Canvass: A Crowd-Sourced, Natural-Product Screening Library for Exploring Biological Space

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Canvass: A Crowd-Sourced, Natural-Product Screening Library for Exploring Biological Space

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Supporting Information
ABSTRACT: Natural products and their derivatives continue to be wellsprings of nascent therapeutic potential. However, many laboratories have limited resources for biological evaluation, leaving their previously isolated or synthesized compounds largely or completely untested. To address this issue, the Canvass library of natural products was assembled, in collaboration with academic and industry researchers, for quantitative high-throughput screening (qHTS) across a diverse set of cell-based and biochemical assays. Characterization of the library in terms of physicochemical properties, structural diversity, and similarity to compounds in publicly available libraries indicates that the Canvass library contains many structural elements in common with approved drugs. The assay data generated were analyzed using a variety of quality control metrics, and the resultant assay profiles were explored using statistical methods, such as clustering and compound promiscuity analyses. Individual compounds were then sorted by structural class and activity profiles. Differential behavior based on these classifications, as well as noteworthy activities, are detailed within. One such highlight is the activity of (−)-2-(S)-cathafoline, which was found to stabilize calcium levels in the endoplasmic reticulum. The workflow described here illustrates a pilot effort to broadly survey the biological potential of natural products by utilizing the power of automation and high-throughput screening.

INTRODUCTION

Throughout history, nature has served as our primary source of medicines and continues to be one of the richest sources of new therapeutics. Either directly or as inspiration, natural products account for 50% to 70% of all small-molecule pharmaceutical agents currently in clinical use. While their influence has been most profound in the treatment of infectious diseases and cancer, natural products have also found utility in other therapeutic arenas such as pain, inflammation, and cardiovascular disorders. Yet, many pharmaceutical companies have diminished or abandoned natural products research throughout recent decades for a variety of reasons, ranging from the promise of emerging technologies (e.g., combinatorial chemistry), to concerns about international regulations of access to natural products and their sources. In parallel, decreasing in funding agency support for natural-products-related research have contributed to this contraction. However, within the same time period, natural compounds still continue to be both a significant source and point of inspiration for new medicines.2

Despite this dichotomy, the pendulum is swinging back toward natural products within both industry and academia. New screening libraries are being designed to incorporate key features of natural products, including scaffold diversity and stereochemistry.2 Strategic prefractionation methods have also facilitated high-throughput screening of natural product extracts.2,3 Genome mining with the goal of discovering “hidden” natural products within microbial genomes4 has fostered a great deal of excitement, and is the foundational approach of a number of pharmaceutical companies. The academic sector has also begun to see a recovery in the funding climate, as reflected in the creation of the Center for High-Throughput Functional Annotation of Natural Products (HiFAN).5 HiFAN is a collaborative, international, multi-institute center established to determine the mechanism of action of natural products and botanicals, with the intention of making platform technologies and data available community-wide. Together, these and other developments bode well for the renewed interest in nature as a rich resource for biologically relevant chemical matter.

While this resurgence has significant potential, especially to address the imminent threat of antibiotic resistance,6 we hypothesize that therapeutic opportunities for natural products across other disease indications have been underexplored. All too often, isolation scientists and synthetic chemists in academic laboratories isolate or synthesize natural products and test them against a single representative cancer cell line or bacterial strain, or in some instances, never test their compounds in any biological assay at all, missing out entirely on the potential to discover a valuable, new therapeutic. Do storage freezers in laboratories engaged in natural product synthesis throughout the world contain the next advancements in human health? Toward realizing the potential of purified natural products, we established the Canvass natural product screening pilot initiative to provide the scientific community with a mechanism to evaluate the biological activities of natural products in a diversity of in vitro assays.

With the Canvass pilot program, we set out to crowd-source a diverse set of purified natural products by inviting academic investigators and companies to submit their natural products to the National Center for Advancing Translational Sciences (NCATS). Upon assembly, the Canvass library was compared to other relevant, well-studied chemical libraries. We then sought to broadly explore, or “canvass”, the library’s biological activity in an assortment of robust assays using quantitative high-throughput screening (qHTS).7 Due to the broad scope of disease-relevant mechanisms investigated by NCATS, we were able to screen the library against a wide range of assays. The resulting data set from 50 different assays was then systematically analyzed to identify overall trends and specific natural products with interesting biological activities. Project teams at NCATS further investigated the activities of several compounds using established workflows, and the full data set was made available through the Canvass website (https://tripod.nih.gov/canvass).

RESULTS

Canvass Library. The Canvass library of 346 natural products was assembled through a broad solicitation of both the academic and private sectors via the Canvass website. The pure (>85% purity by liquid chromatography/mass spectrometry [LC/MS]) compounds were submitted by 45 academic laboratories or companies around the world. We collected pure natural products, rather than natural product extracts, to circumvent deconvolution and structure elucidation due to time and resource limitations. The library was formatted into 1536-well plates and evaluated in 50 assays by qHTS in an 11-point concentration series.8 We manually classified9 the Canvass compounds using a set of 12 well-known structural classes, the distribution of which is summarized in Figure 1a.

Physicochemical Property Distributions. To ascertain the similarity of the Canvass library to existing drug collections, we analyzed and compared the structural features and physicochemical properties of the compound collection to publicly available compound collections known to contain druglike compounds or natural products. We first examined the physicochemical properties of the library in comparison to three well-known small-molecule libraries: the DrugBank Approved Drugs (2073 compounds, database version: 2.0.9),10 the ChEMBL11
natural product set (1921 compounds, database version: ChEMBL 23), and a random subset of 3000 molecules from the Life Chemicals Diversity Set of 50K molecules (LC50K, 50 240 compounds). Selecting a subset of the LC50K library was necessary to reduce the dominance of the chemical space of such a large and diverse library. These libraries, spanned by compounds representing the entry-points and end-points of the drug discovery pipeline, helped us evaluate how the Canvass collection fits within drug discovery space. Specifically, we used the collections of ChEMBL natural products and LC50K molecules to represent the entry-points of drug discovery space. The ChEMBL collection generally represented natural products which historically have served as a rich source of drugs or starting points in lead-optimization efforts, while the LC50K molecules exemplified the engineered libraries of diverse compounds characterized by desirable properties for lead-discovery purposes. Meanwhile, since the DrugBank set covers the majority of approved small-molecule drugs, it represented the end-points space.

We computed seven physicochemical properties: molecular weight (MW), H-bond acceptor (HBA) and H-bond donor (HBD) counts, XLogP,\(^1\) the number of rotatable bonds (RotB), the plane of best fit (PBF),\(^1\) and fraction of rotatable bonds (flexibility).\(^1\) While the first five are relevant in a drug discovery setting, the PBF and flexibility descriptors characterize the three-dimensionality of the molecules. As shown by Meyers et al.,\(^1\) many synthetic scaffolds tend toward flatness, and there

![Image](image_url)
has been increasing interest to enhance the three-dimensionality of molecules in screening libraries. Figure 1b summarizes the distribution of these properties for the Canvass collection versus those of the other libraries. While the medians of the properties are well-aligned between the Canvass and LC50K collections, it should be noted that Canvass compounds represent a more diverse physicochemical space. The accordance of median property values toward the ChEMBL and DrugBank collections shows a mixed picture. There is an almost perfect split in the number of cases where the Canvass physicochemical properties are more closely aligned with either the ChEMBL or DrugBank collections. Surprisingly, the distribution of PBF is similar between the four libraries.

**Chemical Space Overlap.** We next examined the chemical space overlap of the Canvass library with the three comparator libraries. We considered two distinct chemical spaces: the physicochemical 7-dimensional descriptor space defined above, and a 1024-dimensional fingerprint space emphasizing structural features. In both cases, we computed a reduced 2-dimensional (2D) space using t-distributed stochastic neighbor embedding (tSNE). The results for the physicochemical space analysis are presented in the Supporting Information (see Figure S1). In the physicochemical space, the Canvass library is very similar to the other libraries, even though they may not be specifically natural-product-like, which is in line with the property distributions summarized in Figure 1b. We quantified the overlap between pairs of libraries using Thornton’s separability index (S), resulting in the following values: 0.81, 0.82, and 0.89 for Canvass versus the ChEMBL natural products, DrugBank, and the LC50K subset, respectively. A larger index represents a larger separation in terms of likeness. These indices support previous observations regarding physicochemical property distributions that Canvass compounds are well-aligned with the other libraries in terms of their physicochemical properties. As expected, the closest set to Canvass in physicochemical space is the ChEMBL natural products set.

We then computed 1024-bit ECFP fingerprints using the Chemistry Development Kit (CDK) for all compounds and examined the overlap in fingerprint space (Figure 1c). We observed that the embedding of Canvass compounds in this chemical space shows a resemblance to that of the ChEMBL natural products, as might be expected. Further, the chemical space occupied by Canvass and the DrugBank compounds shows a significant overlap. The quantitated overlap (using the separability index) reflects similar observations that we made regarding the physicochemical space, only, in this chemical space, Canvass overlaps to the highest degree with the DrugBank library (0.93), followed by ChEMBL natural product (0.95) and the LC50K (0.99) libraries. While this is somewhat unexpected, it may indicate that the Canvass library contains a number of structural elements in common with approved drugs.

**Summary of the Assay Panel.** The Canvass library was screened in qHTS format with 11-point dose–response against 50 assays covering a variety of readouts, modalities, and targets (either specific protein targets or biological processes) in both cell-based and biochemical assays. The bulk (33) of the assays focused on viability (e.g., cytotoxicity, cell proliferation, or membrane integrity), while 11 assays probed specific pathways (e.g., hypoxia-inducible factor 1-alpha [HIF1] signaling, or calcium modulation), and the remaining 6 assays were designed for specific biochemical targets (e.g., mutant isocitrate dehydrogenase 1 [mIDH1] or ATPase family AAA domain-containing protein 5 [ATAD5]). All cell-based assays were measured at a single end-point, with the exception of the apoptosis assays using Caspase-Glo, which were measured at three time-points (12, 18, and 24 h). This screen generated over 210,000 data points. Though it is worth noting that, while we ran 50 individual assays, this number includes counter-screens associated with other assays. An example is the secreted endoplasmic reticulum calcium-monitoring proteins (SERCaMP) assay designed to detect endoplasmic reticulum (ER) calcium dysfunction. The primary assay identifies compounds that prevent depletion of the ER calcium store and is accompanied by secretion and viability counter-assays, each of which is designed to eliminate false positives from the primary assay.

A variety of quality control (QC) metrics were computed for each assay (focusing on the primary readout only) including Z’-factor, signal-to-background (S/B), and the coefficient of variation (CV). QC measures associated with plates of Z’ ≤ 10 were treated as outliers and accordingly excluded from the analysis. Figure 2a–c summarizes the Z’, S/B, and CV for all assays, grouped by their type (pathway, target, and viability). In general, assay performance was good (0 < Z’ ≤ 0.5) to excellent (Z’ > 0.5) for all the assays in the panel, with a few exceptions. For instance, in the caspase-HEK293 apoptosis assay, the control compound (doxorubicin) did not elicit sufficient signal, which necessitated normalization using the maximum value from the sample wells. As a result, the Z’ is not relevant for this particular assay. For assays with multiple readouts, we report only the median QC measure for the main readout. Except for the apoptosis assays, the viability assays tended to exhibit slightly better performance across all metrics than the other two assay classes (Figure 2d). A separate plot was made for better visibility of QC measure values for assays of Z’ ≥ 0 and of SB < 60 (see Figure S2).

**Clustering Assays.** Several features stand out from a pairwise correlation matrix of a vector representation of the assays (Figure 3, see the Experimental Methods section for details). At a high level, four clusters of assays are apparent. The largest cluster is composed mainly of cytotoxicity assays (purple) with several pathways-specific (green) and one target-specific (red) assays. The cytotoxicity assays exhibit a negative correlation with a number of other assays (e.g., apoptotic assays), which can be largely attributed to the normalization scheme. Agonist assays have positive normalized areas under the curve (nAUCs), whereas antagonist assays have negative nAUCs. However, the observed negative correlations are modest. The second major cluster is composed of the three target-specific (red), two pathway and two cytotoxicity assays that exhibit overall poor correlation with any other. This is likely indicative of the orthogonal nature of biological or chemical processes captured by these assays. For instance, counter-assays associated with different screening technologies, such as AlphaLISA or fluorescence, are poorly correlated with each other, as expected. However, the diaphorase and redox counter-assays that are also in this cluster are correlated to some degree as one would expect; the negative correlation in this case is due to the normalization schemes. Two smaller clusters are characterized by a high correlation among the associated assays. One cluster includes the apoptotic assays, membrane integrity, protease, and HIF1 assays. The other cluster is composed of the p53, ATAD5, and CAR assays. Similar observations can be made when clustering is performed with the help of log AC50 and efficacy values of samples (see Figures S5 and S6). Overall, the screening results in the Canvass assay panel confirm general expectations based on the nature of the individual assays.
Promiscuity Analysis. Promiscuous compounds can pose challenges in screening campaigns. Promiscuity can be due to assay interference (such as quenching and autofluorescence) or intractable mechanisms of action, including nonspecific
reactivity, redox, or aggregation by pan-assay interference compounds (PAINS),\textsuperscript{25} and can be evaluated from the hit-rate among all high-throughput screens run during a given period.\textsuperscript{24,26,27} Given that we screened the Canvass library in 50 assays, we characterized the promiscuity of these compounds based on their nAUC values. We considered the absolute value of nAUCs and ignored the pharmacological action (inhibitor, agonist, or antagonist) of the individual compounds. Using this parameter, we defined a compound as promiscuous using two rules: (i) the transformed nAUC value falls into the 90th percentile in a given assay, and (ii) the first condition holds true for at least 40% of the assays. This rule identified 49 compounds as promiscuous, and these are summarized in Figure S3 (compounds listed in Table S2). However, given that the majority of the assays in which these compounds are active are cytotoxicity assays, rather than target-specific assays, the commonality of the assay end-points may unfairly emphasize their promiscuity. A number of these compounds, however, do appear as hits in target-specific assays (e.g., ATAD5, constitutive androstane receptor [CAR], SERCaMP), but their activity could have been driven by toxicity. To identify compounds not captured by the use of nAUC values, similar promiscuity analyses were carried out using the log AC\textsubscript{50} data and the absolute value of efficacy data. For the promiscuity analysis based on log AC\textsubscript{50} data, compounds with a log AC\textsubscript{50} value lower than the 10th percentile were considered, and the analysis did not reveal additional promiscuous compounds. The promiscuity analysis of the efficacy data was performed in an analogous manner to the nAUC analysis, and it revealed 12 additional promiscuous compounds that might be associated with cytotoxicity or aggregation at high concentrations (Figure S3).
Cytotoxicity Panel Overview. We profiled the cytotoxicity of the Canvass library against a collection of 16 cell lines representing a range of malignancies. The primary motivation for assessing cell killing was the well-known contribution of natural product sources to the chemotherapeutic pharmacopeia. A secondary goal was to identify cytotoxic compounds that may produce artifacts in other cell-based assays performed as part of the library profiling. Sensitivity varied across all cell lines (Figure 4a), though some compounds demonstrated near pan-activity: herboxidiene (NCGC00488492), strophanthidin 3-O-β-glucopyranosyl-(1,2)-O-β-diginopyranosyl-(1,4)-O-β-cymaropyranosyl-(1,4)-O-β-digitoxopyranoside (NCGC00488465), and lactimidomycin (NCGC00488635). There was no clear clustering of sensitivity by tissue-of-origin, although the sensitivity of the canine glioma cell lines G06 and SDT closely correlated. Of the compounds in the library, 49% demonstrated

Figure 4. (a) Comparison of the cytotoxicity of each compound (rows) in 16 cancer cell lines (columns). The heatmap was generated based on the area under the dose–response curve (AUC). Dark red indicates a more potent and efficacious compound. (b) AUC correlation plot of KB-8-5-11 vs KB-3-1. AUC for each Canvass compound is represented by a dot with prospective P-gp substrates highlighted (pink) above the unity line (dashed). (c) Dose–response activity of (+)-chamaecyparosine C, a prospective P-gp substrate identified in the Canvass library screen. This compound showed selective killing against KB-3-1 (black), resistance in KB-8-5-11 (gray), and reversal of resistance to levels approaching that of KB-3-1 in KB-8-5-11 + 1 μM tariquidar (pink).
class 1 or 2 curves with maximum response over 50% against at least one cell line. Natural product cytotoxins are susceptible to efflux by multidrug-resistance transporters. To identify P-glycoprotein (P-gp) substrates, we tested compounds against a P-gp overexpressing cell line, KB-8-5-11, and its nonexpressing parental counterpart, KB-3-1. Inhibition of P-gp reverses resistance to P-gp substrates. KB-8-5-11 cells cotreated with 1 μM tariquidar (KB-8-5-11 + tariquidar), a known P-gp inhibitor, were also tested to confirm the P-gp substrates. P-gp substrates demonstrate reduced cell killing against KB-8-5-11 cells, and greater activity against the parent KB-3-1 cell line. Comparison of activity (AUC) against the two cell lines revealed a small number of compounds less active against the P-gp-expressing cell line (above the unity line, Figure 4b), and most compounds did not demonstrate significant cytotoxicity (clustered at the origin, Figure 4b). Confirmation retesting revealed 4 substrates among 40 compounds demonstrating cytotoxicity (pink spheres, Figure 4b): batzelladine D (NCGC00488661), (+)-chamaecyparone C (NCGC00488556, Figure 4c), apicidin (NCGC00165733, Figure S4), and an isomigrastatin derivative (NCGC00488640). Concentration–response curves show that KB 8-5-11 cells were resistant to cell killing, but were sensitized by the P-gp inhibitor tariquidar.

Apoptotic Behavior. The Canvass assay panel enables us to probe a wide variety of pharmacological responses, but at fixed time-points. The inclusion of the apoptosis assays (measured using Caspase-Glo) read at three time-points (12, 18, and 24 h) allowed us to explore the apoptotic response to the compounds over time. The majority of the 346 Canvass compounds did not induce apoptosis at any of the time-points (just 33 compounds exhibited an active dose–response at one or more time-points), and only 19 compounds induced caspase activity in a dose–response manner at all three time-points in any of the two assays (Figure 5a). In general, these 19 compounds did not exhibit high potencies, with the exception of 15-deoxygoyazensolide (NCGC00488496) (see Figure 5b). Nevertheless, we categorized the observed activities in relation to the two cell lines (HPAF-II and HEK293) for compounds based on the detected maximal response signal (“Max. Data”). This schema gave rise to four categories. Compounds characterized by “high” apoptotic activity in HPAF-II but “low” in HEK293 assays may have implications for biasing apoptosis induction toward cancer as opposed to normal cells. Three compounds were found that fall under this group of interest (NCGC00488600, NCGC00488498, NCGC00488506).

We also examined the correlation between the 19 compounds inducing apoptosis at all three time-points and their activities in the remaining 27 viability assays. We computed the fraction of assays in which each of these compounds exhibited an active curve class. These values ranged from 0.18 (chatancin, NCGC00488505)
Nuclear receptors (NRs) are proteins that regulate physiological homeostasis, metabolism, and development. One crucial NR is the constitutive androstane receptor (CAR, NR1I3), which regulates many drug metabolizing enzymes and transporters while also playing an essential role in energy homeostasis. In this study, we screened the Canvass library of natural products for activation/deactivation of CAR employing a double stable reporter cell line (HepG2-CYP2B6-hCAR). To identify activators, we added 0.75 μM 1-(2-chlorophenyl)methylpropyl)-3-isoquinoline-carboxamide (PK11195), a known CAR inhibitor, while adding 50 nM 6-(4-chlorophenyl)imidazol[1,3]-2,1-thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime (CITCO, a known CAR activator), to compound and CITCO for 24 h in 1536-well plates was treated with ONE-Glo, and the luminescence intensity was calculated (black). The efficacies were compared to the CITCO positive controls, and the viability was also detected using fluorescence in the same well (pink). Data are expressed as mean ± standard error of the mean for triplicate assays. (b) Dose–response curves for the CAR activator (NCGC00094872) using same method in part a, except cells were cotreated with PK11195 instead of CITCO, and PK11195 was used as positive controls. (c) 2(S)-Cathafoline is active in the secreted ER calcium-monitoring protein (SERCaMP) assay, indicating the compound stabilizes ER calcium. Activity was examined in the primary SERCaMP assay (pink), a secretion counter-screen (gray), and a viability counter-screen (black). Mean activity ± SD (n = 3) is shown.

Figure 6. (a) Dose–response curves for the CAR activator (NCGC00094872) identified in the Canvass library. The stable cell line treated with compound and CITCO for 24 h in 1536-well plates was treated with ONE-Glo, and the luminescence intensity was calculated (black). The efficacies were compared to the CITCO positive controls, and the viability was also detected using fluorescence in the same well (pink). Data are expressed as mean ± standard error of the mean for triplicate assays. (b) Dose–response curves for the CAR deactivator (NCGC00488482) using same method in part a, except cells were cotreated with PK11195 instead of CITCO, and PK11195 was used as positive controls. (c) 2(S)-Cathafoline is active in the secreted ER calcium-monitoring protein (SERCaMP) assay, indicating the compound stabilizes ER calcium. Activity was examined in the primary SERCaMP assay (pink), a secretion counter-screen (gray), and a viability counter-screen (black). Mean activity ± SD (n = 3) is shown.

Figure 6. (a) Dose–response curves for the CAR activator (NCGC00094872) identified in the Canvass library. The stable cell line treated with compound and CITCO for 24 h in 1536-well plates was treated with ONE-Glo, and the luminescence intensity was calculated (black). The efficacies were compared to the CITCO positive controls, and the viability was also detected using fluorescence in the same well (pink). Data are expressed as mean ± standard error of the mean for triplicate assays. (b) Dose–response curves for the CAR activator (NCGC00094872) using same method in part a, except cells were cotreated with PK11195 instead of CITCO, and PK11195 was used as positive controls. (c) 2(S)-Cathafoline is active in the secreted ER calcium-monitoring protein (SERCaMP) assay, indicating the compound stabilizes ER calcium. Activity was examined in the primary SERCaMP assay (pink), a secretion counter-screen (gray), and a viability counter-screen (black). Mean activity ± SD (n = 3) is shown.
DISCUSSION

With generous contributions from laboratories throughout the world, we assembled a diverse set of 346 natural products for biological evaluation. Comparison of the Canvass library to three other chemical libraries (DrugBank, LC50K, and ChEMBL natural products) revealed that all four inhabit very similar physicochemical property space, though the LC50K subset tends to be focused with respect to most parameters. Given that DrugBank is a drug library and LC50K is designed for lead discovery purposes, their overlap with ChEMBL natural products and the Canvass libraries supports the observation that natural products are the origins for many drugs. Considering both the physicochemical space and fingerprint structural comparisons, Canvass compounds are most similar to druglike molecules in the DrugBank library. Despite some degree of structural overlap with the ChEMBL natural product set, it is evident that the Canvass set samples novel structural space compared to the other three libraries. Though the Canvass compounds comprised of natural products, their properties are more aligned with drugs than with the ChEMBL natural products, earning them a unique position among chemical collections. This suggests that the Canvass library is a good complement to the ChEMBL natural product set for drug discovery purposes.

The Canvass natural product library was screened in 50 distinct assays to broadly canvass the pharmacological activity of the compounds. The assortment of assays utilized in this pilot study cover a wide biological scope, and they were selected because they have been successfully implemented for hHTS at NCATS. Cytotoxicity was heavily emphasized given the history of natural products as anticancer and anti-infective agents. Targets with unique pharmacological potential, including CAR and Nrf2/ARE, were also examined. The balance of the screen probed developing pharmacological mechanisms in other areas such as inflammation (inflamasome) and cardiovascular as well as rare diseases (SERCaMP).

Beyond the facets highlighted through the analyses, notable activities also emerged for a small subset of compounds. We discovered three natural products that modulate the nuclear receptor CAR. Interestingly, the molecules highlighted above are structurally quite distinct from one another; one of them, piperine, is a component of the dietary spice pepper, and its observed activity has brought to light an unappreciated pharmacological impact of this widely consumed natural product.

The strategic utility of specific counter-screens facilitated the identification of selectively-active compounds, as exemplified by \((-\cdot)\cdot2(S)\)-cathafoline in the SERCaMP assay. The calcium-stabilizing effect of this molecule is significant, especially given that cathafoline was highly selective across our panel; it was not active in any other Canvass assays. Though it is a member of a large family of indole alkaloids, little was known about the biological activity of cathafoline prior to this Canvass study. Fortuitously, the Garg laboratory’s prior total synthesis campaign \(^\) provided access to synthetic cathafoline, which, when submitted in response to our call for natural products, enabled the discovery of cathafoline’s promising \textit{in vitro} activity. Studies are underway to elucidate the biological target of cathafoline and to realize the full potential of its calcium modulatory effects.

Investigations of potent natural products continue to reveal key biological targets with therapeutic relevance. For example, the isolation and biological evaluation of peptides isolated from viper snake venom helped bring to light the importance of the angiotensin converting enzyme (ACE) in regulating blood pressure. This discovery inspired the design of the drug captopril for the treatment of hypertension.\(^3\) There is no doubt that natural products will continue to impact the numerous unmet medical needs of both ubiquitous illnesses and rare diseases alike, especially as we have only just begun to understand new and developing areas of disease biology, such as the human microbiome. It is our hope that screening natural product libraries for a wide range of biological activities, as with the Canvass pilot program, will accelerate the development of therapeutics in many disease areas. The Canvass pilot program serves as an example of how to successfully leverage resources and expertise throughout the scientific community for the evaluation of natural products in drug discovery, and it will serve as a framework for larger scale investigations in the future.

EXPERIMENTAL METHODS

The Canvass library of 346 natural products was assembled through a broad solicitation of both the academic and private sectors via the Canvass website, with heavy emphasis on natural product isolation and total synthesis laboratories. To streamline the pursuit of biological discoveries and minimize deconvolution, only pure natural products were accepted. The NCATS Compound Management team provided our collaborators with tared, barcoded vials for natural product submissions to facilitate both the receipt and processing of samples. Quality control (QC) for purity > 85% was managed in two ways: by requiring the cosubmission by PIs of recent analyses of submitted natural products utilizing either LC/MS or \(^1\)H NMR, and subsequently upon receipt of samples, NCATS performing LC/MS analyses of all submissions. By implementing two QC checks, we minimized QC failures for natural products which are unstable to our standard LC/MS QC protocols. In total, 45 PIs or institutions submitted a sum of 346 natural products to assemble the Canvass library, which was subsequently evaluated in 50 whole-cell or biochemical assays. This library continues to serve as a valuable resource in screening campaigns following the work described herein.

Data generated by the Canvass program has been made available to our collaborators via the website, located at https://tripod.nih.gov/canvass/. The website provides open access to both general and detailed overviews of the program as a whole, as well as a subset of the assays which were planned at the outset, and a portal for questions and answers. By registering, collaborators’ accounts allowed access to secure and private interfaces to expedite compound submissions and to simplify data browsing. Importantly, data generated for a given set of compound submissions were initially only made accessible to the submitters. The application provides the traditional tabular view of compound activities, with a variety of visualizations to provide high-level summaries of activity in the assay panel, along with detailed views for individual compounds. With this publication, the full data set presented herein is now available at the Canvass website (https://tripod.nih.gov/canvass/).

DATA SETS AND COMPUTATIONAL METHODS

Chemical Libraries. The Canvass library consists of 346 compounds that can be described as natural products. To better characterize the chemical space represented by these compounds, we included three additional chemical libraries in our analysis: the DrugBank Approved Drugs (2073 compounds,
database version: 2.0.9), the ChEMBL natural products (1921 compounds, database version: ChEMBL 23), and the Life Chemicals Diversity Set of 50K molecules (LC50K, 50 240 compounds). The LC50K collection was included to provide a baseline for the comparison of molecular properties. To this end, a subset consisting of 3000 molecules was sampled randomly from the LC50K collection and used in subsequent analyses. Chemical libraries were subjected to the same standardization scheme as part of a KNIME workflow including community nodes originating from CDK cheminformatics suites.36–39 Standardization steps involved keeping only the largest component of compounds.

qHTS Data Processing Pipeline. While the data processing details for individual assays can be found in their respective references, we briefly outline the processing pipeline here. Plate data were normalized to the per-plate positive and negative controls, and dose—response curves were fitted using a grid-based algorithm.40,41 Dose—response curves were then assigned a curve class (see Seethala and Zhang40,6 for more detailed definitions of curve classes), which is a heuristic classification that allows us to easily identify good quality versus poor quality dose—response curves. To assign one of seven possible classes, the curve is evaluated in terms of features like asymptote definition, the R² of the curve fit, and efficacy. Furthermore, for assays measuring an increase in signal (e.g., agonist assays), curve classes are positive, and for those measuring a decrease in signal (e.g., inhibitor or antagonist assays), curve classes are negative. Broadly, we consider curve classes of 1.1, 1.2, 2.1, and 2.2 (and the corresponding negative values) as good quality active dose—responses, curve class of 4 as inactive (i.e., no dose response), and all others as inconclusive dose—responses. We only deviated from this classification in the case of Caspase assays, where good quality active dose—responses curves were considered those that are associated with a curve class value different from 4.

AUC Computation. In the Canvass assays, the more potent and efficacious a compound is, the larger its absolute area under the curve (AUC) value. Moreover, the sign of the AUC reflects the pharmacological action of a compound (e.g., inhibitors can be distinguished from activators). It should be noted that automatically generated raw AUC values can be misleading in certain cases. For the case of an inhibitory dose—response curve, which starts in a positive response range, the absolute AUC can be small due to the nature of numerical integration. A similarly misleading absolute AUC value is obtained for activation dose—response curves. The resultant normalized AUC values are denoted by nAUC to distinguish them from raw AUC values.

Assay Clustering. To cluster the assays, we first represented each assay as a 346-element vector of compound nAUC values. Next, we computed the pairwise Pearson correlation matrix, which was then clustered using hierarchical clustering with complete linkage. We also computed clusterings using potency (setting it to 10 μM for compounds that were inactive in an assay) and efficacy (Figures S5 and S6).

Descriptor Computation. Physicochemical descriptors (molecular weight, rotatable bond number, flexibility,14 number of hydrogen-bond donors/acceptors, clogP,42 TPSA43) and ECFP6 fingerprints were computed using CDK nodes for KNIME (version 3.4.2) and processed in R statistical suite (version 3.4.4) using the fingerprint package.

Library Overlap. Thornton’s separability index (S)18 is a class separability measure designed to discriminate between objects from different classes and is commonly used to characterize the quality of clustering. We applied this measure to quantify the degree of overlap of two libraries in a predefined chemical space. S is defined as the fraction of compounds for which their nearest neighbor is not from the same library as themselves. Thus, S ranges from 1 (two libraries completely overlap with each other) to 0 (two libraries have no overlap). Note that these limits are somewhat dependent on the actual spatial distribution of compounds in a given chemical space. Nonetheless, for the purposes of comparing library overlap in a relative manner, the use of S is sufficient. We implemented S using R 3.4.4 (code available at https://spotlite.nih.gov/gzahoranszky/CANVASS.git).

Quantifying 3D-Likeness. The 3D-likeliness of compounds was characterized with the help of a plane of best fit (PBF)13 measure implemented in the RDKit Python Application Programming Interface (API) (version 2017.09.1).37 To generate PBF values, a low energy conformer needs to be generated for each compound. Low energy conformers were generated by a KNIME workflow.36–39

Embedding Compounds into 2D Chemical Space. We defined a physicochemical property space using molecular weight (MW), H-bond donor count, H-bond acceptor count, XLogP,12 PBF,13 and flexibility.14 We then employed tSNE (as implemented in the Rtsne package44 for R) to perform dimension reduction to 2D,17 using the Euclidean metric for distance computations. Apart from setting θ = 0.1, all other parameters were set at their default values. For the case of fingerprints, we first computed a pairwise similarity matrix using the Tanimoto metric45,46 and converted it to a distance matrix (using D = 1 − similarity) for input to the tSNE algorithm, using the same parameters as used for the physicochemical property space. The 2D data sets were visualized using R 3.4.4. All workflow files, Python source code, computed descriptor, and fingerprint data are available at https://spotlite.nih.gov/gzahoranszky/CANVASS.git.

Novelty Assessment of Canvass Compound Structures. The novelty of the Canvass compounds was assessed on the basis of the Bemis–Murcko scaffold (BMS)46 of compounds. First, the BMSs of compounds were determined in all molecular libraries. Next, a unique set of BMSs were derived library-wise. Finally, the overlap of pairs of unique BMS sets was quantified relative to the number of unique BMSs in each set. Of note, the rest of the analyses in this study involved only a random subset of the LC50K library whereas this assessment was performed on the entire LC50K library (Table S1).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscentsci.8b00747.

Additional figures depicting more detailed analyses of the assay data (including assay QC measurement, additional heatmaps and clustering analyses, additional dose—response
Note: The authors declare no competing financial interest. 
Safety statement: no unexpected or unusually high safety hazards were encountered in this work.

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(9) Natural products were categorized with varying levels of specificity granularity to reasonably distribute compounds into classes.


