Chemistry & Biology
Article

Compound Prioritization Methods Increase Rates of Chemical Probe Discovery in Model Organisms

Iain M. Wallace,[1,2,7] Malene L. Urbanus,[1,3,7] Genna M. Luciani,[1,4] Andrew R. Burns,[1,4] Mitchell K.L. Han,[3] Hao Wang,[5] Kriti Arora,[1] Lawrence E. Heisler,[1,3] Michael Proctor,[6] Robert P. St. Onge,[6] Terry Roemer,[5] Peter J. Roy,[1,4] Carolyn L. Cummins,[3] Gary D. Bader,[1,2,4] Corey Nislow,[1,2,4] and Guri Giaever[1,3,4,*]
[1]Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario M5S 3E1, Canada
[2]Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario M5G 1L6, Canada
[3]Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario M5S 3M2, Canada
[4]Department of Molecular Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada
[5]Infectious Disease Research, Merck, Kenilworth, NJ 07033, USA
[6]Stanford Genome Technology Center, Palo Alto, CA 94304, USA
[7]These authors contributed equally to this work
*Correspondence: g.giaever@utoronto.ca
DOI 10.1016/j.chembiol.2011.07.018

SUMMARY

Preselection of compounds that are more likely to induce a phenotype can increase the efficiency and reduce the costs for model organism screening. To identify such molecules, we screened ~81,000 compounds in *Saccharomyces cerevisiae* and identified ~7500 that inhibit cell growth. Screening these growth-inhibitory molecules across a diverse panel of model organisms resulted in an increased phenotypic hit-rate. These data were used to build a model to predict compounds that inhibit yeast growth. Empirical and *in silico* application of the model enriched the discovery of bioactive compounds in diverse model organisms. To demonstrate the potential of these molecules as lead chemical probes, we used chemogenomic profiling in yeast and identified specific inhibitors of lanosterol synthase and of stearoyl-CoA 9-desaturase. As community resources, the ~7500 growth-inhibitory molecules have been made commercially available and the computational model and filter used are provided.

INTRODUCTION

Current methods for identifying lead chemical probes frequently rely on high-throughput screening against select targets of interest. This approach assumes that in vitro high potency of small molecules will translate to low-dose efficacy in vivo. However, this is often not the case (Gleeson et al., 2011). In contrast, in vivo model organism screening provides a direct measure of cellular potency, bypassing the bias of target preselection typically used in modern drug discovery. A growing number of academic laboratories are pursuing model organism screens to identify chemical probes for use as powerful molecular tools to probe biological function (Frearson and Collie, 2009). Chemical probes complement standard genetic approaches to elucidate gene function while offering distinct advantages. For example, when applied to a cell or whole organism, the effects induced by chemical probes are often rapid, reversible, and tuneable (Morgan et al., 2008; Oprea et al., 2009; Workman and Collins, 2010). Moreover, chemical probes can often be transferred across model organisms, regardless of their genetic tractability (Specht and Shokat, 2002). One drawback of chemical and chemical-genetic screens is that the percentage of compounds that results in a desired phenotype is often small; for example, in a *Caenorhabditis elegans* study, only 2% of pharmacologically active compounds resulted in a phenotype (screened at 25 μM) (Kwok et al., 2006) and in a study using a hyperpermeable *Escherichia coli* strain, only 3.5% of compounds (screened at 50 μM) resulted in growth inhibition (Li et al., 2004). These observations, combined with the fact that model organism screening can be both compound-intensive and time-consuming (Burns et al., 2010; Wheeler and Brändli, 2009) places an emphasis on compound selection before screening in contrast to typical in vitro high-throughput screening campaigns (Agresti et al., 2010; Lipinski and Hopkins, 2004) in which the number of total hits is higher and compound consumption is lower. Such prescreening compound selection strategies may include enriching for known active substructures against multiple targets ("privileged structures") (Klekota and Roth, 2008) and/or enriching for compounds most likely to accumulate in the organism of interest (Burns et al., 2010).

The preselection strategy described here is aimed at increasing the discovery rate of lead chemical probes in model organisms by first identifying small molecules that inhibit yeast growth. Growth is a comprehensive phenotype, combining multiple effects on cellular physiology into a single quantitative metric (Botstein and Fink, 1988). Moreover, growth measurements can be made in a rapid, high-throughput, and low-cost manner (Paixão et al., 2008; Proctor et al., 2011). Here, we first screened ~81,000 commercially available synthetic compounds and identified ~7500 compounds that inhibit growth of *S. cerevisiae*. It is noteworthy that yeast screens often require significantly higher doses (approximately 5–10x) compared with typical mammalian cell culture screens or in vitro assays (see Figure S1 available online) (Blackburn and Avery, 2003;

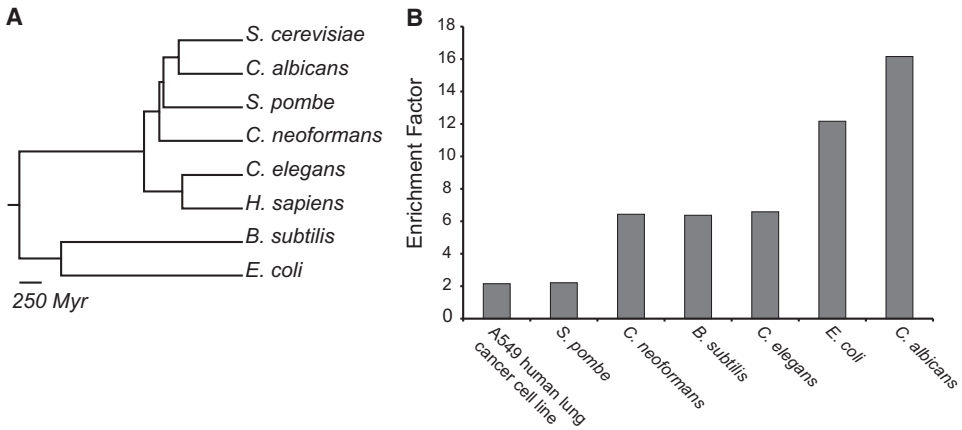

Figure 1. Yactives Enrich for Phenotypes in Diverse Organisms

(A) Phylogenetic tree of the eight organisms screened in this study. Yeast generally requires higher screening concentrations than mammalian screens for similar inhibition levels, as shown in Figure S1.

(B) Enrichment factor; ratio of hit-rate for yactive compounds compared with randomly selected compounds empirically determined by screening seven organisms.

Buurman et al., 2005; Ericson et al., 2008; Kwak et al., 2011). Although our initial yeast screening concentrations are relatively high (maximum 200 μM), this high dose does not sacrifice specificity (Blackburn and Avery, 2003; Botet et al., 2007; Dias et al., 2010; Dorer et al., 2005; Ericson et al., 2008; Giaever et al., 2004; Khozoie et al., 2009; Kwak et al., 2011; Murén et al., 2001). Several biological factors also contribute to yeast's ability to resist chemical perturbation, including the physical barrier of the yeast cell wall (Dielbandhoesing et al., 1998) and a dynamic defense known as the pleiotropic drug response (PDR). The PDR is comprised of efflux pumps that reduce the intracellular dose of a broad spectrum of diverse small molecules (Ernst et al., 2010; Kolaczkowski et al., 1998; Rogers et al., 2001).

Once we had identified the ~7500 yeast bioactives or "yactives," we then tested the set on a diverse set of model organisms for bioactivity. We found that the yactives significantly increased phenotypic hit-rates compared with randomly selected compounds. Using the physicochemical properties of the yactives, we designed a two-property compound filter based on a simple modification of the Lipinski's rule-of-five (Lipinski et al., 2001) and in addition, we built a Naïve Bayes model to identify substructures present in yactives. We demonstrate both empirically and in silico (using publicly available datasets) that application of the two-property filter and the Naïve Bayes model result in an enrichment for phenotype-inducing compounds in diverse model organisms. Finally, we address the question of whether growth inhibitory compounds have the potential to become specific chemical probe leads by testing twenty of the most potent growth-inhibitory compounds in vivo against all ~1100 essential yeast proteins using our well-validated HaploInsufficiency Profiling (HIP) assay (Baetz et al., 2004; Giaever et al., 1999; Giaever et al., 2004; Lain et al., 2008; Lum et al., 2004; Xu et al., 2007). Several of these compounds exhibit specific genome-wide profiles, identifying candidates for the most likely protein target(s). We pursued two of the most promising target candidates: one supporting lanosterol synthase (Erg7 in yeast, mammalian homolog LSS) and the second supporting fatty acid desaturase (Ole1 in yeast, mammalian homolog SCD) as the primary targets. We confirmed these two targets genetically and in independent secondary assays. Taken together, our results demonstrate that preselection and prioritization of compound libraries increase the likelihood of identifying specific chemical probe leads for model organisms while decreasing overall costs. To disseminate these tools, the yactives have been made available through ChemBridge, Inc. (San Diego, CA) and we provide a prioritized list of compounds generated by applying our model to all commercially available small molecules (Irwin and Shoichet, 2005) on our supplementary website (http://chemogenomics.med.utoronto.ca/supplemental/bioactive/).

RESULTS

Small Molecules that Inhibit Yeast Growth Increase the Phenotypic Hit-Rate in Other Model Organisms

To identify small molecules that decrease yeast fitness or growth, we screened 81,320 commercially available synthetic compounds (Table S1) and identified 7476 small molecules that inhibit wild-type (WT) *S. cerevisiae* growth by at least 30% (IC_{30}) (see Experimental Procedures). We next asked whether this set of yactives was enriched for molecules that induce a phenotype when tested across a diverse set of model organisms, spanning substantial evolutionary distance (Figure 1A). Subsets of the 7476 yactives were screened against our panel of model organisms (as well as a human cell line) and the results compared with those obtained from screening random compounds (Figure 1B; Table S2). Yactives significantly enriched for compounds that inhibited growth (IC_{50} or greater) in human A549 non–small-cell lung carcinoma cells, *Schizosaccharomyces pombe*, *Cryptococcus neoformans*, *E. coli*, *Bacillus subtilis*, and *Candida albicans*. The increase in phenotypic hit-rate was independent of evolutionary distance. Notably, in the model metazoan *C. elegans* (where hit-rate was determined by visual inspection) the yactives increased the discovery rate 6.6x over random compounds (Figure 1B).

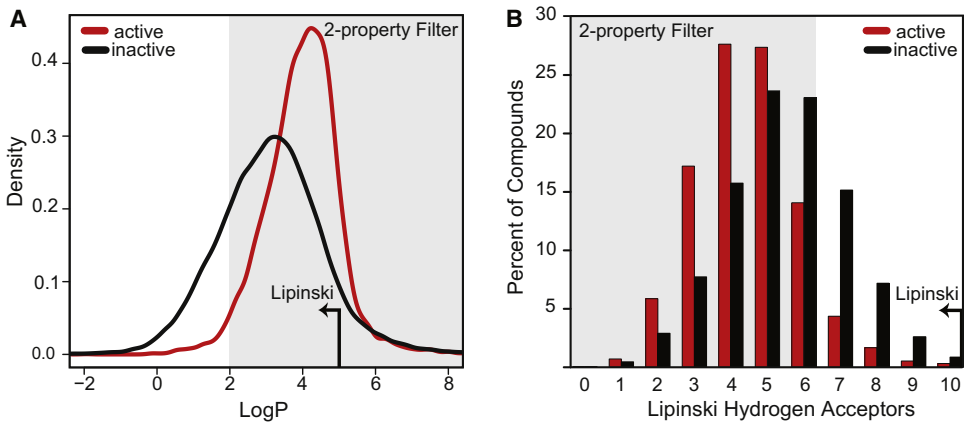

Figure 2. A Two-Property Filter Enriches for Yactive Compounds

(A) Distribution plot of LogP for active compounds versus inactive compounds. The shaded region indicates compounds that pass the two-property filter of $\geq$2 LogP, significantly increasing the percentage of phenotypic-inducing compounds. For comparison, the Lipinski limit of $\leq$5 is indicated by an arrow.

(B) Histogram showing the percentage of compounds of active compounds versus inactive compounds. The shaded region indicates compounds that pass the two-property filter of $\leq$6 hydrogen acceptors, significantly increasing the fraction of phenotypic-inducing compounds. For comparison, the Lipinski limit of $\leq$10 is indicated by an arrow.

Physicochemical Properties of Growth-Inhibitory Compounds

Because the majority of the 81,320 synthetic compounds screened adhere to Lipinski's rule-of-five (intended to define chemical properties that reflect oral bioavailability) (Lipinski et al., 2001), we asked whether a simple modification of these rules could be used as a yactive filter. Two of four physicochemical properties that comprise Lipinski's rule-of-five were significantly different (p value $< 1 \times 10^{-15}$) in yactive compounds versus inactive compounds (Table S3). First, Lipinski's rule states that compounds should have a calculated octanol-water partition coefficient LogP $\leq$5. In contrast, yactive compounds are more lipophilic (mean LogP = 4.0) than inactive compounds (mean LogP = 3.1) (Figure 2A). This observation suggests that they are more likely to be passively transported into the cell because their solubility in a lipid rich-environment would be expected to contribute to cell permeability (Al-Awqati, 1999; Gamo et al., 2010; Hacker et al., 2009). Second, Lipinski's rule includes compounds that have $\leq$10 hydrogen acceptors, whereas yactives are best described using a limit of $\leq$6 hydrogen acceptors (Figure 2B). This decreased number of hydrogen acceptors also reflects the likelihood that such compounds can be passively transported across the cell membrane (Muegge, 2003). The increased hit-rate achieved by applying a two-property filter based on these observations (compounds pass if they have a LogP $\geq$2 and hydrogen acceptors $\leq$6) (12.7% compared with 9.2%, p value $< 1 \times 10^{-15}$, Table S2) prior to purchase would have reduced the number of compounds screened from 81,320 to 53,480 (a 30% cost savings) while still identifying 91% of the original 7475 yactives, demonstrating that even such a modest increase in hit-rate can result in substantial cost savings.

Application of a Naïve Bayes Model Allows Prediction of Yactives

Encouraged by the increased hit-rate resulting from our two-property modification of Lipinski's rule-of-five, we built a Naïve Bayes model to better enable prediction of yactives. Specifically, in this model, substructures in active compounds are weighted higher than those found in inactive compounds, resulting in a prioritized list of compounds for screening. The Naïve Bayes model was built using the data from our original 81,320 compounds as a training set. ECFP_4 topological fingerprints (Rogers et al., 2005) were selected to represent substructures because it outperformed three other representational methods (Figure 3A). Five-fold cross validation was used, with four-fifths of the original screening data used as the training set, and the remaining one-fifth used to test the model's performance. This procedure was repeated five times, and the model's performance reported as the average over the iterations. At a cutoff of the top 10% of ranked compounds, the ECFP_4 model resulted in an enrichment factor of ~4.5, defined as ~4.5-fold the number of yactives compared with a random set of compounds (Figure 3A).

To address potential overestimation bias from using the same library for model building and testing, we assessed the performance of the model on an independent chemical library. Because this library has different structural property distributions than the training set, it better represents real-world performance. To generate this dataset, the Spectrum Library of ~2000 compounds was screened against *S. cerevisiae* to identify yactives followed by application of the Naïve Bayes model. The top 10% of compounds ranked by the model showed an enrichment factor of ~3.5 for the yactives (Figure 3B). Extending these tests, we applied our model to publically available chemical screening data. Specifically, the model was applied to the data from 29 yeast assays available from PubChem (Wang et al., 2010); 23 of the assays were designed to identify modulators of yeast growth (in mutant backgrounds or in WT strains), and six relied on readouts other than growth inhibition (see Table S4 for assay details, Table S5 for two-property filter results, and Table S6 for Naïve Bayes model results). Our model performed well across nearly all of these diverse assays, achieving a median enrichment factor 1.85 in the top 10% of compounds ranked (Figure 3C).

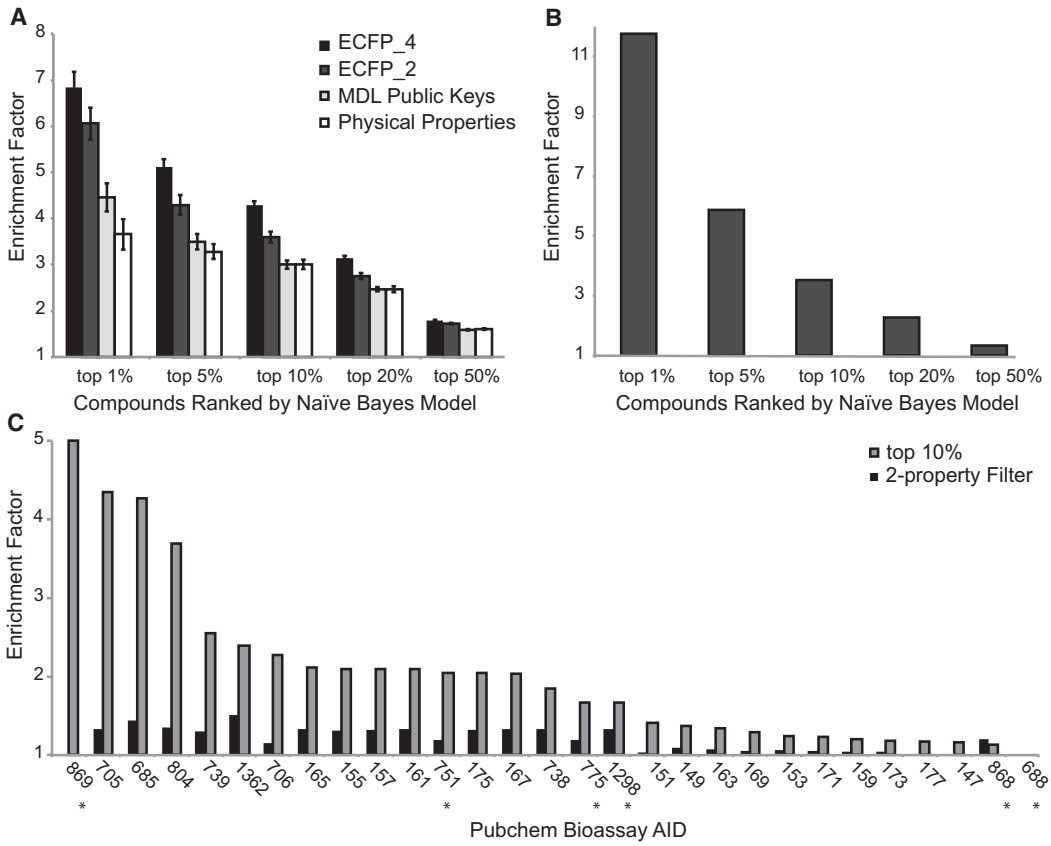

Figure 3. Application of a Naïve Bayes Model Enriches for Yactive Compounds

(A) Cross-validation results for four different structure encoding schemes. The error bars represent the standard deviation obtained by repeating the procedure five times.

(B) Performance of the Naïve Bayes model on the Spectrum Library, a library independent of that used for training the model.

(C) The median enrichment of the Naïve Bayes model and the two-property testing using 29 publicly available yeast BioAssays available from PubChem. Assay details are available in Table S4, results for the two-property filter in Table S5, and the Naïve Bayes model results in Table S6. Absent bars indicate an enrichment factor <1. Assays marked with an asterisk (*) used readouts that were not based on growth inhibition.

Predictive Approaches Enrich for Phenotype-inducing Compounds in Diverse Organisms

We next asked how well the Naïve Bayes model and the two-property filter enriched for phenotype-inducing compounds when applied to our data for other model organisms (*S. pombe*, *B. subtilis*, *E. coli*, and *C. elegans*). Comparison of the performance of three approaches (yactives/Naïve Bayes/two-property filter) revealed that empirical screening of the yactives gave the best performance (median enrichment factor 5.95), the Naïve Bayes model performed nearly as well (median enrichment factor 4.30), whereas the two-property filter performed appreciably lower (median enrichment factor 1.64) (Figure 4A; see also Table S2 and Table S7). To avoid overestimating the level of performance of the Naïve Bayes model as a result of the model being tested on the same library as the training set, we tested the performance using results from nine publicly available small-molecule screens performed in four organisms (*E. coli, C. elegans, Chlamydomonas reinhardtii,* and *Danio rerio)* from PubChem (Wang et al., 2010). As was the case in the yeast assays, the Naïve Bayes model performed best (median enrichment factor 2.10), whereas the two-property filter exhibited only modest improvement (enrichment factor 1.28) (Figure 4B; see also Table S8 and Table S9 for individual results). The increase in enrichment factors observed across such diverse model organisms (see also Figures 4A and 4B) demonstrates that these approaches are broadly generalizable across a very wide range of model organisms and are therefore valuable methods for compound selection and prioritization.

Yactives Are a Rich Source of Lead Probes

To be useful as a chemical probe, a compound should act in a specific manner to inhibit a protein or cellular activity. We therefore tested the twenty most potent yactives using our well-validated genome-wide HaploInsufficiency Profiling (HIP) assay (Baetz et al., 2004; Giaever et al., 1999, 2004; Lain et al., 2008; Lum et al., 2004; Xu et al., 2007) to identify candidate protein targets. The HIP assay allows an unbiased, in vivo quantitative measure of the relative drug sensitivity of all ~1100 essential yeast proteins in a single assay and results in a list of candidate protein targets ranked in order of compound sensitivity. The profiles of the 20 yactives revealed that 13 of the 20 tested exhibited a degree of specificity for an essential protein or

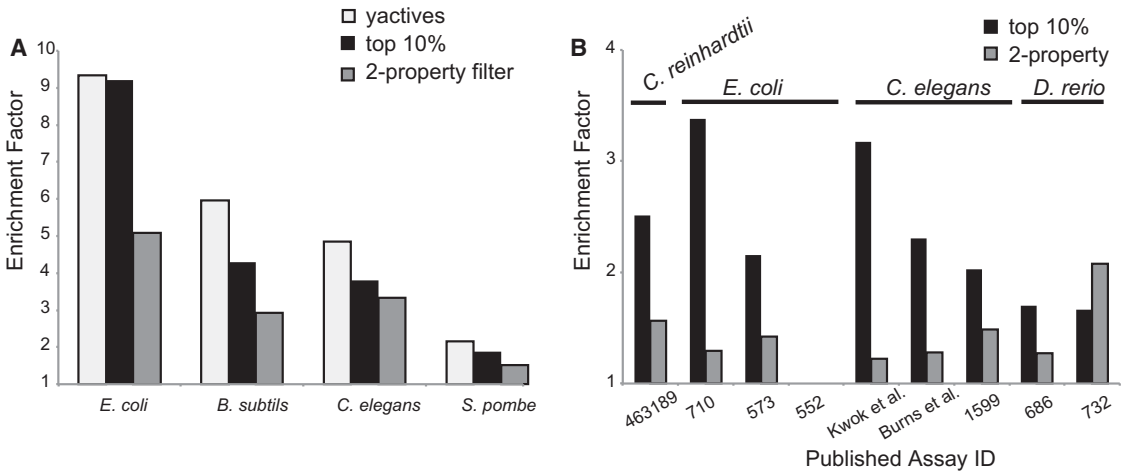

Figure 4. Yactives, Application of the Naïve Bayes Model, and a Two-Property Filter Enrich for Compounds that Induce Phenotypes in Model Organisms

(A) Comparison of the performance of the yactives/Naïve Bayes model/two-property filter. Each bar represents the average performance over two test sets, one containing all of the compounds screened (see Table S2) and a subset containing only random compounds (see Table S7).

(B) The median performance of the Naïve Bayes model and the two-property filter applied to nine publicly available assays, grouped by model organism. Assay details are available in Table S4, results for the two-property filter are in Table S8, and the Naïve Bayes model results are in Table S9.

protein(s) in the HIP profile and the remaining 7 compounds did not (Figure S2). We chose the two compounds that exhibited the highest degree of specificity for detailed follow-up studies. Our data suggest these compounds target Erg7, lanosterol synthase, and Ole1, fatty acid desaturase, respectively.

The HIP profile of ChemBridge 95809153 (ERG7.153, Figure 5A) supports Erg7 as the most likely target. *ERG7* encodes lanosterol synthase, an essential protein involved in ergosterol biosynthesis (Lees et al., 1995), a pathway exhibiting structural and functional conservation with the biosynthesis of cholesterol in human. Erg7 performs an essential step in ergosterol biosynthesis and holds promise as an antifungal target based on the success of antifungal agents that target other steps of this pathway (Jolidon et al., 1990; Voyron et al., 2010). In addition, the human homolog of Erg7 (LSS, lanosterol synthase BLASTP e-value 5e-148) has potential therapeutic relevance as a cholesterol-lowering agent (Charlton-Menys and Durrington, 2007). Two supporting studies demonstrated that compounds sharing structural similarity to ERG7.153 inhibit lanosterol synthase (Figure S3A). One of these compounds was demonstrated to inhibit lanosterol synthase (Erg7) in *C. albicans* (Buurman et al., 2005), whereas the other was shown to inhibit the human lanosterol synthase, LSS (Fouchet et al., 2008) (Figure S3A). To genetically confirm that ERG7.153 inhibits Erg7, we tested the individual *erg7* heterozygous deletion for the expected compound hypersensitivity to the WT (Figure S3B). Because ERG7.153 was not available for resupply, we carried out further testing with a close analog, CB 83425298 (ERG7.298), which induced similar hypersensitivity in the *S. cerevisiae erg7Δ* heterozygous deletion strain (Figure 5B). Analogous growth assays of an *erg7Δ* heterozygous deletion mutant and a conditional promoter shut-off allele in the human fungal pathogen *C. albicans* also exhibited hypersensitivity to compound, providing several lines of gene-dose support for Erg7 as the drug target of ERG7.153 and ERG7.298 (Figure 5B). Two additional heterozygous deletion strains, *neo1Δ* and *pik1Δ*, encoding a putative aminophospholipid translocase (flippase) (Paulusma and Oude Elferink, 2005) and a phosphatidylinositol 4-kinase (Flanagan et al., 1993), respectively, are also sensitive to ERG7.153. Both of these genes have been previously classified as multidrug-resistant (MDR) (Hillenmeyer et al., 2008). In this study, *neo1Δ* heterozygous deletion strain was sensitive in 14 of 20 profiles (70%) and the *pik1Δ* heterozygous deletion strain sensitive to 7 of 20 (35%) compounds tested.

To independently test whether Erg7 is the target of ERG7.298, we analyzed the lipid metabolites from cells grown in the presence of this inhibitor (Figure 5C) by mass spectrometry. As predicted for a bona fide Erg7 inhibitor, the substrate of Erg7 (oxidosqualene) showed significant accumulation in the presence of inhibitor compared with vehicle alone. As a positive control, the level of oxidosqualene was measured in cells treated with cerivastatin, which inhibits HMG-CoA reductase, the rate-limiting step of the ergosterol and cholesterol biosynthetic pathways (Endo, 1988). As expected, cells treated with cerivastatin did not accumulate oxidosqualene. Relative measurements of ergosterol, the end product of the ergosterol biosynthetic pathway, showed depletion in cells treated with the Erg7 inhibitor and with cerivastatin. Finally, we were able to partially rescue the growth defect caused by cerivastatin and ERG7.298 by adding ergosterol to the growth medium. Although yeast does not typically incorporate exogenously supplied lipids, we used a *S. cerevisiae* strain carrying the *upc2-1* mutation (Li and Prinz, 2004) (Figure 5D; Figure S3C), which allows cells to take up exogenously supplied sterols under aerobic growth conditions (Crowley et al., 1998).

A second HIP profile supports Ole1 as a likely protein target (Figure 6A) of a novel compound. Ole1 encodes the yeast delta(9) fatty acid desaturase, which converts stearic acid to oleic acid and which has previously been proposed as a potential antifungal target (Hu et al., 2007). Furthermore, the human homolog

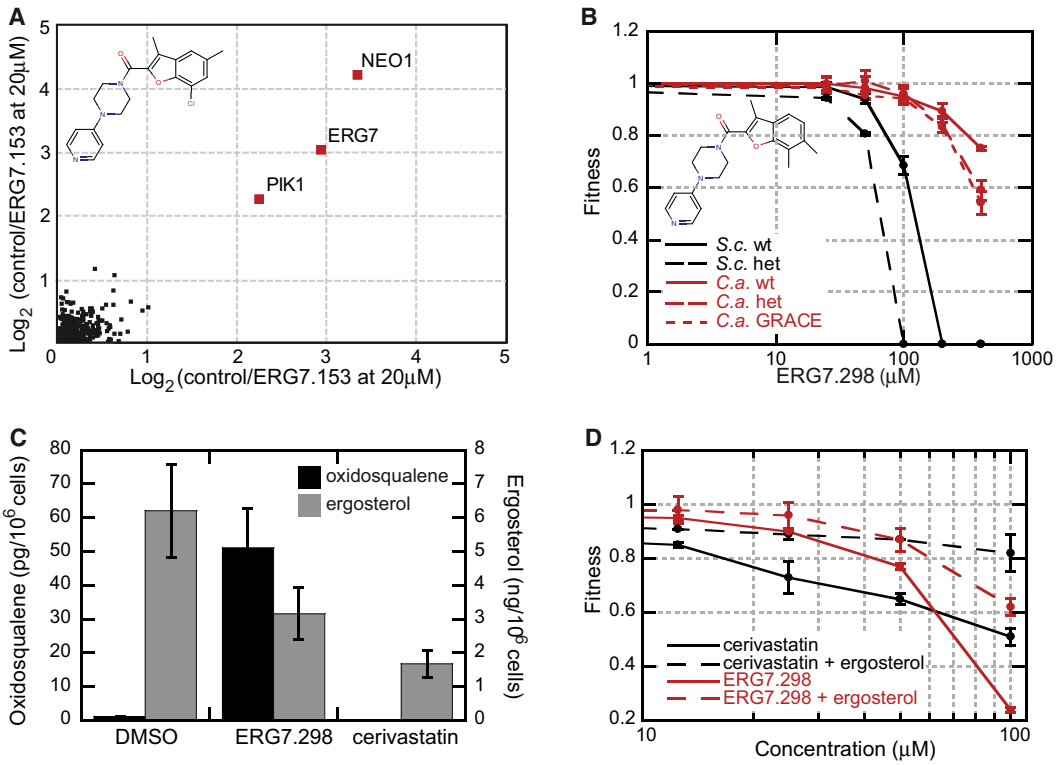

Figure 5. Identification of a Novel Inhibitor of Lanosterol Synthase (Erg7)

(A) Chemical structure of ERG7.153 and the HIP results for this compound. Sensitivity of a deletion strain to compound is expressed as the $\log_2$ ratio of control intensity/treatment intensity. The graph shows the results from two replicate experiments performed using 20 μM ERG7.153. Deletion strains are labeled with their gene name if the $\log_2$ ratio is above one in both replicates.

(B) Dose-response curve and chemical structure for ERG7.298 (a close analog of ERG7.153, original compound no longer available), a putative Erg7 inhibitor, in WT strain of *S. cerevisiae* (*S.c.*) and the *erg7* heterozygous deletion strain (het) as well as for *C. albicans* (*C.a.*) and the tet-repressible *erg7* GRACE mutant (Roemer et al., 2003). The fitness of a strain in compound was calculated from high-resolution growth curves as the ratio (control/treatment) of the average doubling time in the first five generations of growth. Experiments were performed in quadruplicate and error bars represent the standard deviation.

(C) Bar graph showing the amount of 2,3-oxidosqualene (black) and ergosterol (gray) in DMSO, ERG7.298- and cerivastatin-treated cells. Experiments were performed in triplicate; the error bars represent the standard deviation.

(D) Dose-response curve of cerivastatin and ERG7.298 in the *upc2-1* strain grown with and without 20 μg/ml ergosterol. The fitness was calculated as in (B), the experiment was performed in triplicate, the error bars represent the standard deviation. Additional information can be found in Figure S3.

of Ole1, SCD (stearoyl-CoA desaturase, BLASTP e-value 3e-52), has attracted interest for its potential modulation for the treatment of diabetes (Lenhard, 2011; Ntambi et al., 2002). Two other heterozygous deletion strains unrelated to fatty acid desaturase inhibition, (*lsg1Δ* and *rpb8Δ*) are sensitive to this compound. Given that both of these genes encode ribosomal components that frequently come up as sensitive in diverse chemical screens (unpublished data), they were not further pursued in this study. We used a *S. cerevisiae ole1* DAmP loss-of-function allele (Schuldiner et al., 2005; Yan et al., 2008) to confirm compound hypersensitivity. Hypersensitivity was also seen with an *ole1* conditional promoter shut-off allele in *C. albicans*, further supporting Ole1 as the target of OLE1.041(CB 11119041) (Xu et al., 2009) (Figure 6B). This compound was also effective in vitro, inhibiting the enzymatic activity of Ole1 in *S. cerevisiae*, *C. albicans*, and human HepG2 cells (Figure 6C). Finally, we found that two *C. elegans* mutants with reduced stearoyl-CoA desaturase activity (*fat-5;fat-7* and *fat-5;fat-6)* (Brock et al., 2006) are hypersensitive to OLE1.041 (Figure 6D). These two examples highlight the ability of the HIP assay to identify additional effects that can be monitored during compound optimization.

DISCUSSION

Compounds that inhibit yeast growth are more likely to induce phenotypes in other model organisms. Modeling the properties of the subset of drug-like compounds that inhibit yeast growth allows prioritization of compounds for model organism screening, reducing screening costs, and increasing efficiency. Over time, as the research community accumulates compound screening data, these models can be refined to be both organism and phenotype-specific, resulting in increasing the predictability and accuracy. As an important first step, we have demonstrated that compounds that inhibit yeast growth are more likely to induce phenotypes of interest in other model organisms and in mammalian cell culture assays. In order to address whether the inhibitory compounds we identified could act in a specific manner, we followed up on two compounds that looked particularly promising based on their genome-wide profile of drug

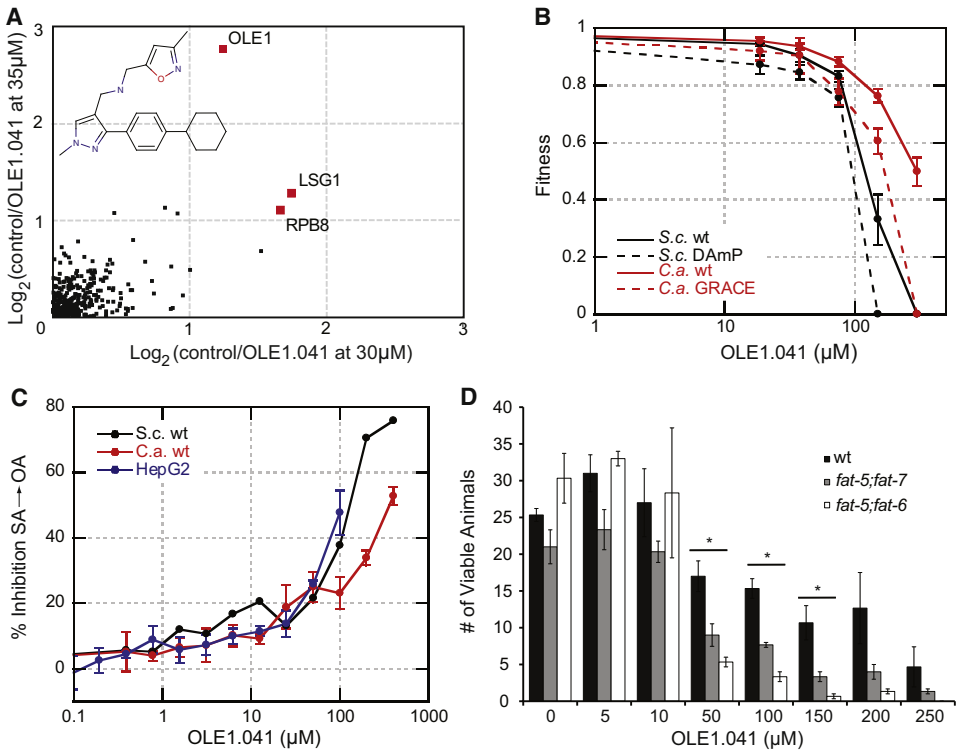

Figure 6. Identification of a Novel Inhibitor of Delta(9) Fatty Acid Desaturase (Ole1)

(A) Chemical structure of OLE1.041 and the HIP results for this compound performed using 30 and 35 μM OLE1.041, details as in Figure 5A. All 20 HIP profiles generated in this study are shown in Figure S2.

(B) Dose-response curve for OLE1.041 in WT strains of *S. cerevisae* (*S.c.*) compared with the *ole1* DAmP strain and *C. albicans* (*C.a.*) WT strain compared with the *ole1* GRACE strain, details as in Figure 5B.

(C) Percent inhibition of radiolabelled stearic acid to oleic acid conversion by OLE1.041 in vivo in *S. cerevisiae*, *C. albicans*, and human HepG2 cells. The experiment was performed in duplicate for *S. cerevisiae* and quadruplicate for *C. albicans* and HepG2; the error bars represent the standard deviation.

(D) Phenotypic analysis of OLE1.041 in *C. elegans* WT, *fat-5;fat-7* and *fat-5;fat-6* mutant strains. The bar graph shows the number of viable animals remaining after treatment with a DMSO control and across a range of OLE1.041 concentrations. Significant differences (p-value < 0.05) between the WT and mutant strains are indicated with an asterisk (*). The error bars represent the standard deviation in repeating the experiment in triplicate.

sensitivity. The mechanism of action of these compounds (ERG7.298, OLE1.041) was shown to be consistent with inhibition of their presumed molecular targets in both genetic and cellular biochemical assays. Both *ERG7* and *OLE1* are highly conserved with human *LSS* and *SCD* and represent recognized targets of medical relevance. *ERG7* and *LSS* inhibitors have clinical relevance as potential antifungal and anticholesterol lowering agents, respectively, while the human homolog of *OLE1*, *SCD*, may represent a potential target for diabetes treatment.

The identification of two compounds that act with a high degree of specificity in a relatively short experimental time frame underscores the benefits of prioritizing compounds. While no filtering or prioritization method can trump an exhaustive screening campaign and perfectly predict all compounds of interest, our results clearly indicate that preselection methods, when applied across diverse assays and organisms, can identify and prioritize those compounds most likely to induce a phenotype. The advantage of using such compounds as starting points for chemical probe discovery in model organisms is that a wide variety of genetic tools in different organisms can be used to validate the mode of action, as well as to identify off-target effects.

To provide a publicly accessible resource, we applied our two-property filter and our Naïve Bayes model to compounds available in (1) the NIH Molecular Screening program (Austin et al., 2004) and (2) the Zinc catalog of approximately 14 million purchasable compounds (Irwin and Shoichet, 2005). These results are available on our website (http://chemogenomics.med.utoronto.ca/supplemental/bioactive/).

Finally, a primary goal of this work was to encourage compound suppliers to provide libraries directed at model organism screening to the research community. Toward this end, ChemBridge, Inc. has agreed to make our ~7500 yactive compounds available for purchase as a preplated compound set. This library should prove a valuable resource for chemical screening labs working to develop chemical probes using model organisms.

SIGNIFICANCE

We have presented three approaches, based on yeast growth inhibition, to guide compound selection to reduce the costs associated with model organism screening programs. First, we have demonstrated that compounds that inhibit yeast growth are enriched for compounds that induce

a variety of phenotypes in diverse model organisms, and these compounds, with further optimization, may yield specific chemical probes. The first approach is then to simply screen compounds for those that inhibit yeast growth. A second approach is to prioritize compounds based on those that pass the two-property filter described here. This approach, depending on the model organism, can decrease costs by ~25% and is straightforward to implement. The third approach is to purchase compounds based on their likelihood to result in a desired phenotype by applying our Naïve Bayes model. This approach can also dramatically reduce costs. Newly generated screening data can be used to rebuild the model described here in the context of the model organism of interest to increase performance. This iterative approach is key because no model will perform optimally in all applications. Finally, as an experimental resource the yactive compounds are available as a preplated collection from ChemBridge, and a list of 14 M purchasable compounds scored by our Naïve Bayes model and two-property filter is available for download from our website (http://chemogenomics.med.utoronto.ca/supplemental/bioactive/).

EXPERIMENTAL PROCEDURES

Reagents, Strains, and Equipment

The chemical libraries screened were obtained from ChemDiv (Divers, San Diego, CA) and ChemBridge (NOVACore and DIVERSet, San Diego, CA) in a 96-well format at 10 mM in DMSO. The Spectrum library (Microsource, Gaylordsville, CT) of 2000 compounds was supplied at 2.5 mM in DMSO and was a gift from D. Desveaux and D. Guttman (University of Toronto).

E. coli strain BW25113 (Datsenko and Wanner, 2000) (*lacI^q rrnB$_{T14}$ ΔlacZ$_{WJ16}$ hsdR514 ΔaraBAD$_{AH33}$ ΔrhaBAD$_{LD78}$*) was a gift from Andrew Emili (University of Toronto), *S. pombe* strain TK1/972 (*h$^-$*) was a gift from Charlie Boone (University of Toronto), and *B. subtilis* strain 168 1A700 (*trpC2*) was a gift from Alex ter Beek and Gertien Smits (University of Amsterdam). *C. albicans* heterozygous deletion (Xu et al., 2007) and conditional shut-off or GRACE mutants (Roemer et al., 2003) in the SC5314 background were a gift from Terry Roemer (Merck-Frosst Canada, Ltd.). *C. neoformans* strain H99 was a gift from Joseph Heitman (Duke University). A549 human lung cancer cells (ATCC number: CCL-185) and HepG2 cells (ATCC number HB-8065) were obtained from American Type Culture Collection (ATCC, Rockville, MD). *S. cerevisiae* WPY361 (*MATa upc2-1 ura3-1 his3-11,-15 leu2-3,-112 trp1-1*) was a gift from William Prinz (NIH, Bethesda, MD) (Li and Prinz, 2004). The BX110 *fat-7(wa36);fat-5(tm420)V* double, the BX160 *fat-6(tm331) IV;fat-5(tm420)V* double, and the WT (N2) worm strains were obtained from the *C. elegans* Genetics Center (University of Minnesota) maintained at 20°C using standard techniques (Lewis and Fleming, 1995).

Growth assays were performed in clear, flat-bottom 48-, 96-, and 384-well microplates (Greiner) sealed with adhesive plate seals (Cat. No. AB-0580, ABgene) using a custom developed platform incorporating microplate readers GENios, Infinite, and Safire^2 (Tecan-US, Durham, NC) and the Packard Multiprobe II four-probe liquid-handling system (PerkinElmer, Waltham, MA). Genome-wide assays were analyzed on Genflex_Tag_16K_dev microarrays (Item No. 511331, Affymetrix, Santa Clara, CA) using GeneChip Fluidics Station 450 and GeneChip Scanner 3000 (Affymetrix). For protocol detail see Pierce et al., (2007).

Screening Chemical Libraries on *S. cerevisiae*

Our WT yeast strain, BY4743 (*MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0*), was grown in YPD medium at 30°C. 20 mM HEPES pH 7.0 was added to the YPD medium as indicated in the Supplemental Information. Cells were diluted from a fresh overnight culture to 0.0625 OD_{595} in a final volume of 100 μl for 96-well and 30 μl for 384-well plates. Compounds were added to the culture using a 2 μl or 600 nl pin tool (V&P Scientific, San Diego, CA) for 96-well or 384-well microplates, respectively, to dilute the compounds 50 times to a final DMSO concentration of 2%. The ChemDiv Diverse, NOVACore SAR, and NOVACore DIVERSet libraries were screened at 200 μM final concentration, and the Spectrum library was screened at 50 μM final concentration. Yeast growth was monitored for up to 24 hr by measuring the OD_{595} every 15 min as described (Giaever et al., 2004; Lee et al., 2005). The majority of the compounds screened were soluble at 200 μM, with less than 8% of compounds having a starting optical density that was significantly different from the DMSO controls, indicating that, at this dose, either the compound had a color, or solubility was an issue.

The fitness of BY4743 in compound was expressed as the ratio of the average generation time (AvgG) (AvgG reference/AvgG compound), where the reference condition was grown on the same plate in 2% DMSO. Average generation time is calculated by (time to five generations/5) (Lee et al., 2005). A compound was scored as active when the ratio AvgG was 0.7 or less, corresponding to an IC_{30} or greater. Automatic flagging of actives was confirmed by visual inspection of the data.

Screening Chemical Libraries on *E. coli*, *B. subtilis*, *S. pombe*, *C. albicans*, *C. neoformans*, *C. elegans*, and Human Cell Line A549

Compounds scored as growth inhibitory were transferred to a new 96-well microplate, the hit-plate. Hit-plates and random naïve plates were screened at 200 μM final concentration on *E. coli* strain BW25113 grown in Luria broth (LB), *B. subtilis* strain 1A700 grown in nutrient broth (NB), *S. pombe* strain TK1 grown in YES medium, *S. cerevisiae* strain BY4743, *C. neoformans* strain H99, and *C. albicans* strain HIS3 grown in YPD medium. All media were buffered with 20 mM HEPES pH 7 and growth temperature was 37°C for bacteria and 30°C for yeast.

A compound was considered active in *S. pombe*, *C. albicans*, *C. neoformans*, *E. coli*, or *B. subtilis* if the area under the growth curve after 20 hr of growth was 50% of that compared with the DMSO control (ratio [compound/control] <0.5). Compounds that showed a high ratio, defined as >1.2, were excluded. We found the area under the growth curve to be a more robust method for measuring growth rate in the other organisms than the ratio AvgG. A value of 0.5 gives a similar hit-rate as a ratio AvgG of 0.7 in *S. cerevisiae*.

Phenotypic screening of *C. elegans* was performed as reported previously (Kwok et al., 2006). In brief, molecules were screened in duplicate in 24-well format at 25 μM concentration. Two L4 stage N2 animals were deposited per well on agar and the progeny were visually assessed for phenotype, including slow growth, egg laying abnormalities, and embryonic lethality, using an MZ12 dissection microscope (Leica Microsystems GmbH, Wetzlar, Germany).

A549 human lung cancer cells were maintained in Dulbecco's Modified Eagle medium (Wisent, St-Bruno, QC, Canada) supplemented with 10% fetal bovine serum (Wisent) and 100 U/ml penicillin/streptomycin (Wisent) in a humidified incubator with 5% CO_2 at 37°C. The cells were seeded in 96-well plates with a density of 2200 cells per well and treated for 48 hr with 50 μM compound in 0.5% DMSO. Cell survival was measured using the Sulforhodamine B (SRB) colorimeteric assay (Vichai and Kirtikara, 2006) and readout using a SpectraMax Plus^{384} (Molecular Devices, Sunnyvale, CA) with the following modification: the cells were stained with 50 μl of 0.4% SRB. Actives for the A549 cells were defined as compounds causing ≤50% viability after 48 hr of growth in the presence of the compound.

HaploInsufficiency Profiling Assay

By serial dilution of 16 hit-plates, each containing ~86 active compounds, 79 compounds were found to completely inhibit the growth of WT *S. cerevisiae* at a four-fold dilution (50 μM). 20 diverse compounds were selected for testing in the HIP assay. The molecular weights of these 20 compounds were verified by liquid chromatography and mass spectrometry to confirm their structure (see Supplementary Methods).

The HIP assay was performed as previously described (Pierce et al., 2007). Two biological replicates were generated for each compound condition. A significant hit is defined as a gene with a $\log_2$ ratio >1 of the intensity of the DMSO control intensity/drug treatment intensity in both replicates. All raw and ratio data files are available on the supplementary website. All HIP profiles

are shown in Figure S2. In addition, the microarray data is available on ArrayExpress (http://www.ebi.ac.uk/microarray-as/ae/).

Computational Analysis

All chemical analysis and Naïve Bayes model building was performed using the cheminformatic package in Pipeline Pilot version 6.1 (Scitegic Inc. Accelyrs, San Diego, CA). Marvin version 5.4.1 (ChemAxon, http://www.chemaxon.com) was used for drawing and displaying chemical structures.

Pipeline Pilot was used to standardize the representation of all compounds studied including removing inorganic compounds, salts, and duplicates. All data used for the model building are available on our supplementary website. Three methods were tested to represent molecules for the Naïve Bayes model using five-fold cross validation: (1) a vector constructed from physical properties for each compound (LogP, Molecular Weight, Molecular Polar Surface Area, Molecular Solvent Accessible Area, the number of Hydrogen acceptors and donors, the number of rotatable bonds, the number of rings, and the number of aromatic rings); (2) a vector based on MDL Public Keys (Durant et al., 2002), where the presence or absence of specific substructures is recorded; and (3) a vector using the Extended Connectivity Fingerprints (Rogers et al., 2005) method, where the compound is represented by overlapping fragments of a diameter of up to two-fourths bond lengths (ECFP_2/ECFP_4). The enrichment factor was used as a measure of accuracy. This is calculated by ranking the library of compounds to be tested by the model score. Next, for different thresholds, the number of observed actives was compared with the number of actives expected by random selection.

Tanimoto coefficient, also known at the Jaccard Coefficient (Rogers and Tanimoto, 1960), was used to calculate the similarity between two compounds and was calculated based on the number of features in common between the compounds divided by the total number of features present.

LogP values were calculated using the Ghose and Crippen algorithm (Viswanadhan et al., 1989).

Statistical Analysis

The significance of the effect of the two-property and Lipinski filters was calculated using the hypergeometric test, termed the phyper function, in R. To test whether the distribution of LogP and the number of hydrogen acceptors for active and inactive compounds was significantly different, a two-sample Kolmogorov-Smirnov test (Durbin, 1973) was implemented using R.

Website

All of the data used in this study is available at http://chemogenomics.med.utoronto.ca/supplemental/bioactive/.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, nine tables, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.chembiol.2011.07.018.

ACKNOWLEDGMENTS

The authors thank Kahlin Cheung-Ong for performing the SRB assay on cultured human cells, Sally Cheung for help with screening, Karen Robinson (Dalton Pharma Services, Toronto, Canada) for verifying the molecular weight of 60 compounds and William Lee for helpful comments on the manuscript. This work was supported by grants from the NHGRI to CN and GG, the CIHR to CN (MOPS-84305) and to GG (MOPS-81340), a grant from the Canadian Cancer Society to GG and CN (#020380), NSERC support to GDB, NSERC (RGPIN 356873-08), CIHR (MOP-97904) and CFI to CLC, CIHR grant (MOP-68813) to PJR, and a Marie Curie fellowship to IMW. GG is a Canada Research Chair (CRC) in Chemical Genetics and PJR a CRC in Molecular Neurobiology.

Received: November 22, 2010
Revised: June 29, 2011
Accepted: July 15, 2011
Published: October 27, 2011

REFERENCES

Agresti, J.J., Antipov, E., Abate, A.R., Ahn, K., Rowat, A.C., Baret, J.-C., Marquez, M., Klibanov, A.M., Griffiths, A.D., and Weitz, D.A. (2010). Ultrahigh-throughput screening in drop-based microfluidics for directed evolution. Proc. Natl. Acad. Sci. USA *107*, 4004–4009.

Al-Awqati, Q. (1999). One hundred years of membrane permeability: does Overton still rule? Nat. Cell Biol. *1*, E201–E202.

Austin, C.P., Brady, L.S., Insel, T.R., and Collins, F.S. (2004). NIH Molecular Libraries Initiative. Science *306*, 1138–1139.

Baetz, K., McHardy, L., Gable, K., Tarling, T., Rebérioux, D., Bryan, J., Andersen, R.J., Dunn, T., Hieter, P., and Roberge, M. (2004). Yeast genome-wide drug-induced haploinsufficiency screen to determine drug mode of action. Proc. Natl. Acad. Sci. USA *101*, 4525–4530.

Blackburn, A.S., and Avery, S.V. (2003). Genome-wide screening of Saccharomyces cerevisiae to identify genes required for antibiotic insusceptibility of eukaryotes. Antimicrob. Agents Chemother. *47*, 676–681.

Botet, J., Mateos, L., Revuelta, J.L., and Santos, M.A. (2007). A chemogenomic screening of sulfanilamide-hypersensitive Saccharomyces cerevisiae mutants uncovers ABZ2, the gene encoding a fungal aminodeoxychorismate lyase. Eukaryot. Cell *6*, 2102–2111.

Botstein, D., and Fink, G.R. (1988). Yeast: an experimental organism for modern biology. Science *240*, 1439–1443.

Brock, T.J., Browse, J., and Watts, J.L. (2006). Genetic regulation of unsaturated fatty acid composition in C. elegans. PLoS Genet. *2*, e108.

Burns, A.R., Wallace, I.M., Wildenhain, J., Tyers, M., Giaever, G., Bader, G.D., Nislow, C., Cutler, S.R., and Roy, P.J. (2010). A predictive model for drug bioaccumulation and bioactivity in Caenorhabditis elegans. Nat. Chem. Biol. *6*, 549–557.

Buurman, E.T., Andrews, B., Blodgett, A.E., Chavda, J.S., and Schnell, N.F. (2005). Utilization of target-specific, hypersensitive strains of Saccharomyces cerevisiae to determine the mode of action of antifungal compounds. Antimicrob. Agents Chemother. *49*, 2558–2560.

Charlton-Menys, V., and Durrington, P.N. (2007). Squalene synthase inhibitors : clinical pharmacology and cholesterol-lowering potential. Drugs *67*, 11–16.

Crowley, J.H., Leak, F.W., Jr., Shianna, K.V., Tove, S., and Parks, L.W. (1998). A mutation in a purported regulatory gene affects control of sterol uptake in Saccharomyces cerevisiae. J. Bacteriol. *180*, 4177–4183.

Datsenko, K.A., and Wanner, B.L. (2000). One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc. Natl. Acad. Sci. USA *97*, 6640–6645.

Dias, P.J., Teixeira, M.C., Telo, J.P., and Sá-Correia, I. (2010). Insights into the mechanisms of toxicity and tolerance to the agricultural fungicide mancozeb in yeast, as suggested by a chemogenomic approach. OMICS *14*, 211–227.

Dielbandhoesing, S.K., Zhang, H., Caro, L.H., van der Vaart, J.M., Klis, F.M., Verrips, C.T., and Brul, S. (1998). Specific cell wall proteins confer resistance to nisin upon yeast cells. Appl. Environ. Microbiol. *64*, 4047–4052.

Dorer, R.K., Zhong, S., Tallarico, J.A., Wong, W.H., Mitchison, T.J., and Murray, A.W. (2005). A small-molecule inhibitor of Mps1 blocks the spindle-checkpoint response to a lack of tension on mitotic chromosomes. Curr. Biol. *15*, 1070–1076.

Durant, J.L., Leland, B.A., Henry, D.R., and Nourse, J.G. (2002). Reoptimization of MDL keys for use in drug discovery. J. Chem. Inf. Comput. Sci. *42*, 1273–1280.

Durbin, J. (1973). Distribution Theory for Tests Based on the Sample Distribution Function (Philadelphia, PA: Society for Industrial and Applied Mathematics).

Endo, A. (1988). Chemistry, biochemistry, and pharmacology of HMG-CoA reductase inhibitors. Klin. Wochenschr. *66*, 421–427.

Ericson, E., Gebbia, M., Heisler, L.E., Wildenhain, J., Tyers, M., Giaever, G., and Nislow, C. (2008). Off-target effects of psychoactive drugs revealed by genome-wide assays in yeast. PLoS Genet. *4*, e1000151.

Ernst, R., Kueppers, P., Stindt, J., Kuchler, K., and Schmitt, L. (2010). Multidrug efflux pumps: substrate selection in ATP-binding cassette multidrug efflux pumps—first come, first served? FEBS J. *277*, 540–549.

Flanagan, C.A., Schnieders, E.A., Emerick, A.W., Kunisawa, R., Admon, A., and Thorner, J. (1993). Phosphatidylinositol 4-kinase: gene structure and requirement for yeast cell viability. Science *262*, 1444–1448.

Fouchet, M.H., Donche, F., Martin, C., Bouillot, A., Junot, C., Boullay, A.B., Potvain, F., Magny, S.D., Coste, H., Walker, M., et al. (2008). Design and evaluation of a novel series of 2,3-oxidosqualene cyclase inhibitors with low systemic exposure, relationship between pharmacokinetic properties and ocular toxicity. Bioorg. Med. Chem. *16*, 6218–6232.

Frearson, J.A., and Collie, I.T. (2009). HTS and hit finding in academia—from chemical genomics to drug discovery. Drug Discov. Today *14*, 1150–1158.

Gamo, F.-J., Sanz, L.M., Vidal, J., de Cozar, C., Alvarez, E., Lavandera, J.-L., Vanderwall, D.E., Green, D.V.S., Kumar, V., Hasan, S., et al. (2010). Thousands of chemical starting points for antimalarial lead identification. Nature *465*, 305–310.

Giaever, G., Flaherty, P., Kumm, J., Proctor, M., Nislow, C., Jaramillo, D.F., Chu, A.M., Jordan, M.I., Arkin, A.P., and Davis, R.W. (2004). Chemogenomic profiling: identifying the functional interactions of small molecules in yeast. Proc. Natl. Acad. Sci. USA *101*, 793–798.

Giaever, G., Shoemaker, D.D., Jones, T.W., Liang, H., Winzeler, E.A., Astromoff, A., and Davis, R.W. (1999). Genomic profiling of drug sensitivities via induced haploinsufficiency. Nat. Genet. *21*, 278–283.

Gleeson, M.P., Hersey, A., Montanari, D., and Overington, J. (2011). Probing the links between in vitro potency, ADMET and physicochemical parameters. Nat. Rev. Drug Discov. *10*, 197–208.

Hacker, M.P., Messer, W.S., and Bachmann, K.A. (2009). Pharmacology: Principles and Practice (Waltham, MA: Academic Press).

Hillenmeyer, M.E., Fung, E., Wildenhain, J., Pierce, S.E., Hoon, S., Lee, W., Proctor, M., St Onge, R.P., Tyers, M., Koller, D., et al. (2008). The chemical genomic portrait of yeast: uncovering a phenotype for all genes. Science *320*, 362–365.

Hu, W., Sillaots, S., Lemieux, S., Davison, J., Kauffman, S., Breton, A., Linteau, A., Xin, C., Bowman, J., Becker, J., et al. (2007). Essential gene identification and drug target prioritization in Aspergillus fumigatus. PLoS Pathog. *3*, e24.

Irwin, J.J., and Shoichet, B.K. (2005). ZINC—a free database of commercially available compounds for virtual screening. J. Chem. Inf. Model. *45*, 177–182.

Jolidon, S., Polak, A.M., Guerry, P., and Hartman, P.G. (1990). Inhibitors of 2,3-oxidosqualene lanosterol-cyclase as potential antifungal agents. Biochem. Soc. Trans. *18*, 47–48.

Khozoie, C., Pleass, R.J., and Avery, S.V. (2009). The antimalarial drug quinine disrupts Tat2p-mediated tryptophan transport and causes tryptophan starvation. J. Biol. Chem. *284*, 17968–17974.

Klekota, J., and Roth, F.P. (2008). Chemical substructures that enrich for biological activity. Bioinformatics *24*, 2518–2525.

Kolaczkowski, M., Kolaczowska, A., Luczynski, J., Witek, S., and Goffeau, A. (1998). In vivo characterization of the drug resistance profile of the major ABC transporters and other components of the yeast pleiotropic drug resistance network. Microb. Drug. Resist. *4*, 143–158.

Kwak, Y.S., Han, S., Thomashow, L.S., Rice, J.T., Paulitz, T.C., Kim, D., and Weller, D.M. (2011). Saccharomyces cerevisiae genome-wide mutant screen for sensitivity to 2,4-diacetylphloroglucinol, an antibiotic produced by Pseudomonas fluorescens. Appl. Environ. Microbiol. *77*, 1770–1776.

Kwok, T.C., Ricker, N., Fraser, R., Chan, A.W., Burns, A., Stanley, E.F., McCourt, P., Cutler, S.R., and Roy, P.J. (2006). A small-molecule screen in C. elegans yields a new calcium channel antagonist. Nature *441*, 91–95.

Lain, S., Hollick, J.J., Campbell, J., Staples, O.D., Higgins, M., Aoubala, M., McCarthy, A., Appleyard, V., Murray, K.E., Baker, L., et al. (2008). Discovery, in vivo activity, and mechanism of action of a small-molecule p53 activator. Cancer Cell *13*, 454–463.

Lee, W., St Onge, R.P., Proctor, M., Flaherty, P., Jordan, M.I., Arkin, A.P., Davis, R.W., Nislow, C., and Giaever, G. (2005). Genome-wide requirements for resistance to functionally distinct DNA-damaging agents. PLoS Genet. *1*, e24.

Lees, N.D., Skaggs, B., Kirsch, D.R., and Bard, M. (1995). Cloning of the late genes in the ergosterol biosynthetic pathway of Saccharomyces cerevisiae—a review. Lipids *30*, 221–226.

Lenhard, J.M. (2011). Lipogenic enzymes as therapeutic targets for obesity and diabetes. Curr. Pharm. Des. *17*, 325–331.

Lewis, J.A., and Fleming, J.T. (1995). Caenorhabditis Elegans: Modern Biological Analysis of an Organism (San Diego, CA: Academic Press).

Li, X., Zolli-Juran, M., Cechetto, J.D., Daigle, D.M., Wright, G.D., and Brown, E.D. (2004). Multicopy suppressors for novel antibacterial compounds reveal targets and drug efflux susceptibility. Chem. Biol. *11*, 1423–1430.

Li, Y., and Prinz, W.A. (2004). ATP-binding cassette (ABC) transporters mediate nonvesicular, raft-modulated sterol movement from the plasma membrane to the endoplasmic reticulum. J. Biol. Chem. *279*, 45226–45234.

Lipinski, C., and Hopkins, A. (2004). Navigating chemical space for biology and medicine. Nature *432*, 855–861.

Lipinski, C.A., Lombardo, F., Dominy, B.W., and Feeney, P.J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. *46*, 3–26.

Lum, P.Y., Armour, C.D., Stepaniants, S.B., Cavet, G., Wolf, M.K., Butler, J.S., Hinshaw, J.C., Garnier, P., Prestwich, G.D., Leonardson, A., et al. (2004). Discovering modes of action for therapeutic compounds using a genome-wide screen of yeast heterozygotes. Cell *116*, 121–137.

Morgan, D.J., Weisenhaus, M., Shum, S., Su, T., Zheng, R., Zhang, C., Shokat, K.M., Hille, B., Babcock, D.F., and McKnight, G.S. (2008). Tissue-specific PKA inhibition using a chemical genetic approach and its application to studies on sperm capacitation. Proc. Natl. Acad. Sci. USA *105*, 20740–20745.

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

Murén, E., Oyen, M., Barmark, G., and Ronne, H. (2001). Identification of yeast deletion strains that are hypersensitive to brefeldin A or monensin, two drugs that affect intracellular transport. Yeast *18*, 163–172.

Ntambi, J.M., Miyazaki, M., Stoehr, J.P., Lan, H., Kendziorski, C.M., Yandell, B.S., Song, Y., Cohen, P., Friedman, J.M., and Attie, A.D. (2002). Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. Proc. Natl. Acad. Sci. USA *99*, 11482–11486.

Oprea, T.I., Bologa, C.G., Boyer, S., Curpan, R.F., Glen, R.C., Hopkins, A.L., Lipinski, C.A., Marshall, G.R., Martin, Y.C., Ostopovici-Halip, L., et al. (2009). A crowdsourcing evaluation of the NIH chemical probes. Nat. Chem. Biol. *5*, 441–447.

Paixão, S.M., Silva, L., Fernandes, A., O'Rourke, K., Mendonça, E., and Picado, A. (2008). Performance of a miniaturized algal bioassay in phytotoxicity screening. Ecotoxicology *17*, 165–171.

Paulusma, C.C., and Oude Elferink, R.P.J. (2005). The type 4 subfamily of P-type ATPases, putative aminophospholipid translocases with a role in human disease. Biochim. Biophys. Acta *1741*, 11–24.

Pierce, S.E., Davis, R.W., Nislow, C., and Giaever, G. (2007). Genome-wide analysis of barcoded Saccharomyces cerevisiae gene-deletion mutants in pooled cultures. Nat. Protoc. *2*, 2958–2974.

Proctor, M., Urbanus, M.L., Fung, E.L., Jaramillo, D.F., Davis, R.W., Nislow, C., and Giaever, G. (2011). The Automated Cell, Compound and Environment Screening System (ACCESS) for Chemogenomic Screening. In Yeast Systems Biology. Methods and Protocols, J.I. Castrillo and S.G. Oliver, eds. (New York: Humana Press, Springer).

Roemer, T., Jiang, B., Davison, J., Ketela, T., Veillette, K., Breton, A., Tandia, F., Linteau, A., Sillaots, S., Marta, C., et al. (2003). Large-scale essential gene identification in Candida albicans and applications to antifungal drug discovery. Mol. Microbiol. *50*, 167–181.

Rogers, B., Decottignies, A., Kolaczkowski, M., Carvajal, E., Balzi, E., and Goffeau, A. (2001). The pleitropic drug ABC transporters from Saccharomyces cerevisiae. J. Mol. Microbiol. Biotechnol. *3*, 207–214.

Rogers, D., Brown, R.D., and Hahn, M. (2005). Using extended-connectivity fingerprints with Laplacian-modified Bayesian analysis in high-throughput screening follow-up. J. Biomol. Screen. *10*, 682–686.

Rogers, D.J., and Tanimoto, T.T. (1960). A Computer Program for Classifying Plants. Science *132*, 1115–1118.

Schuldiner, M., Collins, S.R., Thompson, N.J., Denic, V., Bhamidipati, A., Punna, T., Ihmels, J., Andrews, B., Boone, C., Greenblatt, J.F., et al. (2005). Exploration of the function and organization of the yeast early secretory pathway through an epistatic miniarray profile. Cell *123*, 507–519.

Specht, K.M., and Shokat, K.M. (2002). The emerging power of chemical genetics. Curr. Opin. Cell Biol. *14*, 155–159.

Vichai, V., and Kirtikara, K. (2006). Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat. Protoc. *1*, 1112–1116.

Viswanadhan, V.N., Ghose, A.K., Revankar, G.R., and Robins, R.K. (1989). Atomic physicochemical parameters for three dimensional structure directed quantitative structure-activity relationships. 4. Additional parameters for hydrophobic and dispersive interactions and their application for an automated superposition of certain naturally occurring nucleoside antibiotics. J. Chem. Inf. Comput. Sci. *29*, 163–172.

Voyron, S., Rocco, F., Ceruti, M., Forni, P., Pla, A.F., Sarpietro, M.G., Varese, G.C., and Marchisio, V.F. (2010). Antifungal activity of bis-azasqualenes, inhibitors of oxidosqualene cyclase. Mycoses *53*, 481–487.

Wang, Y., Bolton, E., Dracheva, S., Karapetyan, K., Shoemaker, B.A., Suzek, T.O., Wang, J., Xiao, J., Zhang, J., and Bryant, S.H. (2010). An overview of the PubChem BioAssay resource. Nucleic Acids Res. *38* (Database issue), D255–D266.

Wheeler, G.N., and Brändli, A.W. (2009). Simple vertebrate models for chemical genetics and drug discovery screens: lessons from zebrafish and Xenopus. Dev. Dyn. *238*, 1287–1308.

Workman, P., and Collins, I. (2010). Probing the probes: fitness factors for small molecule tools. Chem. Biol. *17*, 561–577.

Xu, D., Jiang, B., Ketela, T., Lemieux, S., Veillette, K., Martel, N., Davison, J., Sillaots, S., Trosok, S., Bachewich, C., et al. (2007). Genome-wide fitness test and mechanism-of-action studies of inhibitory compounds in Candida albicans. PLoS Pathog. *3*, e92.

Xu, D., Sillaots, S., Davison, J., Hu, W., Jiang, B., Kauffman, S., Martel, N., Ocampo, P., Oh, C., Trosok, S., et al. (2009). Chemical genetic profiling and characterization of small-molecule compounds that affect the biosynthesis of unsaturated fatty acids in Candida albicans. J. Biol. Chem. *284*, 19754–19764.

Yan, Z., Costanzo, M., Heisler, L.E., Paw, J., Kaper, F., Andrews, B.J., Boone, C., Giaever, G., and Nislow, C. (2008). Yeast Barcoders: a chemogenomic application of a universal donor-strain collection carrying bar-code identifiers. Nat. Methods *5*, 719–725.