

Halogen. There are 5 subclasses and 11 rules. Bromine is given 34 demerits, adding up to a rejection (≥100 demerits) with three or more under the regular rules. Chlorine and fluorine begin accruing demerits at three and five incidences, respectively. Other rules reflect oxidizing capability and/or unusual valence.

Miscellaneous. There are 6 subclasses and 14 rules. Compounds containing neither nitrogen nor oxygen are rejected ("no_interesting_atoms"). Compounds with multiple charges often have poor membrane permeability. After the assignment of predicted charges at neutral pH (R.F.B. and I.A.W., manuscript in preparation), each positive or negative charge above 1 is counted as 50 demerits; triple positive or negative charges (or doubles of both) are therefore rejected under the regular rules.

Nitrogen. With 9 subclasses and 40 rules, the nitrogen class is the second-largest family of rules. Aniline groups are known to predispose toward problems with genetic toxicity.[18] Electron-rich anilines are more subject to chemical or enzymatic oxidation reactions. Eight rules for anilines are given different demerit values calibrated roughly by electron density. Aliphatic nitrogen attached to a heteroatom (N, O, or S) tends to result in metabolic instability and/or toxicity; 10 rejection rules and 6 demerit rules are related to this theme.

Nuisance. The 4 subclasses and 12 rules of the nuisance class cover structural themes that are known to be troublesome but that fall outside other rule classes. Several (azapteridine, naphthalene_sulfonate, benzocyclopentenone) capture chemotypes that have shown promiscuous activity in previous Lilly screening campaigns. The rules governing number of successive methylenes (C4 through C7) relate to lack of druggability attendant on excessive lipophilicity and flexibility.

Phosphorus. There are 4 subclasses and 10 rules. The 2 rules related to phosphate esters capture chemical series that are often nonspecific inhibitors of serine hydrolases[15] such as acetylcholinesterase. Most of the remaining phosphorus rules relate to the poor oral bioavailability of phosphates.

Protecting Group. The four protecting group rules (BOC, FMOC, phthalimide, trityl) are markers for chemical intermediates and predictors of excessive lipophilicity.

Quat. Quaternary amines usually do not cross the intestinal wall and therefore are not useful as oral drugs. However, if a quaternary amine is active, it is often possible to find the same activity with a tertiary amine; hence, this motif is not rejected but is penalized with 40 demerits. Aryl compounds with quaternary nitrogens (e.g., *N*-alkylpyridinium) are often toxic, for instance because of DNA intercalation,[19] and are therefore rejected.

Redox. Six of the seven redox rules refer to reducing agents that can form peroxides by redox cycling;[20] the peroxide rule captures direct oxidants.

Ring. The 23 rules related to ring systems are divided into 5 subclasses. Most of these rules relate to druggability.[21] Rejection reasons can include too many lipophilic rings (cyclohexane, isolated_aromatic), excessively large ring systems that can engender poor solubility (ring_system_too_large), and ring systems with a propensity for promiscuity (pyrrole_vinylidene, Table S1). Several rules for polyaromatic ring systems flag potential mutagens and/or fluors (anthracene_het). Compounds without any rings are more common in nondrug data sets such as ACD compared to drug data sets

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such as MDDR (Table S1). The no_rings rule is a marker for compounds that were not intended to be drugs (30 demerits).

Sulfur. Nineteen rules span nine subclasses. Sulfonic acids usually have poor oral absorption;[22] a single sulfonic acid incurs 40 demerits (sulfonic_acid rule) and the presence of two in the same molecule triggers a rejection (too_many_sulfonate rule). Thiols (70 demerits) bind nonspecifically to the metals in metalloenzymes and can be unstable. Thiones (40 demerits) are unstable and can hydrolyze into hydrogen sulfide.

Vinyl. There are 9 subclasses and 30 rules. Ten of the rules relate to Michael acceptors, reverse Michael acceptors, or their sulfur equivalents. For instance, the reverse_michael rule matches an amine separated from a carbonyl by an ethane linker. When protonated, the amine can dissociate, leaving a vinyl ketone that can act as a Michael acceptor. Several vinyl halide rules flag compounds with toxicity or promiscuity issues.

General Comments on the Queries. A common pattern of many of the above rules is the identification of a functional group likely to be reactive or unstable, the creation of a query to identify such groups, and the refinement of the query to exclude variations expected to be less troublesome. For instance, the michael_rejected query excludes acrylic acids (but not amides or esters) because the negative charge of the carboxylate at neutral pH should ameliorate the electron-withdrawing activity of the carbonyl, rendering the group much less reactive as a Michael acceptor. Such exceptions can be based on chemical theory (as in the previous example) or on empirical evidence that particular molecules do not show objectionable behavior. Although such refinements have been made for several rules, it is simply not practical to enumerate a detailed list of exceptions for every rule. For this reason, the rules will occasionally be overaggressive. An interesting example is the widely used antimalarial artemisinin[23] (Figure 2). This

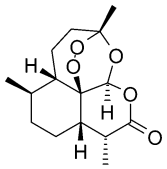

Figure 2. Artemisinin, a stable peroxide failing the rejection rules.

natural product contains an endoperoxide that is stabilized by the surrounding cyclic structure. Unless an institution has a specialized interest in such stabilized peroxides, it would not be a productive use of time to create an exception to the peroxide rejection rule to handle such cases.

Profile of the Structural Filters. The rules showed distinctive patterns when profiled against five molecule databases and against promiscuous and nonpromiscuous compounds from the Lilly screening database (Table 2). Three versions of the rules were used in the profiling: outright rejections computed without the demerit rules, the relaxed rules with a 160-demerit cutoff, and the regular rules with a 100-demerit cutoff. Of the five data sets, the Lilly database showed the lowest rate of outright rejections; this was not surprising, considering that nearly half of Lilly inventory had its origin in third-party purchases, almost all of which were filtered through some version of the structural rules. ACD 2005 showed the highest rate of outright rejections, mainly due to reactive compounds used as synthetic reagents. Interestingly, the 2010 version of ACD had a much lower rejection rate, suggesting that the rapid growth of that data set was fueled by addition of innocuous compounds, presumably various simple building blocks. The percentage of compounds failing on demerits varied from 4.5% for ACD up to 21% for MDDR. These results mostly reflect the relative content of larger compounds incurring demerits for atom count.

The relatively high rate of outright rejections in the MDDR data set is particularly noteworthy. Some of these include analogues of drugs with "rough" mechanisms: platinum complexes, alkylating antineoplastic agents, covalent-binding anti-infectives, etc. These would not be considered useful leads in contemporary drug screening programs. Others include rationally designed chemical probes. Many of these contain covalently binding electrophiles (aldehydes, electron-deficient ketones, boronic acids) or metal-binding "warheads" (hydroxamic acids, phosphonates). In many cases, the warheads dominate the energetics of inhibition to the point that variations in potency arise from structure–*reactivity* relationships rather than structure–*activity* relationships. With this type of mechanism, it is difficult to build in the exquisite selectivity needed for a safe drug. Utility as a pharmacological tool can also be compromised.[24]

Although commercially available "third-party" compounds also had a high rate of outright rejections, vendors are increasingly designing new additions to their collections with druggability in mind.[25]

Rejection rates for promiscuous compounds were higher than for nonpromiscuous compounds for all three levels of the filters. However, even the more-stringent regular rules only rejected about 36% of promiscuous compounds, suggesting that promiscuity may not be easily predictable from structural cues (see below for additional discussion of this issue). Conversely,

Table 2. Profiles of Various Data Sets against Three Versions of the Structural Filters[a]

		% fail				
data set	no. compds	outright rejection	relaxed	regular	no. rules matched	% fail on demerits
third-party	4256596	25.2	37.4	42.4	257	17.2
ACD 2010.3	2242400	20.1	24.0	24.5	269	4.5
ACD 2005	341214	39.1	45.9	47.6	261	8.5
Lilly	873801	17.7	23.3	27.1	271	9.4
MDDR	191778	27.7	40.9	48.6	250	20.9
promiscuous	6165	24.5	31.2	35.7	166	11.1
nonpromiscuous	300877	10.9	16.4	20.0	255	9.0

[a]"Outright rejection" refers to structures that fail at least one rejection rule without respect to demerits. The relaxed and regular rules include the effect of demerits, with cutoffs of 160 and 100 demerits, respectively. The promiscuous list was derived as described in the section Promiscuity Index. The nonpromiscuous list consisted of compounds with at least 100 screening results that were active at two or fewer target subfamilies.

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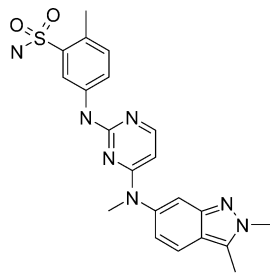
many of the rules related to chemical reactivity are not associated with elevated levels of promiscuity (Table S1). The chemical groups in question may not be reactive enough, may react with protein sites that are not critical for activity, or may be so reactive that they break down in storage or during aqueous dilution. Babaoglu et al.[26] found that only about 1% of the false positives in a β-lactamase screen were due to covalent binders.

Best-Selling Drugs. Of a set of 123 best-selling drugs,[27] 19 failed the rules as an outright rejection and an additional 18 failed on demerits (Table S3). Many of the outright rejections represented chemotypes that would not be considered useful screening hits under current practices. For instance, penicillins and cephalosporins are rejected because of their instability and acylating activity, as discussed previously. A few rejected drugs may represent examples where the rules are too aggressive, for instance, the vinyl_nitro rule causing the rejection of ranitidine (e.g., Zantac). Several steroids failed the Michael acceptor rule. These are probably too sterically hindered to act as strong Michael acceptors, but it would be difficult to devise a query to exclude them. Many of the drugs that fail on demerits do so because of molecular size. On the basis of the distinction between leadlike and druglike compounds,[24,28] these would not be considered promising screening hits.

Promiscuity Index. Two recent publications[4,5] focused on creation of rejection rules to identify compounds that show promiscuity in biological assays. Although some of the Lilly rules were inspired by promiscuous compounds (see section History of the Lilly Rules), these were based on a list of nuisance hits compiled in 2000. Biological data in the Lilly data warehouse have increased by nearly an order of magnitude since then, providing an opportunity to broaden our understanding of promiscuity and assay interference.

The first task was to define promiscuity. It was immediately apparent that simply counting the number of active targets could give misleading results. For instance, compound **3** (Figure 3), example 69 from a VEGFR2 inhibitor patent,[29] was



3

Figure 3. Example of a compound active at multiple targets within a single target subfamily (kinases).

active in 86 kinase assays but did not show activity at any of several dozen non-kinase targets. We would argue that **3** is not promiscuous, as it interacts specifically with the hinge-binding region of the kinase ATP binding site. The high number of active targets is solely due to the fact that it binds to a motif that exists in more than 500 copies in the human genome.

Compounds like **3** can radically skew an index of promiscuity if not compensated for in some way. We chose a definition of promiscuity that is insensitive to cross-activity at closely related targets. The 584 targets with defined gene identifiers in the Lilly data warehouse were divided into 17 target subfamilies: for instance, GPCR-A, GPCR-B, metalloprotease, cysteine protease, etc. (see Table 3 for names of subfamilies). Because of the high cross-correlations in activity between different kinase clades, all kinases were aggregated into a single subfamily. The promiscuity index was defined as the number of subfamilies at which a compound was active. Generous cutoffs of $IC_{50} < 10$ μM or single-point inhibition of >90% were used to define active targets. This allowed detection of promiscuous compounds active in the low micromolar range; our experience suggested that many of these will eventually be active in the submicromolar range when tested against a particularly sensitive target. Single-point inhibition of >90% was used because in assays with high hit rates, many single-point hits were never tested in concentration−response mode because of resource constraints.

Compounds with results in at least 100 assays were binned according to the number of subfamilies at which they were active (Figure 4). The number of compounds per bin in this histogram dropped off exponentially with the number of active subfamilies. Despite a conscious effort to devise rules that would exclude promiscuous compounds, failure rates never exceeded 50% except for the three compounds that were active at 12 subfamilies. Those three all failed the bis_aryl_maleimide rule, as discussed below. Nevertheless, failure rates rose with the number of subfamilies active, and this trend was seen for both outright rejections and failures for demerits.

Examples of compounds active at six or more subfamilies are given in Table 3 and Figure 5. Most of the compounds passing the structural filters (columns 1 and 3 of Figure 5) seem too simple to be causing widespread cross-activity, and it is likely that the activity is due to contaminants. For instance, a close analogue of **10** was active at a cysteine protease; an X-ray structure after cocrystallization with the enzyme revealed an empty binding site except for two atoms of a heavy metal, probably palladium or zinc left over from a catalytic coupling (Shane Atwell, personal communication). We have seen several other examples of undocumented or residual heavy metals in compound samples and speculate that this phenomenon may be far more common than generally realized. At the cysteine protease mentioned above, we saw several signs that many of the hits may have been due to contaminants. For instance, simple, innocuous structures with no obvious reactive groups produced irreversible inhibition. On the basis of these observations, the team decided to repurify a diverse selection of the hits and found that 38 out of 40 were inactive after repurification (R.F.B., Michael R. Wiley, and Douglas R. Stack, unpublished observations). The remaining two were irreversible; one had a potential reactive group (carbamate) and the other had a structure capable of carrying a tightly bound heavy metal through the purification. Contaminants may also be responsible for other types of interference. For instance, reduction of resazurin to resorufin is a sensitive method for detecting redox cycling.[30,31] However, when we attempted to model 3000 data points from this assay, we achieved a q^2 of only 0.1, suggesting that activity was unrelated to structure and possibly due to contaminants. Leaching of polyanions from cation exchange resins (SCX) used to purify final products was a frequent source of false hits in the 1990s; more recently, better practices including extensive prewashing of the columns and better quality control by manufacturers have reduced the problem. However, our recent experience suggests that this problem persists to some extent even in newer samples, and in

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Table 3. Activity Profiles of Randomly Chosen Compounds Active at Six or More Target Subfamilies

ID	assays tested	assays active	subfamilies active	cyp450	gpcr_a	gpcr_b	gpcr_c	gpcr_u	ion channel	kinase	lipid metab	nuclear receptor	other	PDE	phosphatase	aspartyl protease	cysteine protease	metalloprotease	serine protease	transporter
4	495	48	11	0	15	0	4	2	2	5	2	6	3	3	0	3	0	0	0	3
5	424	80	11	0	14	0	2	0	1	49	1	3	0	3	0	3	2	1	0	1
6	326	48	10	0	3	0	7	0	3	16	3	1	2	0	0	0	6	2	5	0
7	687	36	10	0	8	0	2	0	3	2	3	10	3	2	0	2	0	0	1	0
8	515	38	9	0	6	0	0	0	0	18	3	0	1	0	0	1	4	3	1	1
9	274	12	9	0	1	1	0	0	0	2	2	1	1	0	0	1	2	1	0	0
10	382	16	8	0	1	2	4	0	0	1	0	2	3	0	0	2	0	0	0	1
11	415	58	8	0	36	0	2	0	2	3	0	2	2	0	0	0	0	0	2	9
12	769	42	7	0	32	0	0	0	3	1	0	0	2	0	0	0	1	1	0	2
13	334	20	7	0	11	1	0	0	0	0	0	0	1	1	0	2	0	2	0	2
14	140	16	6	0	0	0	0	0	0	2	0	1	8	1	0	0	3	1	0	0
15	156	11	6	0	1	0	1	0	2	2	0	1	4	0	0	0	0	0	0	0

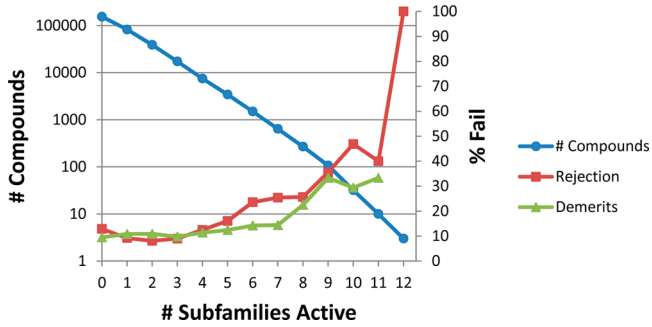

Figure 4. Failure rates for structural filters as a function of promiscuity. Only compounds with at least 100 screening results were included in the analysis (306 461 compounds). Since demerit queries are only run on compounds passing the outright rejection rules, the failure rate for demerit filters was defined relative to number of compounds passing the outright rejection rules.

addition many of the older samples remain in the compound collection and may cause false hits if they enter the screening queue. It should be noted that neither heavy metals nor polymeric contaminants will show up in LC/MS and NMR analyses. Baell[32] reported that carry-through of synthetic intermediates can result in promiscuous activity; the tetrahydroquinoline series mentioned by Baell has shown widespread activity at Lilly targets and is now the subject of a rejection query (see section Evaluation of PAINS Queries).

The results with regard to contamination cited above show that there are limits to the extent that promiscuous compounds can be removed by substructure filters alone. For this reason, we decided to create a "no-screen" list of compounds that showed promiscuity in biological assays. An immediate concern was where to draw the line between promiscuous and nonpromiscuous. Checking of activity profiles and structures indicated that compounds active at six or more subfamilies were unlikely to be innocent. This list included known nonproductive hits from several historical screening campaigns. The same was true for compounds active at five subfamilies, with one exception: compounds with a positive charge that were active at type A GPCR, ion channel, and transporter subfamilies often showed typical pharmacophoric features for activity at biogenic amine targets.[33] An example is the antipsychotic chlorpromazine (Figure 6). Taking this into account, compounds active at exactly five subfamilies were included in the promiscuous list except when they had a basic group and were active in at least two of the following subfamilies: type A GPCR, ion channel, and transporter. Compounds active at four or fewer subfamilies were excluded from the promiscuous list. Since active compounds from previous projects are often purposely over-represented in screening decks, it is not uncommon to see a chemical series recycled into two or even three projects. For instance, we are aware of a series that produced a clinical candidate for a type A GPCR target, then later produced hits for an ion channel target, and subsequently yielded hits at an enzyme target. The specificity of interactions was confirmed by achievement of single-digit nanomolar affinity for all three targets as well as production of a protein−ligand X-ray structure for the enzyme target.

In chemical databases, compounds are characterized by serial number, linked uniquely to chemical structure, and by lot number, linked uniquely to the batch of compound that was synthesized or purchased. Since promiscuity often originates

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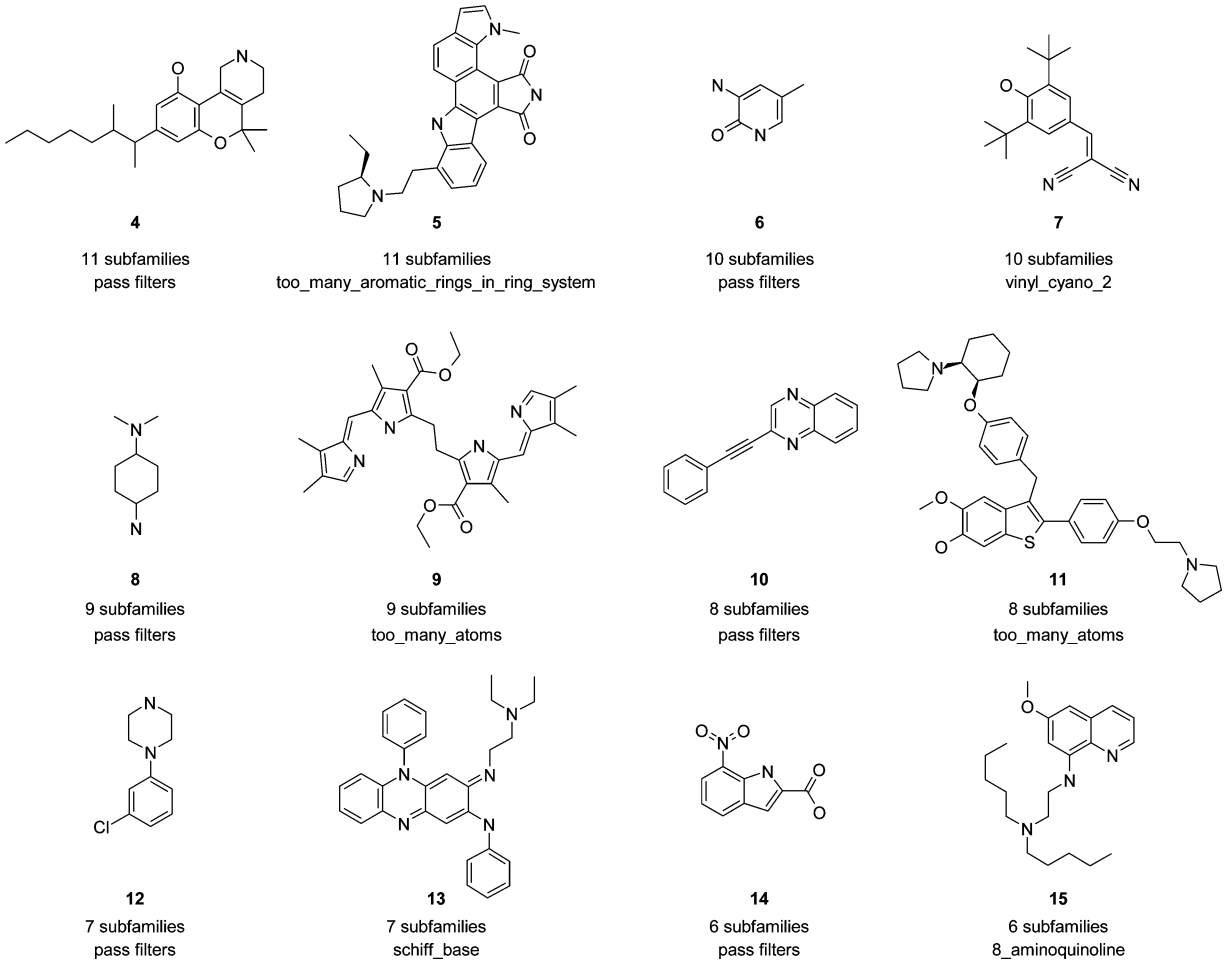
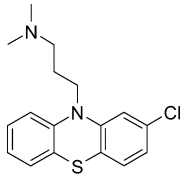


Figure 5. Structures of compounds from Table 3.



16

Figure 6. Chlorpromazine. This compound is active at five subfamilies, including biogenic amine related targets in the type A GPCR, ion channel, and transporter subfamilies.

from impurities in a given batch of a compound, promiscuity should ideally be computed versus lot number. Although most large biological databases do link activity to lot number, in many cases the tools for data retrieval key on serial number; these tools will need to be modified to assign promiscuity scores to chemical lot numbers.

Evaluation of PAINS Queries. Baell and Holloway[5] recently reported a series of structural filters based on promiscuous activity at six screening targets for a test set of 93 000 compounds. We wished to evaluate the PAINS queries against our large internal results database. Because the main purpose was to identify gaps in the Lilly filters, we began with two lists of compounds that passed the regular Lilly rules: 4351 promiscuous compounds as defined above and 242 466 nonpromiscuous compounds, defined as those tested at least 100 times that were active versus two or fewer subfamilies. An enrichment factor was defined as the match rate for the promiscuous set divided by the match rate for the nonpromiscuous set. The PAINS queries matched 286 promiscuous compounds that passed the Lilly rules, compared to 3986 in the nonpromiscuous set, for an enrichment factor of 4.0. Although 67 PAINS queries matched at least one promiscuous compound, only nine queries matched at least five promiscuous compounds and had an enrichment of at least 5. Compounds matching these nine queries were examined in detail, and new rules or modifications of existing rules were created for six of them (Table 4). (We were unable to incorporate the PAINS queries directly into our filters because our substructure engine cannot read the Tripos query language used for the PAINS queries.)

Evaluation of BMS Queries. A set of 191 structural filters coded in smarts was reported by investigators from Bristol-Myers Squibb.[4] The filters were motivated in part by promiscuity data distilled from about 60 million biological assay results. Three of the queries matched at least five promiscuous compounds with an enrichment factor of 5 or better (Table 5). The smarts for these three queries were incorporated directly into the Lilly rules.

Additional Promiscuity-Related Rules. Promiscuous compounds passing the Lilly rules were clustered by scaffold, and clusters with at least six members were examined. By far the most prominent cluster was a set of 370 bis-arylmaleimides

Table 4. PAINS Rules Matching at Least Five Promiscuous Compounds and Having at Least 5-Fold Enrichment for Promiscuous over Nonpromiscuous Compounds[a]

PAINS rule	no. not promiscuous	no. promiscuous	enrichment	outcome
quinone_A	7	5	39.80	broaden existing quinone_para rule
quinone_B	13	7	30.01	new rule anthra_ketone
naphth_amino_A	73	18	13.74	new rule perimidine
anil_alk_ene	119	25	11.71	new rule fused_tetrahydroquinoline
imidazole_A	85	17	11.15	skip; several literature kinase inhibitors fail
cyano_pyridone_A	31	5	8.99	matches milrinone (marketed PDE inhibitor)
anil_di_alk_B	105	14	7.43	4-vinylaniline query not specific enough; dye marker?
ene_one_hal	48	5	5.80	change existing anthracene_het rule to rejection
thiophene_amino_Aa	49	5	5.69	boost existing thiophene_furan_nh rule to 70 demerits
all matches	3986	286	4.00	

[a]The PAINS rules were run under Sybyl using the original queries from Baell and Holloway.[5] Enrichment is the fraction matching in the promiscuous set divided by the fraction matching in the nonpromiscuous set.

Table 5. BMS Rules Matching at Least Five Promiscuous Compounds and Having at Least 5-Fold Enrichment for Promiscuous over Nonpromiscuous Compounds

BMS rule	no. not promiscuous	no. promiscuous	enrichment	outcome
quinone_methide	33	10	17.16	create new rule from smarts
rhodanine	43	5	6.59	create new rule from smarts
non_ring_ketal	101	10	5.61	create new rule from smarts
all matches	12245	191	0.88	

(BAMs). The BAMs are potent, broadly active kinase inhibitors related to the natural product staurosporine.[34] Widespread activity at non-kinase targets is probably related to aggregation.[35] Lilly's experience with following up on BAM hits at non-kinase targets has not been productive. At the same time, representative BAMs have already been profiled at a wide variety of kinase targets, diminishing the value of additional screening. For these reasons, a rejection rule for BAMs was created.

Several other large clusters consisted of small, apparently innocuous compounds. It is likely that promiscuity of these compounds is due to contaminants.

Several publications from the Shoichet group describe aggregation as a source of promiscuous activity.[3,36−38] We checked for exact matches in the Lilly collection of compounds listed as aggregators in Table S5 from Feng et al.[36] Selection criteria were >50% inhibition of β-lactamase at 30 μM and SCATTER or SCATTER_80 rating in dynamic light scattering. Eleven exact matches had at least 100 testing results, but none were active at more than two subfamilies. Although based on a limited sample size, these results suggest that aggregation is not a major source of promiscuity under current screening practices, in which detergent is added to prevent aggregation-based inhibition.[39]

CONCLUSIONS

In this paper, we provide an account of structural queries designed to remove compounds that are likely to provide invalid or misleading screening results. These include compounds that are unstable, whose screening results might not be attributable to the nominal structure; reactive compounds whose activity could be due to undesirable covalent modification of proteins; compounds that interfere with activity measurements; promiscuous compounds that are active at multiple target families; and compounds that would not be considered for follow-up because of toxicity, poor stability in vivo, etc. The rules are used to filter primary and follow-up screensets, to triage compounds being considered for purchase, and as alerts for compounds being proposed for synthesis. Details of the queries and search engine are included, along with experience motivating the rules. We also present a novel index of promiscuity based on the number of target subfamilies at which a compound is active. We find that the application of these tools significantly reduces the amount of time devoted to following up on unproductive screening actives.

ASSOCIATED CONTENT

Supporting Information

A pdf file containg detailed description of the substructure search engine, legend to Table S1 (individual rules), Table S2 (smiles for an example structure for each rule), Table S3 (outcomes of rules for best-selling drugs), and text of substructure queries; an Excel file containing the contents of Table S1 (individual rules and information on number of matches). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

ACD, Available Chemicals Directory; BAM, bis-arylmaleimide; BMS, Bristol-Myers Squibb; MACCS, molecular access system; MDDR, MDL Drug Data Report; OIDD, Open Innovation Drug Discovery; PAINS, pan assay interference compounds; VEGFR2, vascular endothelial growth factor receptor 2

■ REFERENCES

(1) Shoichet, B. K. Screening in a spirit haunted world. *Drug Discovery Today* **2006**, *11*, 607−615.

(2) Thorne, N.; Auld, D. S.; Inglese, J. Apparent activity in high-throughput screening: origins of compound-dependent assay interference. *Curr. Opin. Chem. Biol.* **2010**, *14*, 315−324.

(3) McGovern, S. L.; Caselli, E.; Grigorieff, N.; Shoichet, B. K. A common mechanism underlying promiscuous inhibitors from virtual and high-throughput screening. *J. Med. Chem.* **2002**, *45*, 1712−1722.

(4) Pearce, B. C.; Sofia, M. J.; Good, A. C.; Drexler, D. M.; Stock, D. A. An empirical process for the design of high-throughput screening deck filters. *J. Chem. Inf. Model.* **2006**, *46*, 1060−1068.

(5) Baell, J. B.; Holloway, G. A. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J. Med. Chem.* **2010**, *53*, 2719−2740.

(6) Rishton, G. M. Reactive compounds and in vitro false positives in HTS. *Drug Discovery Today* **1997**, *2*, 382−384.

(7) Hann, M.; Hudson, B.; Lewell, X.; Lifely, R.; Miller, L.; Ramsden, N. Strategic pooling of compounds for high-throughput screening. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 897−902.

(8) Walters, W. P.; Namchuk, M. Designing screens: how to make your hits a hit. *Nat. Rev. Drug Discovery* **2003**, *2*, 259−266.

(9) Rishton, G. M. Nonleadlikeness and leadlikeness in biochemical screening. *Drug Discovery Today* **2003**, *8*, 86−96.

(10) Open Innovation Drug Discovery. Connecting Compounds to Patients. https://openinnovation.lilly.com/dd/.

(11) MSI Cerius2. http://www.chem.cmu.edu/courses/09-560/docs/msi/modenv/D_Files.html#948951

(12) Garofalo, A. W.; Wone, D. W. G.; Phuc, A.; Audia, J. E.; Bales, C. A.; Dovey, H. F.; Dressen, D. B.; Folmer, B.; Goldbach, E. G.; Guinn, A. C.; Latimer, L. H.; Mabry, T. E.; Nissen, J. S.; Pleiss, M. A.; Sohn, S.; Thorsett, E. D.; Tung, J. S.; Wu, J. A series of C-terminal amino alcohol dipeptide A[beta] inhibitors. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3051−3053.

(13) Lajiness, M. S.; Maggiora, G. M.; Shanmugasundaram, V. Assessment of the consistency of medicinal chemists in reviewing sets of compounds. *J. Med. Chem.* **2004**, *47*, 4891−4896.

(14) OIDD Chemoinformatics Structure Evaluation. https://openinnovation.lilly.com/dd/docs/oidd_cheminformatics_structure_evaluation.pdf.

(15) Powers, J. C.; Asgian, J. L.; Ekici, Ö. D.; James, K. E. Irreversible inhibitors of serine, cysteine, and threonine proteases. *Chem. Rev.* **2002**, *102*, 4639−4750.

(16) Otto, H.-H.; Schirmeister, T. Cysteine proteases and their inhibitors. *Chem. Rev.* **1997**, *97*, 133−172.

(17) Doose, C. A.; Ranke, J.; Stock, F.; Bottin-Weber, U.; Jastorff, B. Structure−activity relationships of pyrithiones: IPC-81 toxicity tests with the antifouling biocide zinc pyrithione and structural analogs. *Green Chem.* **2004**, *6*, 259−266.

(18) Committee on Amines. *Aromatic Amines: An Assessment of the Biological and Environmental Effects*; National Academy Press: Washington, DC, 1981.

(19) Waring, M. J. Complex formation between ethidium bromide and nucleic acids. *J. Mol. Biol.* **1965**, *13*, 269−282.

(20) Bova, M. P.; Mattson, M. N.; Vasile, S.; Tam, D.; Holsinger, L.; Bremer, M.; Hui, T.; McMahon, G.; Rice, A.; Fukuto, J. M. The oxidative mechanism of action of ortho-quinone inhibitors of protein-tyrosine phosphatase α is mediated by hydrogen peroxide. *Arch. Biochem. Biophys.* **2004**, *429*, 30−41.

(21) Lovering, F.; Bikker, J.; Humblet, C. Escape from Flatland: increasing saturation as an approach to improving clinical success. *J. Med. Chem.* **2009**, *52*, 6752−6756.

(22) Hogben, C. A. M.; Schanker, L. S.; Tocco, D. J.; Brodie, B. B. Absorption of drugs from the stomach. II. The human. *J. Pharmacol. Exp. Ther.* **1957**, *120*, 540−545.

(23) Qinghaosu Antimalarial Coordinating Research Group.. Antimalaria studies on qinghaosu. *Chin. Med. J.* **1979**, *92*, 811−816.

(24) Rishton, G. M. Molecular diversity in the context of leadlikeness: compound properties that enable effective biochemical screening. *Curr. Opin. Chem. Biol.* **2008**, *12*, 340−351.

(25) Verheij, H. Leadlikeness and structural diversity of synthetic screening libraries. *Mol. Diversity* **2006**, *10*, 377−388.

(26) Babaoglu, K.; Simeonov, A.; Irwin, J. J.; Nelson, M. E.; Feng, B.; Thomas, C. J.; Cancian, L.; Costi, M. P.; Maltby, D. A.; Jadhav, A.; Inglese, J.; Austin, C. P.; Shoichet, B. K. Comprehensive mechanistic analysis of hits from high-throughput and docking screens against $\hat{I}^2$-lactamase. *J. Med. Chem.* **2008**, *51*, 2502−2511.

(27) The Research Group of Jon Tryggvi Njardarson at the University of Arizona. http://cbc.arizona.edu/njardarson/group/.

(28) Oprea, T. I.; Davis, A. M.; Teague, S. J.; Leeson, P. D. Is there a difference between leads and drugs? A historical perspective. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 1308−1315.

(29) Boloor, A. C., M.; Davis, R.; Harris, P. A.; Hinkle, K.; Mook, R. A., Jr.; Stafford, J. A.; Veal, J. M. Chemical Compounds. US 2007/0015756 A1, 2007.

(30) Ali-Vehmas, T.; Louhi, M.; Sandholm, M. Automation of the resazurin reduction test using fluorometry of microtitration trays. *J. Vet. Med., Ser. B* **1991**, *38*, 358−372.

(31) Lor, L. A.; Schneck, J.; McNulty, D. E.; Diaz, E.; Brandt, M.; Thrall, S. H.; Schwartz, B. A simple assay for detection of small-molecule redox activity. *J. Biomol. Screening* **2007**, *12*, 881−890.

(32) Baell, J. B. Observations on screening-based research and some concerning trends in the literature. *Future Med. Chem.* **2010**, *2*, 1529−1546.

(33) Peters, J.-U.; Hert, J.; Bissantz, C.; Hillebrecht, A.; Gerebtzoff, G.; Bendels, S.; Tillier, F.; Migeon, J.; Fischer, H.; Guba, W.; Kansy, M. Can we discover pharmacological promiscuity early in the drug discovery process? *Drug Discovery Today* **2012**, *17*, 325−335.

(34) Omura, S. I., Y.; Hirano, A.; Nakagawa, A.; Awaya, J.; Tsuchiya, H.; Takahashi, Y.; Masuma, R. A new alkaloid AM-2282 of Streptomyces origin: taxonomy, fermentation, isolation and preliminary characterization. *J. Antibiot.* **1977**, *30*, 275−282.

(35) McGovern, S. L.; Shoichet, B. K. Kinase inhibitors: not just for kinases anymore. *J. Med. Chem.* **2003**, *46*, 1478−1483.

(36) Feng, B. Y.; Shelat, A.; Doman, T. N.; Guy, R. K.; Shoichet, B. K. High-throughput assays for promiscuous inhibitors. *Nat. Chem. Biol.* **2005**, *1*, 146−148.

(37) McGovern, S. L.; Helfand, B. T.; Feng, B.; Shoichet, B. K. A specific mechanism of nonspecific inhibition. *J. Med. Chem.* **2003**, *46*, 4265−4272.

(38) Giannetti, A. M.; Koch, B. D.; Browner, M. F. Surface plasmon resonance based assay for the detection and characterization of promiscuous inhibitors. *J. Med. Chem.* **2008**, *51*, 574−580.

(39) Ryan, A. J.; Gray, N. M.; Lowe, P. N.; Chung, C.-w. Effect of detergent on "promiscuous" inhibitors. *J. Med. Chem.* **2003**, *46*, 3448−3451.