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NPL-800, Natural Products Library in Publication.

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Luteolin peracetate and gossypolone inhibit immune complex-mediated neutrophil activation in vitro and dermal-epidermal separation in an ex vivo model of epidermolysis bullosa acquisita
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PMID: 37720234 PMCID: PMC10503437 DOI: 10.3389/fimmu.2023.1196116
Abstract
Introduction: Natural products have been shown to an important source of therapeutics for human disease. In this study, we aimed to identify natural compounds as potential therapeutics for epidermolysis bullosa acquisita (EBA), an autoimmune disease caused by autoantibodies to type VII collagen (COL7).
Methods: Utilizing an in vitro experimental system, we screened a natural product library composed of 800 pure compounds for their inhibitory effect on COL7-anti-COL7 IgG immune complex (IC)-mediated neutrophil activation and on neutrophil-mediated tissue damage.
Results: Three natural compounds, namely luteolin peracetate, gossypol, and gossypolone were capable in inhibiting the IC-induced neutrophil adhesion and oxygen burst in vitro. Furthermore, luteolin peracetate and gossypolone were able to inhibit the anti-COL7 IgG induced dermal-epidermal separation in an ex vivo model for EBA.
Discussion: In summary, this study demonstrates that luteolin peracetate and gossypolone are potential therapeutics for experimental EBA, which deserves further investigation.

Novel BH4-BCL-2 Domain Antagonists Induce BCL-2-Mediated Apoptosis in Triple-Negative Breast Cancer
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PMID: 36358660 PMCID: PMC9657696 DOI: 10.3390/cancers14215241
Abstract
Targeting the challenging tumors lacking explicit markers and predictors for chemosensitivity is one of the major impediments of the current cancer armamentarium. Triple-negative breast cancer (TNBC) is an aggressive and challenging molecular subtype of breast cancer, which needs astute strategies to achieve clinical success. The pro-survival B-cell lymphoma 2 (BCL-2) overexpression reported in TNBC plays a central role in deterring apoptosis and is a promising target. Here, we propose three novel BH4 mimetic small molecules, SM396, a covalent binder, and two non-covalent binders, i.e., SM216 and SM949, which show high binding affinity (nM) and selectivity, designed by remodeling the existing BCL-2 chemical space. Our mechanistic studies validate the selectivity of the compounds towards cancerous cells and not on normal cells. A series of functional assays illustrated BCL-2-mediated apoptosis in the tumor cells as a potent anti-cancerous mechanism. Moreover, the compounds exhibited efficacious in vivo activity as single agents in the MDA-MB-231 xenograft model (at nanomolar dosage). Overall, these findings depict SM216, SM396, and SM949 as promising leads, pointing to the clinical translation of these compounds in targeting triple-negative breast cancer.

Two Approaches to Drug Discovery in SOD1-Mediated ALS
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Volume 11, Issue 7
https://doi.org/10.1177/1087057106290937
Abstract
Familial amyotrophic lateral sclerosis (ALS) accounts for 10% of all ALS cases; approximately 25% of these cases are due to mutations in the Cu/Zn superoxide dismutase gene (SOD1). To date, 105 different mutations spanning all 5 exons have been identified in the SOD1 gene. Mutant SOD1-associated ALS is caused by a toxic gain of function of the mutated protein. Therefore, regardless of the specific mechanism whereby mutant SOD1 initiates motor neuron death, the authors hypothesize that measures that decrease levels of mutant SOD1 protein should ameliorate the phenotype in transgenic mice and potentially in patients with SOD1-mediated disease. They have designed 2 cell-based screening assays to identify small, brain-permeant molecules that inactivate expression of the SOD1 gene or increase the degradation of the SOD1 protein. Here they describe the development and optimization of these assays and the results of high-throughput screening using a variety of compound libraries, including a total of more than 116,000 compounds. The majority of the hit compounds identified that down-regulated SOD1 were shown to be toxic in a cell-based viability assay or were nonselective transcription inhibitors, but work is continuing on a number of nonspecific inhibitors of SOD1 expression. Ultimately, the authors believe that these 2 cell-based assays will provide powerful strategies to identify novel therapies for the treatment of inherited SOD1-associated forms of ALS.

Data Resources for the Computer-Guided Discovery of Bioactive Natural Products
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Abstract
Natural products from plants, animals, marine life, fungi, bacteria, and other organisms are an important resource for modern drug discovery. Their biological relevance and structural diversity make natural products good starting points for drug design. Natural product-based drug discovery can benefit greatly from computational approaches, which are a valuable precursor or supplementary method to in vitro testing. We present an overview of 25 virtual and 31 physical natural product libraries that are useful for applications in cheminformatics, in particular virtual screening. The overview includes detailed information about each library, the extent of its structural information, and the overlap between different sources of natural products. In terms of chemical structures, there is a large overlap between freely available and commercial virtual natural product libraries. Of particular interest for drug discovery is that at least ten percent of known natural products are readily purchasable and many more natural products and derivatives are available through on-demand sourcing, extraction and synthesis services. Many of the readily purchasable natural products are of small size and hence of relevance to fragment-based drug discovery. There are also an increasing number of macrocyclic natural products and derivatives becoming available for screening.

Identification of catechols as histone–lysine demethylase inhibitors
Edited by Ned Mantei
Author links open overlay panelAnders L. Nielsen a b 1, Line H. Kristensen a c 1, Karen B. Stephansen a, Jan B.L. Kristensen a, Charlotte Helgstrand a, Michael Lees c, Paul Cloos c d, Kristian Helin c d, Michael Gajhede a, Lars Olsen
Abstract
Identification of inhibitors of histone–lysine demethylase (HDM) enzymes is important because of their involvement in the development of cancer. An ELISA-based assay was developed for identification of inhibitors of the HDM KDM4C in a natural products library. Based on one of the hits with affinity in the

low μM range (1, a catechol), a subset of structurally related compounds was selected and tested against a panel of HDMs. In this subset, two inhibitors (2 and 10) had comparable affinities towards KDM4C and KDM6A but no effect on PHF8. One inhibitor restored H3K9me3 levels in KDM4C transfected U2-OS cells.

Artesunate Inhibits Graft-Versus-Host Disease in Mice Via a Mechanism of Inducing Mitochondrial Calcium Overloading in Activated T Cells

Author links open overlay panelYing Wang PhD * 1, Shan He PhD 2, Yongnian Liu * 2, Robert Hooper PhD * 3, Hongshuang Yu * 1, Yuanyuan Tian * 1 1, Bui Tien * 3, Bryant M Schultz * 3, John W Elrod PhD * 3, Jonathan Soboloff * 4, Yi Zhang MD PhD 1

Abstract

Despite pharmacological prophylaxis using calcineurin (CN) inhibitors (i.e., cyclosporin A and Tacrolimus), graft-versus-host disease (GVHD) remains a major barrier to the success of allogeneic hematopoietic stem cell transplantation (allo-HSCT). Upon antigen stimulation, activated T cell receptor (TCR) signaling triggers rapid Ca2+ influx. This induces both CN-mediated NFAT activation, and increase of mitochondrial Ca2+ content, a major driver of metabolic activity. However, mitochondrial Ca2+ overload triggers opening of the mitochondrial permeability transition pore and cell death. We hypothesize that pharmacologically increasing mitochondrial Ca2+ load may decrease T cell survival capability, thereby reducing the GVH reaction. If this can be accomplished, it may lead to new strategies for inhibition of GVHD, which is conceptually different from the use of CN inhibitors. To test this hypothesis, we employed a high throughput drug screening system with the proliferation and cytotoxicity of TCR-activated human T cells as the readout, and screened the NPL-800 library (https://www.timtec.net) composed of 800 pure natural compounds. With a stringent criterion in consideration of dose-dependent effect, 26 compounds stood out for reducing the count of activated human T cells with a reduction rate of at least 30% at both 1.0uM and 10.0uM. Positive hits included inhibitors of DNA synthesis, the Na-K-ATPase and mitochondrial metabolism. We were particularly interested in artesunate (ART), which is a derivative of artemisinin that has been used for treating malaria in patients. While artemisinin acts by inhibiting sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) in P. falciparum malaria, which causes passive endoplasmic reticulum (ER) Ca2+ depletion and the subsequent cytosolic Ca2+ influx, ART did not inhibit SERCA in T cells. Ex vivo culture assays showed that ART dose-dependently reduced the survival of TCR-activated murine T cells. This inhibitory effect of ART was abrogated by inhibiting Ca2+ influx using BTP2, a potent inhibitor of store-operated Ca2+ channels. Furthermore, treatment of murine CD8 T cells with ART induced significant increases in mitochondrial Ca2+ loading upon TCR activation. These data suggest that inhibition of T cell survival by ART was dependent on TCR activation-induced Ca2+ influx and associated with enhanced mitochondrial Ca2+ uptake. We examined the impact of ART on GVHD in Balb/c mice receiving C57BL/6 (B6) mouse T cell-depleted bone marrow (TCD-BM) and CD4+ T cells. Intraperitoneal injection of ART (10 mg/kg, every other day) from day 1 to day 28 after transplantation reduced clinical signs of GVHD in these recipients and significantly improved their overall survival. Similar inhibition effects of ART on GVHD were observed in miHA-mismatched B6 anti-Balb/b and haplo-identical B6 anti-BDF1 mouse models of GVHD. Further investigations showed that in vivo administration of ART caused significant decreases in the number of host-reactive donor T cells in the spleen and liver of Balb/c mice 7 days after transfer of B6 TCD-BM plus CD4+ T cells. ART treatment did not affect the capacity of donor T cells to produce effector cytokines (e.g., IFN-g and TNF-α) in individual cells. Importantly, in vivo administration of ART preserved anti-leukemia activity of donor T cells and did not impair the reconstitution of hematopoiesis and lymphocytes. Collectively, our findings indicate that pharmacologically increasing mitochondrial Ca2+ loading may have significant implications in the development of novel strategies to prevent GVHD and other T cell-mediated inflammatory disorders in a broad context. Since ART therapy has been clinically approved, this work could be immediately translated into patients.

Combinatorial Efficacy of Nanoliposomal Ceramide and the Antioxidant 7,8- Benzoflavone for Acute Myeloid Leukemia Brian M. Barth1,2,3*, Timothy J. Brown1,2, Matthew T. Adams1,2, Aileen M. Garcia3,4, Lindsey N. Fisher1,2, Jennifer L. Fritz5 , Adam J. Beck5 , Colin M. McGill6 , Mark Kester2,3,7, Melissa A. Tran5 and David F. Claxton1,2 1Department of Medicine, Division of Hematology and Oncology, Penn State College of Medicine, Hershey, PA, USA 2Penn State Hershey Cancer Institute, Penn State College of Medicine, Hershey, PA, USA 3Department of Pharmacology, Penn State College of

Medicine, Hershey, PA, USA 4Department of Biomedical Science, Inter American University of Puerto Rico, Ponce, PR, USA 5Drug Discovery, Development, and Delivery Core, Penn State College of Medicine, Hershey, PA, USA 6Department of Chemistry, University of Alaska-Anchorage, Anchorage, AK, USA 7University of Virginia Cancer Center, Charlottesville, VA, USA *Corresponding author: Dr. Brian M Barth, Department of Medicine, Division of Hematology and Oncology, Penn State Hershey Cancer Institute, Penn State College of Medicine, 500 University Drive, PO Box 850, CH46 Hershey, PA 17033, USA, Tel: 717-531-0003 (289457); E-mail: bmb14@psu.edu Rec date: Aug 28, 2014, Acc date: Sep 18, 2014; Pub date: Sep 23, 2014

Abstract

Ceramide-based therapeutics have gained recent attention as anti-neoplastic therapeutics. These include standard of care therapeutics that in part exert efficacy through the generation of ceramide, as well as new therapeutics that seek to specifically deliver or augment ceramide levels in malignant cells. Ceramide is a bioactive sphingolipid involved in apoptotic and stress cellular signaling pathways. It has also been shown to regulate oxidative stress, which may negate its otherwise anti-neoplastic effects by promoting the proliferation of leukemia cells. Metabolism of ceramide to neutral or pro-oncogenic metabolites can serve as a further pathway of therapeutic resistance. In this study, the antioxidant 7,8-benzoflavone (BF) was identified through a natural products chemical library screening process as a compound that can augment the efficacy of nanoliposomal C6-ceramide (Lip-C6) in cellular models of Acute Myeloid Leukemia (AML). This study demonstrates that BF exerts an antioxidant effect in AML, which likely refines the bioactivity of ceramide as an anti-leukemic agent. Intriguingly, BF has been shown to block drug efflux pumps, such as P-glycoprotein, allowing BF to also impede P-glycoprotein-mediated ceramide glycosylation. In this study, BF was further formulated into nanoliposomes for in vivo studies using two murine models of AML. Treatment of C3H/HeJ mice engrafted with a FLT3-ITD driven AML with a combinatorial nanoliposomal formulation of BF and Lip-C6 significantly augmented the survival of mice beyond that of nanoliposomal formulations containing either agent alone. This was in contrast to the modest extension of survival of C57BL/6J mice engrafted with C1498 AML cells utilizing either single agent or combinatorial nanoliposomal formulations. Altogether this study demonstrates that the anti-AML efficacy of Lip-C6 as a ceramide-based therapeutic can be augmented for particular types of AML, such as that driven by FLT3-ITD, by combinatorial treatment with the antioxidant BF.

Watson VG, Drake KM, et.al. Development of a high-throughput screening-compatible assay for the discovery of inhibitors of the AF4-AF9 interaction using AlphaScreen technology. Assay Drug Dev Technol. 2013 May;11(4):253-68. doi: 10.1089/adt.2012.495.

Abstract

Rearrangements of the mixed-lineage leukemia (MLL) gene occur predominately in pediatric leukemia cases and are generally predictors of a poor prognosis. These chromosomal rearrangements result in fusion of the protein MLL to one of more than 60 protein partners. MLL fusions are potent inducers of leukemia through activation of oncogene expression; therefore, targeting this transcriptional activation function may arrest MLL-rearranged (MLL-R) leukemia. Leukemic cell lines harboring the most common fusion protein, MLL-AF4, require the direct interaction of AF4 with the transcription factor AF9 to survive and self-renew; disrupting this interaction with a cell-penetrating AF4- derived peptide results in cell death, suggesting that the AF4-AF9 interaction could be a viable target for a novel MLL-R leukemia therapy. Here we describe the use of AlphaScreen technology to develop a high-throughput screening (HTS) assay to detect nonpeptidic inhibitors of AF4-AF9 binding. The assay is economical, requiring only low nanomolar concentrations of biotinylated AF4-derived peptide and FLAG-tagged AF9 in low-volume 384-well plates. A Z'-factor of 0.71 and a signal-to-background ratio of 21.3 showed the assay to be robust, and sensitivity to inhibition was demonstrated with competing AF4-derived peptides. Two pilot screens comprising 5,680 compounds served as validation for HTS at Nemours and the Broad Institute. Assay artifacts were excluded using a counterscreen comprising a biotinylated FLAG peptide. This is the first reported HTS-compatible assay to identify compounds that inhibit a key binding interaction of an MLL fusion partner, and the results presented here demonstrate suitability for screening large chemical libraries in high-density, low-volume plate formats.

NPL800

Schuster D, Wolber G. Identification of bioactive natural products by pharmacophore-based virtual screening. Curr Pharm Des. 2010 May;16 (15):1666-81.

Abstract
Natural products have been exposed to a long selection process to interact with biological targets and are therefore a valuable source for ideas for novel chemical entities in drug development. However, the process to determine activities of natural products is mainly based on serendipity, and can thus become time- and cost-intensive. In this review we present strategies on how modern in-silico molecular modeling techniques can be used to make this process more efficient and discuss how to discover and optimize drug candidates inspired by nature. Focusing on 3D pharmacophore modeling techniques, we provide an overview of virtual screening and modeling methods, review available in silico databases as sources for chemical structures of natural products, discuss techniques for biological activity profiling, and summarize recent success stories for the combination of in-silico approaches and pharmacognosy. Montague CR, Fitzmaurice A,et.al. Screen for Small Molecules Increasing the Mitochondrial Membrane Potential. J Biomol Screen. 2013 Jul 18.

Abstract
The identification of small molecules that positively modulate the mitochondrial respiratory function has broad applications in fundamental research, therapeutic target validation, and drug discovery. We present an approach in which primary screens for mitochondrial function in yeast are used to efficiently identify a subset of high-value compounds that can in turn be rapidly tested against a broad range of mammalian cell lines. The ability of the yeast assay to successfully identify in a high-throughput format hit compounds that increase the mitochondrial membrane potential and adenosine triphosphate (ATP) levels by as little as 15% was demonstrated. In this study, 14 hits were identified from a collection of 13,680 compounds. Secondary testing with myotubes, fibroblasts, and PC-12 and HepG2 cells identified two compounds increasing ATP levels in hepatocytes and two other compounds increasing ATP in fibroblasts. The effect on hepatocytes was further studied using genomic and mitochondrial proteomic tools to characterize the changes induced by the two compounds. Changes in the accumulation of a series of factors involved in early gene response or apoptosis or linked to metabolic functions (i.e., -Klotho, RORa, PGC-1a, G6PC, IGFBP1, FTL) were discovered.

Simon E Alfred, Anuradha Surendra, et.al. A phenotypic screening platform to identify small molecule modulators of Chlamydomonas reinhardtii growth, motility and photosynthesis. Genome Biol. 2012; 13(11): R105. doi: 10.1186/gb-2012-13-11-r105

Abstract
Chemical biology, the interfacial discipline of using small molecules as probes to investigate biology, is a powerful approach of developing specific, rapidly acting tools that can be applied across organisms. The single-celled alga Chlamydomonas reinhardtii is an excellent model system because of its photosynthetic ability, cilia-related motility and simple genetics. We report the results of an automated fitness screen of 5,445 small molecules and subsequent assays on motility/phototaxis and photosynthesis. Cheminformatic analysis revealed active core structures and was used to construct a na ve Bayes model that successfully predicts algal bioactive compounds.

Dik-Lung Ma, Daniel Shiu-Hin Chana and Chung-Hang Leung. Molecular docking for virtual screening of natural product databases. Chem. Sci., 2011,2, 1656-1665. DOI: 10.1039/C1SC00152C

Abstract
Molecular docking enables the extraordinary structural diversity of natural products to be harnessed in an efficient manner. In this mini- review, we highlight recent examples of the use of molecular docking in virtual screening for the identification of bioactive molecules from natural product databases.

Austin B. Yongye, Jacob Waddell, Jos L. Medina-Franco. Molecular Scaffold Analysis of Natural Products Databases in the Public Domain. Chem Biology & Drug Design. Vol 80, 5, pages 717 724, November 2012. DOI: 10.1111/cbdd.12011

Abstracts

Natural products represent important sources of bioactive compounds in drug discovery efforts. In this work, we compiled five natural products databases available in the public domain and performed a comprehensive chemoinformatic analysis focused on the content and diversity of the scaffolds with an overview of the diversity based on molecular fingerprints. The natural products databases were compared with each other and with a set of molecules obtained from in-house combinatorial libraries, and with a general screening commercial library. It was found that publicly available natural products databases have different scaffold diversity. In contrast to the common concept that larger libraries have the largest scaffold diversity, the largest natural products collection analyzed in this work was not the most diverse. The general screening library showed, overall, the highest scaffold diversity. However, considering the most frequent scaffolds, the general reference library was the least diverse. In general, natural products databases in the public domain showed low molecule overlap. In addition to benzene and acyclic compounds, flavones, coumarins, and flavanones were identified as the most frequent molecular scaffolds across the different natural products collections. The results of this work have direct implications in the computational and experimental screening of natural product databases for drug discovery.

Ma C, Lazo JS, Xie XQ. Compound acquisition and prioritization algorithm for constructing structurally diverse compound libraries. ACS Comb Sci. 2011 May 9;13(3):223-31. doi: 10.1021/co100033m

Abstract

In the present study, we report a compound acquisition and prioritization algorithm established for rational chemical library purchasing or compound synthesis to increase the diversity of an existing compound collection. This method was established based on a chemistry-space calculation using BCUT (Burden CAS University of Texas) descriptors. To identify the acquisition of compounds from candidate collections into the existing collection, a derived distance-based selection rule was applied, and the results were well supported by pairwise similarity calculations and cell-partition statistics in chemistry space. The correlation between chemistry-space distance and Tanimoto similarity index was also studied to justify the compound acquisition strategy through weighted linear regression. As a rational approach for library design, the distance-based selection rule exhibits certain advantages in prioritizing compound selection to enhance the overall structural diversity of an existing in-house compound collection or virtual combinatorial library for in silico screening, diversity oriented synthesis, and high-throughput screening.

Shaneyfelt ME, Burke AD, et.al. Natural products that reduce rotavirus infectivity identified by a cell-based moderate-throughput screening assay. Virol J. 2006 Sep 1;3:68.

Abstract

BACKGROUND: There is widespread interest in the use of innate immune modulators as a defense strategy against infectious pathogens. Using rotavirus as a model system, we developed a cell-based, moderate-throughput screening (MTS) assay to identify compounds that reduce rotavirus infectivity in vitro, toward a long-term goal of discovering immunomodulatory agents that enhance innate responses to viral infection. RESULTS: A natural product library consisting of 280 compounds was screened in the assay and 15 compounds that significantly reduced infectivity without cytotoxicity were identified. Time course analysis of four compounds with previously characterized effects on inflammatory gene expression inhibited replication with pre-treatment times as minimal as 2 hours. Two of these four compounds, alpha-mangostin and 18-beta- glycyrrhetinic acid, activated NFkappaB and induced IL-8 secretion. The assay is adaptable to other virus systems, and amenable to full automation and adaptation to a high-throughput format. CONCLUSION: Identification of several compounds with known effects on inflammatory and antiviral gene expression that confer resistance to rotavirus infection in vitro suggests the assay is an appropriate platform for discovery of compounds with potential to amplify innate antiviral responses.

Bologa CG, Olah MM, Oprea TI.Chemical database preparation for compound acquisition or virtual screening.Methods Mol Biol. 2006;316:375-88.

Abstract Virtual and high-throughput screening are time-saving techniques that have been successfully applied to identify novel chemotypes in biologically active molecules. Both methods require the ability to

aptly handle large numbers of chemicals prior to an experiment or acquisition. We describe a step-by-step preparation procedure for handling large collections of existing or virtual compounds prior to virtual screening or acquisition.

Feng BY, Simeonov A, et.al. A high-throughput screen for aggregation-based inhibition in a large compound library. J Med Chem. 2007 May 17;50(10):2385-90

Abstract
High-throughput screening (HTS) is the primary technique for new lead identification in drug discovery and chemical biology. Unfortunately, it is susceptible to false-positive hits. One common mechanism for such false-positives is the congregation of organic molecules into colloidal aggregates, which nonspecifically inhibit enzymes. To both evaluate the feasibility of large-scale identification of aggregate-based inhibition and quantify its prevalence among screening hits, we tested 70,563 molecules from the National Institutes of Health Chemical Genomics Center (NCGC) library for detergent-sensitive inhibition. Each molecule was screened in at least seven concentrations, such that dose-response curves were obtained for all molecules in the library. There were 1274 inhibitors identified in total, of which 1204 were unambiguously detergent-sensitive. We identified these as aggregate-based inhibitors. Thirty-one library molecules were independently purchased and retested in secondary low-throughput experiments; 29 of these were confirmed as either aggregators or nonaggregators, as appropriate. Finally, with the dose-response information collected for every compound, we could examine the correlation between aggregate-based inhibition and steep dose-response curves. Three key results emerge from this study: first, detergent-dependent identification of aggregate-based inhibition is feasible on the large scale. Second, 95% of the actives obtained in this screen are aggregate-based inhibitors. Third, aggregate-based inhibition is correlated with steep dose-response curves, although not absolutely. The results of this screen are being released publicly via the PubChem database.

Shelat AA, Guy RK. The interdependence between screening methods and screening libraries. Curr Opin Chem Biol. 2007 Jun;11(3):244-51. Epub 2007 May 23.

Abstract
The most common methods for discovery of chemical compounds capable of manipulating biological function involves some form of screening. The success of such screens is highly dependent on the chemical materials - commonly referred to as libraries - that are assayed. Classic methods for the design of screening libraries have depended on knowledge of target structure and relevant pharmacophores for target focus, and on simple count-based measures to assess other properties. The recent proliferation of two novel screening paradigms, structure-based screening and high-content screening, prompts a profound rethink about the ideal composition of small-molecule screening libraries. We suggest that currently utilized libraries are not optimal for addressing new targets by high-throughput screening, or complex phenotypes by high-content screening.

Broom WJ, Auwarter KE,et.al. Two approaches to drug discovery in SOD1-mediated ALS.J Biomol Screen. 2006 Oct;11(7):729-35.

Abstract
Familial amyotrophic lateral sclerosis (ALS) accounts for 10% of all ALS cases; approximately 25% of these cases are due to mutations in the Cu/Zn superoxide dismutase gene (SOD1). To date, 105 different mutations spanning all 5 exons have been identified in the SOD1 gene. Mutant SOD1-associated ALS is caused by a toxic gain of function of the mutated protein. Therefore, regardless of the specific mechanism whereby mutant SOD1 initiates motor neuron death, the authors hypothesize that measures that decrease levels of mutant SOD1 protein should ameliorate the phenotype in transgenic mice and potentially in patients with SOD1-mediated disease. They have designed 2 cell-based screening assays to identify small, brain-permeant molecules that inactivate expression of the SOD1 gene or increase the degradation of the SOD1 protein. Here they describe the development and optimization of these assays and the results of high-throughput screening using a variety of compound libraries, including a total of more than 116,000 compounds. The majority of the hit compounds identified that down- regulated SOD1 were shown to be toxic in a cell-based viability assay or were nonselective transcription inhibitors, but work is continuing on

a number of nonspecific inhibitors of SOD1 expression. Ultimately, the authors believe that these 2 cell-based assays will provide powerful strategies to identify novel therapies for the treatment of inherited SOD1-associated forms of ALS.

Buckner D, Wilson S, et.al. Use of early passage fetal intestinal epithelial cells in semi-high-throughput screening assays: an approach to identify new innate immune system adjuvants.J Biomol Screen. 2006 Sep;11(6):664-71.

Abstract
Innate immune system stimulants (innate adjuvants) offer complementary approaches to vaccines and antimicrobial compounds to increase host resistance to infection. The authors established fetal bovine intestinal epithelial cell (BIEC) cultures to screen natural product and synthetic compound libraries for novel mucosal adjuvants. They showed that BIECs from fetal intestine maintained an in vivo phenotype as reflected in cytokeratin expression, expression of antigens restricted to intestinal enterocytes, and induced interleukin-8 (IL-8) production. BIECs could be infected by and support replication of bovine rotavirus. A semi-high-throughput enzyme-linked immunosorbent assay-based assay that measured IL-8 production by BIECs was established and used to screen commercially available natural compounds for novel adjuvant activity. Five novel hits were identified, demonstrating the utility of the assay for selecting and screening new epithelial cell adjuvants. Although the identified compounds had not previously been shown to induce IL-8 production in epithelial cells, other known functions for 3 of the 5 were consistent with this activity. Statistical analysis of the throughput data demonstrated that the assay is adaptable to a high-throughput format for screening both synthetic and natural product derived compound libraries.

Nielsen AL, Kristensen LH,et.al.Identification of catechols as histone-lysine demethylase inhibitors.FEBS Lett. 2012 Apr 24;586(8):1190-4. doi: 10.1016/j.febslet.2012.03.001

Abstract
Identification of inhibitors of histone-lysine demethylase (HDM) enzymes is important because of their involvement in the development of cancer. An ELISA-based assay was developed for identification of inhibitors of the HDM KDM4C in a natural products library. Based on one of the hits with affinity in the low M range (1, a catechol), a subset of structurally related compounds was selected and tested against a panel of HDMs. In this subset, two inhibitors (2 and 10) had comparable affinities towards KDM4C and KDM6A but no effect on PHF8. One inhibitor restored H3K9me3 levels in KDM4C transfected U2-OS cells.

Simeonov A, Yasgar A,et.al.Dual-fluorophore quantitative high-throughput screen for inhibitors of BRCT-phosphoprotein interaction.Anal Biochem. 2008 Apr 1;375(1):60-70. Epub 2007 Dec 5.

Abstract
Finding specific small-molecule inhibitors of protein-protein interactions remains a significant challenge. Recently, attention has grown toward "hot spot" interactions where binding is dominated by a limited number of amino acid contacts, theoretically offering an increased opportunity for disruption by small molecules. Inhibitors of the interaction between BRCT (the C-terminal portion of BRCA1, a key tumor suppressor protein with various functions) and phosphorylated proteins (Abraxas/BACH1/CtIP), implicated in DNA damage response and repair pathways, should prove to be useful in studying BRCA1's role in cancer and in potentially sensitizing tumors to chemotherapeutic agents. We developed and miniaturized to a 1536-well format and 3-mul final volume a pair of fluorescence polarization (FP) assays using fluorescein- and rhodamine-labeled pBACH1 fragment. To minimize the effect of fluorescence artifacts and to increase the overall robustness of the screen, the 75,552 compound library members all were assayed against both the fluorescein- and rhodamine-labeled probe-protein complexes in separate but interleaved reactions. In addition, every library compound was tested over a range of concentrations following the quantitative high-throughput screening (qHTS) paradigm. Analyses of the screening results led to the selection and subsequent confirmation of 16 compounds active in both assays. Faced with a traditionally difficult protein-protein interaction assay, by performing two-fluorophore qHTS, we were able to confidently select a number of actives for further studies.

Simeonov A, Jadhav A,et.al.Fluorescence spectroscopic profiling of compound libraries.J Med Chem. 2008 Apr 24;51(8):2363-71. doi: 10.1021/jm701301m

Abstract
Chromo/fluorophoric properties often accompany the heterocyclic scaffolds and impurities that comprise libraries used for high-throughput screening (HTS). These properties affect assay outputs obtained with optical detection, thus complicating analysis and leading to false positives and negatives. Here, we report the fluorescence profile of more than 70,000 samples across spectral regions commonly utilized in HTS. The quantitative HTS paradigm was utilized to test each sample at seven or more concentrations over a 4-log range in 1,536-well format. Raw fluorescence was compared with fluorophore standards to compute a normalized response as a function of concentration and spectral region. More than 5% of library members were brighter than the equivalent of 10 nM 4-methyl umbelliferone, a common UV-active probe. Red-shifting the spectral window by as little as 100 nm was accompanied by a dramatic decrease in autofluorescence. Native compound fluorescence, fluorescent impurities, novel fluorescent compounds, and the utilization of fluorescence profiling data are discussed.

Anton Simeonov, Ajit Jadhav, et.al. Quantitative High-Throughput Screen Identifies Inhibitors of the Schistosoma mansoni Redox Cascade. PLoS Negl Trop Dis. 2008 January; 2(1): e127.Published online 2008 January 2. doi: 10.1371/journal.pntd.0000127

Abstract
Schistosomiasis is a tropical disease associated with high morbidity and mortality, currently affecting over 200 million people worldwide. Praziquantel is the only drug used to treat the disease, and with its increased use the probability of developing drug resistance has grown significantly. The Schistosoma parasites can survive for up to decades in the human host due in part to a unique set of antioxidant enzymes that continuously degrade the reactive oxygen species produced by the host's innate immune response. Two principal components of this defense system have been recently identified in S. mansoni as thioredoxin/glutathione reductase (TGR) and peroxiredoxin (Prx) and as such these enzymes present attractive new targets for anti-schistosomiasis drug development. Inhibition of TGR/Prx activity was screened in a dual-enzyme format with reducing equivalents being transferred from NADPH to glutathione via a TGR-catalyzed reaction and then to hydrogen peroxide via a Prx-catalyzed step. A fully automated quantitative high-throughput (qHTS) experiment was performed against a collection of 71,028 compounds tested as 7- to 15-point concentration series at 5 L reaction volume in 1536-well plate format. In order to generate a robust data set and to minimize the effect of compound autofluorescence, apparent reaction rates derived from a kinetic read were utilized instead of end-point measurements. Actives identified from the screen, along with previously untested analogues, were subjected to confirmatory experiments using the screening assay and subsequently against the individual targets in secondary assays. Several novel active series were identified which inhibited TGR at a range of potencies, with IC50s ranging from micromolar to the assay response limit (~25 nM). This is, to our knowledge, the first report of a large-scale HTS to identify lead compounds for a helminthic disease, and provides a paradigm that can be used to jump-start development of novel therapeutics for other neglected tropical diseases.

Kabbani N, Woll MP,et.al.Dopamine receptor interacting proteins: targeting neuronal calcium sensor-1/D2 dopamine receptor interaction for antipsychotic drug development.Curr Drug Targets. 2012 Jan;13(1):72-9.

Abstract
D2 dopamine receptors (D2Rs) represent an important class of receptors in the pharmacological development of novel therapeutic drugs for the treatment of schizophrenia. Recent research into D2R signaling suggests that receptor properties are dependent on interaction with a cohort of dopamine receptor interacting proteins (DRIPs) within a macromolecular structure termed the signalplex. One component of this signalplex is neuronal calcium sensor 1 (NCS-1) a protein found to regulate the phosphorylation, trafficking, and signaling profile of the D2R in neurons. It has also been found that NCS-1 can contribute to the pathology of schizophrenia and may play a role in the efficacy of antipsychotic drug medication in the brain. In this review we discuss how the selective targeting of a DRIP, such as NCS-1, can be utilized as a novel strategy of drug design for the creation of new therapeutics for a disease such as schizophrenia.

Using a fluorescence polarization assay we explore how the ability to detect changes in D2R/NCS-1 interaction can be exploited as an effective screening tool in the isolation and development of lead compounds for antipsychotic drug development. This line of work explores a novel direction in targeting D2Rs via their signalplex components and supports the notion that receptor interacting proteins represent an emerging new class of molecular targets for pharmacological drug development.

Honson NS, Johnson RL,et.al.Differentiating Alzheimer disease-associated aggregates with small molecules.Neurobiol Dis. 2007 Dec;28(3):251- 60. Epub 2007 Jul 28.

Abstract
Alzheimer disease is diagnosed postmortem by the density and spatial distribution of beta-amyloid plaques and tau-bearing neurofibrillary tangles. The major protein component of each lesion adopts cross-beta-sheet conformation capable of binding small molecules with submicromolar affinity. In many cases, however, Alzheimer pathology overlaps with Lewy body disease, characterized by the accumulation of a third cross-beta-sheet forming protein, alpha-synuclein. To determine the feasibility of distinguishing tau aggregates from beta-amyloid and alpha-synuclein aggregates with small molecule probes, a library containing 72,455 small molecules was screened for antagonists of tau- aggregate-mediated changes in Thioflavin S fluorescence, followed by secondary screens to distinguish the relative affinity for each substrate protein. Results showed that >10-fold binding selectivity among substrates could be achieved, with molecules selective for tau aggregates containing at least three aromatic or rigid moieties connected by two rotatable bonds.

Gallo-Ebert C, Donigan M,et.al. Novel Antifungal Drug Discovery Based On Targeting Pathways Regulating The Fungal-Conserved Upc2 Transcription Factor.Antimicrob Agents Chemother. 2013 Oct 21.

Abstract
Infections by Candida albicans and related fungal pathogens pose a serious health problem for immune compromised patients. Azole drugs, the most common agents used to combat infections, target the sterol biosynthetic pathway. Adaptation to azole therapy develops as drug stressed cells compensate by up-regulating several genes in the pathway, a process mediated in part by the Upc2 transcription factor. We have implemented a cell-based high throughput screen to identify small molecule inhibitors of Upc2-dependent induction of sterol gene expression in response to azole drug treatment. The assay is designed to identify not only Upc2 DNA binding inhibitors, but also compounds impeding the activation of gene expression by Upc2. An AlphaScreen assay was developed to determine if the compounds identified interact directly with Upc2 and inhibit DNA binding. Three compounds identified by the cell-based assay inhibited Upc2 protein level and UPC2-LacZ gene expression in response to a block in sterol biosynthesis. The compounds were growth inhibitory and attenuated antifungal-induced sterol gene expression in vivo. They did so by reducing the level of Upc2 protein and Upc2 DNA binding in the presence of drug. The mechanism by which the compounds restrict Upc2 DNA binding is not through a direct interaction, as demonstrated by a lack of DNA binding inhibitory activity using the AlphaScreen assay. Rather, they likely inhibit a novel pathway activating Upc2 in response to a block in sterol biosynthesis. We suggest the compounds identified represent potential precursors for the synthesis of novel antifungal drugs.

Auld DS, Southall NT,et.al. Characterization of chemical libraries for luciferase inhibitory activity. J Med Chem. 2008 Apr 24;51(8):2372- 86. doi: 10.1021/jm701302v. Epub 2008 Mar 26.

Abstract
To aid in the interpretation of high-throughput screening (HTS) results derived from luciferase-based assays, we used quantitative HTS, an approach that defines the concentration-response behavior of each library sample, to profile the ATP-dependent luciferase from Photinus pyralis against more than 70,000 samples. We found that approximately 3% of the library was active, containing only compounds with inhibitory concentration-responses, of which 681 (0.9%) exhibited IC 50 < 10 microM. Representative compounds were shown to inhibit purified P. pyralis as well as several commercial luciferase-based detection reagents but were found to be largely inactive against Renilla reniformis luciferase. Light attenuation by the samples was also examined and found to be more prominent in the blue-shifted

bioluminescence produced by R. reniformis luciferase than in the bioluminescence produced by P. pyralis luciferase. We describe the structure-activity relationship of the luciferase inhibitors and discuss the use of this data in the interpretation of HTS results and configuration of luciferase-based assays.

Auld DS, Zhang YQ,et.al.A basis for reduced chemical library inhibition of firefly luciferase obtained from directed evolution.J Med Chem. 2009 Mar 12;52(5):1450-8. doi: 10.1021/jm8014525.

Abstract
We measured the "druggability" of the ATP-dependent luciferase derived from the firefly Photuris pennsylvanica that was optimized using directed evolution (Ultra-Glo, Promega). Quantitative high-throughput screening (qHTS) was used to determine IC(50)s of 198899 samples against a formulation of Ultra-Glo luciferase (Kinase-Glo). We found that only 0.1% of the Kinase-Glo inhibitors showed an IC(50) < 10 microM compared to 0.9% found from a previous qHTS against the firefly luciferase from Photinus pyralis (lucPpy). Further, the maximum affinity identified in the lucPpy qHTS was 50 nM, while for Kinase-Glo this value increased to 600 nM. Compounds with interactions stretching outside the luciferin binding pocket were largely lost with Ultra-Glo luciferase. Therefore, Ultra-Glo luciferase will show less compound interference when used as an ATP sensor compared to lucPpy. This study demonstrates the power of large-scale quantitative analysis of structure-activity relationships (>100K compounds) in addressing important questions such as a target's druggability.

Suvi Manner, Malena Skogman,et.al. Systematic Exploration of Natural and Synthetic Flavonoids for the Inhibition of Staphylococcus aureus Biofilms. Int J Mol Sci. 2013 October; 14(10): 19434 19451.

Abstract
When single-cell (or suspended) bacteria switch into the biofilm lifestyle, they become less susceptible to antimicrobials, imposing the need for anti-biofilms research. Flavonoids are among the most extensively studied natural compounds with an unprecedented amount of bioactivity claims. Most studies focus on the antibacterial effects against suspended cells; fewer reports have researched their anti- biofilm properties. Here, a high throughput phenotypic platform was utilized to screen for the inhibitory activity of 500 flavonoids, including natural and synthetic derivatives, against Staphylococcus aureus biofilms. Since discrepancies among results from earlier antibacterial studies on flavonoids had been noted, the current study aimed to minimize sources of variations. After the first screen, flavonoids were classified as inactive (443), moderately active (47) or highly active (10). Further, exclusion criteria combining bioactivity and selectivity identified two synthetic flavans as the most promising. The body of data reported here serves three main purposes. First, it offers an improved methodological workflow for anti-biofilm screens of chemical libraries taking into account the (many times ignored) connections between anti-biofilm and antibacterial properties. This is particularly relevant for the study of flavonoids and other natural products. Second, it provides a large and freely available anti-biofilm bioactivity dataset that expands the knowledge on flavonoids and paves the way for future structure-activity relationship studies and structural optimizations. Finally, it identifies two new flavans that can successfully act on biofilms, as well as on suspended bacteria and represent more feasible antibacterial candidates.

Schreiber KJ, Nasmith CG,et.al.Found in translation: high-throughput chemical screening in Arabidopsis thaliana identifies small molecules that reduce Fusarium head blight disease in wheat.Mol Plant Microbe Interact. 2011 Jun;24(6):640-8. doi: 10.1094/MPMI-09-10-0210

Abstract
Despite the tremendous economic impact of cereal crop pathogens such as the fungus Fusarium graminearum, the development of strategies for enhanced crop protection is hampered by complex host genetics and difficulties in performing high-throughput analyses. To bypass these challenges, we have developed an assay in which the interaction between F. graminearum and the model plant Arabidopsis thaliana is monitored in liquid media in 96-well plates. In this assay, fungal infection is associated with the development of dark lesion-like spots on the cotyledons of Arabidopsis seedlings by 4 days postinoculation. These symptoms can be alleviated by the application of known defense- activating small molecules and in previously described resistant host genetic backgrounds. Based on this infection phenotype, we conducted a small-scale chemical screen to identify small molecules that protect

Arabidopsis seedlings from infection by F. graminearum. We identified sulfamethoxazole and the indole alkaloid gramine as compounds with strong protective activity in the liquid assay. Remarkably, these two chemicals also significantly reduced the severity of F. graminearum infection in wheat. As such, the Arabidopsis-based liquid assay represents a biologically relevant surrogate system for high-throughput studies of agriculturally important plant-pathogen interactions.

Zhu PJ, Hobson JP,et.al. Quantitative high-throughput screening identifies inhibitors of anthrax-induced cell death.Bioorg Med Chem. 2009 Jul 15;17(14):5139-45. doi: 10.1016/j.bmc.2009.05.054. Epub 2009 May 29.

Abstract
Here, we report the results of a quantitative high-throughput screen (qHTS) measuring the endocytosis and translocation of a beta- lactamase-fused-lethal factor and the identification of small molecules capable of obstructing the process of anthrax toxin internalization. Several small molecules protect RAW264.7 macrophages and CHO cells from anthrax lethal toxin and protected cells from an LF-Pseudomonas exotoxin fusion protein and diphtheria toxin. Further efforts demonstrated that these compounds impaired the PA heptamer pre-pore to pore conversion in cells expressing the CMG2 receptor, but not the related TEM8 receptor, indicating that these compounds likely interfere with toxin internalization.

Yamanaka K, Dorjsuren D,et.al.A comprehensive strategy to discover inhibitors of the translesion synthesis DNA polymerase ?.PLoS One. 2012;7(10):e45032. doi: 10.1371/journal.pone.0045032. Epub 2012 Oct 8.

Abstract
Human DNA polymerase kappa (pol ?) is a translesion synthesis (TLS) polymerase that catalyzes TLS past various minor groove lesions including N(2)-dG linked acrolein- and polycyclic aromatic hydrocarbon-derived adducts, as well as N(2)-dG DNA-DNA interstrand cross-links introduced by the chemotherapeutic agent mitomycin C. It also processes ultraviolet light-induced DNA lesions. Since pol ? TLS activity can reduce the cellular toxicity of chemotherapeutic agents and since gliomas overexpress pol ?, small molecule library screens targeting pol ? were conducted to initiate the first step in the development of new adjunct cancer therapeutics. A high-throughput, fluorescence-based DNA strand displacement assay was utilized to screen ~16,000 bioactive compounds, and the 60 top hits were validated by primer extension assays using non-damaged DNAs. Candesartan cilexetil, manoalide, and MK-886 were selected as proof-of-principle compounds and further characterized for their specificity toward pol ? by primer extension assays using DNAs containing a site-specific acrolein-derived, ring-opened reduced form of ?-HOPdG. Furthermore, candesartan cilexetil could enhance ultraviolet light-induced cytotoxicity in xeroderma pigmentosum variant cells, suggesting its inhibitory effect against intracellular pol ?. In summary, this investigation represents the first high-throughput screening designed to identify inhibitors of pol ?, with the characterization of biochemical and biologically relevant endpoints as a consequence of pol ? inhibition. These approaches lay the foundation for the future discovery of compounds that can be applied to combination chemotherapy.

Tradtrantip L, Zhang H,et.al.Small-molecule inhibitors of NMO-IgG binding to aquaporin-4 reduce astrocyte cytotoxicity in neuromyelitis optica.FASEB J. 2012 May;26(5):2197-208. doi: 10.1096/fj.11-201608. Epub 2012 Feb 8.

Abstract
Neuromyelitis optica (NMO) is an inflammatory demyelinating disease of spinal cord and optic nerve caused by pathogenic autoantibodies (NMO-IgG) against astrocyte aquaporin-4 (AQP4). We developed a high-throughput screen to identify blockers of NMO-IgG binding to human AQP4 using a human recombinant monoclonal NMO-IgG and transfected Fisher rat thyroid cells stably expressing human M23-AQP4. Screening of ~60,000 compounds yielded the antiviral arbidol, the flavonoid tamarixetin, and several plant-derived berbamine alkaloids, each of which blocked NMO-IgG binding to AQP4 without affecting AQP4 expression, array assembly, or water permeability. The compounds inhibited NMO-IgG binding to AQP4 in NMO patient sera and blocked NMO-IgG-dependent complement- and cell-mediated cytotoxicity with IC(50) down to ~5 M. Docking computations identified putative sites of blocker binding at the extracellular surface of AQP4. The blockers did not affect complement- dependent cytotoxicity

caused by anti-GD3 antibody binding to ganglioside GD3. The blockers reduced by >80% the severity of NMO lesions in an ex vivo spinal cord slice culture model of NMO and in mice in vivo. Our results provide proof of concept for a small-molecule blocker strategy to reduce NMO pathology. Small-molecule blockers may also be useful for other autoimmune diseases caused by binding of pathogenic autoantibodies to defined targets.

Hong CC.Large-scale small-molecule screen using zebrafish embryos.Methods Mol Biol. 2009;486:43-55. doi: 10.1007/978-1-60327-545-3_4.

Abstract
Zebrafish represents a versatile model organism with many molecular, morphological, and physiological similarities to mammals. Importantly, zebrafish are readily susceptible to perturbations by small molecules, including numerous pharmaceuticals in clinical use. Given these qualities, plus their small size and transparency, zebrafish embryos can be utilized for large-scale phenotype-based screens for small-molecule modifiers of biological processes. Thus, in a manner analogous to classical genetic screens, zebrafish chemical screens have the potential to reveal novel insights into complex biological pathways, as well as to identify lead compounds for novel therapeutics.

R. Eric Davis, Ya-Qin Zhang, et.al. ASSAY and Drug Development Technologies. February 2007, 5(1): 85-104. doi:10.1089/adt.2006.048.

Abstract
A cell-sensor assay for stabilization of I?Ba was developed in the activated B cell-like diffuse large B-cell lymphoma cell line OCI-Ly3. This cell line expresses known nuclear factor ?B (NF?B) target genes due to high constitutive activity of I?B kinase (IKK), which phosphorylates the protein I?Ba leading to proteasomal degradation of I?Ba and activation of NF?B. The cell-sensor assay uses green and red light-emitting beetle luciferases, with the green luciferase fused to I?Ba (I?Ba-CBG68) and the red luciferase (CBR) present in its native state. The I?Ba-CBG68 reporter functions as a sensor of IKK and proteasome activity, while CBR serves to normalize for cell number and nonspecific effects. Both reporter constructs were stably integrated and placed under the control of an inducible promoter system, which increased fold responsiveness to inhibitors when assay incubations were performed simultaneous to reporter induction by doxycycline. The assay was miniaturized to a 1,536-well plate format and showed a Z' of 0.6; it was then used to panel 2,677 bioactive compounds by a concentration response-based screening strategy. The concentration effect curves for the I?Ba-CBG68 and CBR signals were then used to identify specific stabilizers of I?Ba, such as IKK inhibitors or proteasome inhibitors, which increased the doxycycline-induced rise in I?Ba-CBG68 without affecting the rise in CBR. Known and unexpected inhibitors of NF?B signaling were identified from the bioactive collection. We describe here the development and performance of this assay, and discuss the merits of its specific features.

Bodner RA, Outeiro TF,et.al.Pharmacological promotion of inclusion formation: a therapeutic approach for Huntington's and Parkinson's diseases.Proc Natl Acad Sci U S A. 2006 Mar 14;103(11):4246-51. Epub 2006 Mar 6.

Abstract
Misfolded proteins accumulate in many neurodegenerative diseases, including huntingtin in Huntington's disease and alpha-synuclein in Parkinson's disease. The disease-causing proteins can take various conformations and are prone to aggregate and form larger cytoplasmic or nuclear inclusions. One approach to the development of therapeutic intervention for these diseases has been to identify chemical compounds that reduce the size or number of inclusions. We have, however, identified a compound that promotes inclusion formation in cellular models of both Huntington's disease and Parkinson's disease. Of particular interest, this compound prevents huntingtin-mediated proteasome dysfunction and reduces alpha-synuclein-mediated toxicity. These results demonstrate that compounds that increase inclusion formation may actually lessen cellular pathology in both Huntington's and Parkinson's diseases, suggesting a therapeutic approach for neurodegenerative diseases caused by protein misfolding.

Narwal M, Haikarainen T,et.al.Screening and structural analysis of flavones inhibiting tankyrases.J Med Chem. 2013 May 9;56(9):3507-17. doi: 10.1021/jm3018783. Epub 2013 Apr 24.

Abstract
Flavonoids are known for their beneficial effects on human health, and therefore the therapeutic potential of these compounds have been extensively studied. Flavone has been previously identified as a tankyrase inhibitor, and to further elucidate whether tankyrases would be inhibited by other flavonoids, we performed a systematic screening of tankyrase 2 inhibitory activity using 500 natural and naturally derived flavonoids covering nine different flavonoid classes. All identified tankyrase inhibitors were flavones. We report crystal structures of all the hit compounds in complex with the catalytic domain of human tankyrase 2. Flavone derivatives in all 10 crystal structures bind to the nicotinamide binding site of tankyrase 2. Potencies of the active flavones toward tankyrases vary between 50 nM and 1.1 M, and flavones show up to 200-fold selectivity for tankyrases over ARTD1. The molecular details of the interactions revealed by cocrystal structures efficiently describe the properties of potent flavone derivatives inhibiting tankyrases.

Ger D, Szoleczky P,et.al.Identification of pharmacological modulators of HMGB1-induced inflammatory response by cell-based screening.PLoS One. 2013 Jun 14;8(6):e65994. doi: 10.1371/journal.pone.0065994. Print 2013.

Abstract
High mobility group box 1 (HMGB1), a highly conserved, ubiquitous protein, is released into the circulation during sterile inflammation (e.g. arthritis, trauma) and circulatory shock. It participates in the pathogenesis of delayed inflammatory responses and organ dysfunction. While several molecules have been identified that modulate the release of HMGB1, less attention has been paid to identify pharmacological inhibitors of the downstream inflammatory processes elicited by HMGB1 (C23-C45 disulfide C106 thiol form). In the current study, a cell- based medium-throughput screening of a 5000+ compound focused library of clinical drugs and drug-like compounds was performed in murine RAW264.7 macrophages, in order to identify modulators of HMGB1-induced tumor-necrosis factor alpha (TNFa) production. Clinically used drugs that suppressed HMGB1-induced TNFa production included glucocorticoids, beta agonists, and the anti-HIV compound indinavir. A re-screen of the NIH clinical compound library identified beta-agonists and various intracellular cAMP enhancers as compounds that potentiate the inhibitory effect of glucocorticoids on HMGB1-induced TNFa production. The molecular pathways involved in this synergistic anti-inflammatory effect are related, at least in part, to inhibition of TNFa mRNA synthesis via a synergistic suppression of ERK/I?B activation. Inhibition of TNFa production by prednisolone+salbutamol pretreatment was also confirmed in vivo in mice subjected to HMGB1 injection; this effect was more pronounced than the effect of either of the agents administered separately. The current study unveils several drug-like modulators of HMGB1-mediated inflammatory responses and offers pharmacological directions for the therapeutic suppression of inflammatory responses in HMGB1-dependent diseases.

Ajit Jadhav, Rafaela S. Ferreira, et.al.Quantitative Analyses of Aggregation, Autofluorescence, and Reactivity Artifacts in a Screen for Inhibitors of a Thiol Protease.J. Med. Chem., 2010, 53 (1), pp 37 51. DOI: 10.1021/jm901070c

Abstract
The perceived and actual burden of false positives in high-throughput screening has received considerable attention; however, few studies exist on the contributions of distinct mechanisms of nonspecific effects like chemical reactivity, assay signal interference, and colloidal aggregation. Here, we analyze the outcome of a screen of 197861 diverse compounds in a concentration-response format against the cysteine protease cruzain, a target expected to be particularly sensitive to reactive compounds, and using an assay format with light detection in the short-wavelength region where significant compound autofluorescence is typically encountered. Approximately 1.9% of all compounds screened were detergent-sensitive inhibitors. The contribution from autofluorescence and compounds bearing reactive functionalities was dramatically lower: of all hits, only 1.8% were autofluorescent and 1.5% contained reactive or undesired functional groups. The distribution of false positives was relatively constant across library sources. The simple step of

including detergent in the assay buffer suppressed the nonspecific effect of approximately 93% of the original hits.

Tan SY, Chua SL,et.al.Identification of five structurally unrelated quorum-sensing inhibitors of Pseudomonas aeruginosa from a natural- derivative database.Antimicrob Agents Chemother. 2013 Nov;57(11):5629-41. doi: 10.1128/AAC.00955-13. Epub 2013 Sep 3.

Abstract
Bacteria communicate by means of small signal molecules in a process termed quorum sensing (QS). QS enables bacteria to organize their activities at the population level, including the coordinated secretion of virulence factors. Certain small-molecule compounds, known as quorum-sensing inhibitors (QSIs), have been shown to effectively block QS and subsequently attenuate the virulence of Pseudomonas aeruginosa, as well as increasing its susceptibility to both antibiotics and the immune system. In this study, a structure-based virtual screening (SB-VS) approach was used for the discovery of novel QSI candidates. Three-dimensional structures of 3,040 natural compounds and their derivatives were obtained, after which molecular docking was performed using the QS receptor LasR as a target. Based on docking scores and molecular masses, 22 compounds were purchased to determine their efficacies as quorum-sensing inhibitors. Using a live reporter assay for quorum sensing, 5 compounds were found to be able to inhibit QS-regulated gene expression in P. aeruginosa in a dose-dependent manner. The most promising compound, G1, was evaluated by isobaric tag for relative and absolute quantitation (iTRAQ)-based proteomic analysis, and it was found to significantly affect the abundance of 46 proteins (19 were upregulated; 27 were downregulated) in P. aeruginosa PAO1. It specifically reduced the expression of several quorum-sensing-regulated virulence factors, such as protease IV, chitinase, and pyoverdine synthetases. G1 was also able to reduce extracellular DNA release and inhibited the secretion of the virulence factor, elastase, whose expression is regulated by LasR. These results demonstrate the utility of SB-VS for the discovery of target-specific QSIs.

Inglese, J. and Auld, D. S. 2008. High Throughput Screening (HTS) Techniques: Applications in Chemical Biology. Wiley Encyclopedia of Chemical Biology. 1 15.

Abstract
The rapid testing of chemical libraries for biological activity is the primary aim of high throughput screening (HTS). Advances in HTS have paralleled those in molecular biology, instrumentation and automation, and informatics, and the increased availability of arrayed compound libraries. Sophisticated high sensitivity assays and the associated technologies required to implement these assays in HTS have been largely developed within the pharmaceutical industry for the identification of new chemical matter for drug development. However, HTS approaches are now widely applied to the broader questions within biological research. By way of introduction, we will describe the components of HTS and provide examples of strategies used to identify novel chemtypes for specific biological targets using large chemical libraries. We then will illustrate how more narrowly defined compound collections (e.g., targeted libraries or bioactive compounds) have been profiled against related targets or cell types for the purpose of discovering or defining, compound class/gene family selectivity, off-target activity or hidden phenotypes , toxic fingerprints, or any other relationship between chemical structure and bioactivity. In this way, HTS systems can expand the scope of an experimental hypothesis to address questions of chemical biology, be they at the level of an isolated enzymatic activity or that of a complex cellular phenotype.

Norton JT, Titus SA, et.al. Automated high-content screening for compounds that disassemble the perinucleolar compartment.J Biomol Screen. 2009 Oct;14(9):1045-53. doi: 10.1177/1087057109343120. Epub 2009 Sep 17.

Abstract
All solid malignancies share characteristic traits, including unlimited cellular proliferation, evasion of immune regulation, and the propensity to metastasize. The authors have previously described that a subnuclear structure, the perinucleolar compartment (PNC), is associated with the metastatic phenotype in solid tumor cancer cells. The percentage of cancer cells that contain PNCs (PNC prevalence) is indicative of the malignancy of a tumor both in vitro and in vivo, and thus PNC prevalence is a marker that reflects

metastatic capability in a population of tumor cells. Although the function of the PNC remains to be determined, the PNC is highly enriched with small RNAs and RNA binding proteins. The initial chemical biology studies using a set of anticancer drugs that disassemble PNCs revealed a direct association of the structure with DNA. Therefore, PNC prevalence reduction as a phenotypic marker can be used to identify compounds that target cellular processes required for PNC maintenance and hence used to elucidate the nature of the PNC function. Here the authors report the development of an automated high-content screening assay that is capable of detecting PNC prevalence in prostate cancer cells (PC-3M) stably expressing a green fluorescent protein (GFP)-fusion protein that localizes to the PNC. The assay was optimized using known PNC- reducing drugs and non-PNC-reducing cytotoxic drugs. After optimization, the fidelity of the assay was probed with a collection of 8284 compounds and was shown to be robust and capable of detecting known and novel PNC-reducing compounds, making it the first reported high- content phenotypic screen for small changes in nuclear structure.

Shah M, Stebbins JL,et.al.Inhibition of Siah2 ubiquitin ligase by vitamin K3 (menadione) attenuates hypoxia and MAPK signaling and blocks melanoma tumorigenesis.Pigment Cell Melanoma Res. 2009 Dec;22(6):799-808. doi: 10.1111/j.1755-148X.2009.00628.x. Epub 2009 Aug 27.

Abstract
The E3 ubiquitin ligase Siah2 has been implicated in the regulation of the hypoxia response, as well as in the control of Ras, JNK/p38/NF- kappaB signaling pathways. Both Ras/mitogen-activated protein kinase (MAPK) and hypoxia pathways are important for melanoma development and progression, pointing to the possible use of Siah2 as target for treatment of this tumor type. In the present study, we have established a high-throughput electro-chemiluninescent-based assay in order to screen and identify inhibitors of Siah2 ubiquitin ligase activity. Of 1840 compounds screened, we identified and characterized menadione (MEN) as a specific inhibitor of Siah2 ligase activity. MEN attenuated Siah2 self-ubiquitination, and increased expression of its substrates PHD3 and Sprouty2, with concomitant decrease in levels of HIF-1alpha and pERK, the respective downstream effectors. MEN treatment no longer affected PHD3 or Sprouty2 in Siah-KO cells, pointing to its Siah- dependent effects. Further, MEN inhibition of Siah2 was not attenuated by free radical scavenger, suggesting it is ROS-independent. Significantly, growth of xenograft melanoma tumors was inhibited following the administration of MEN or its derivative. These findings reveal an efficient platform for the identification of Siah inhibitors while identifying and characterizing MEN as Siah inhibitor that attenuates hypoxia and MAPK signaling, and inhibits melanoma tumorigenesis.

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