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Modern Phenotypic Drug Discovery Is a Viable, Neoclassic Pharma Strategy

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INTRODUCTION

The pharmaceutical industry (Pharma) is currently facing unprecedented challenges. In addition to strategic patent expirations, the rate of drug launches has been essentially constant for 60 years[1] with overall productivity falling since the 1970s.[2] Similarly, the appearance of novel targets to FDA approved drugs, a measure of industry innovation, has not dramatically improved since the 1980s.[3] Although Pharma productivity is a multifaceted problem, detailed analyses of comprehensive, industry-wide data indicate that late stage clinical failures are a major contributor that has been attributed to poor target validation (TV) and the lack of predictive biomarkers that translate to the clinic.[4] In the spirit of "reinventing innovation"[5] Pharma needs to introspectively identify areas for improvement. This communication considers how choices in drug screening strategies may relate to target validation issues and influence the probability of identifying novel medicines.

Target-directed drug discovery (TDD) and phenotypic drug discovery (PDD) are Pharma strategies that have roots in distinct but complementary philosophies. Advantages of gene-specific, reductionist approaches include the formulation and testing of specific molecular hypotheses. TDD approaches utilize advances in automation, biochemistry, structural biology, and chemistry related technologies to provide efficient and high capacity testing of unprecedented numbers of compounds and molecular targets.[6] In addition, deep mining of cDNA expressed sequence tags (ESTs)[7] and subsequently entire genomes[8] led to the discovery of thousands of unknown genes and the potential for deep insight into novel drug target biology. Taken together, these advances in science and technology, in conjunction with the innate human desire to seek new challenges, contributed to the rapid adoption of molecular-reductionist views of biology. In contrast, PDD tests compounds in complex biological systems and monitors physiological responses with minimal assumptions concerning the participation of specific molecular targets and/or signaling pathways.[9] Analyses of new molecular entities (NMEs) approved by the FDA between 1999 and 2008 indicate that for first in class molecules, 37% resulted from projects that used phenotypic screening whereas target based screening identified 23%; moreover, the discovery rate of PDD NMEs was greater than TDD NMEs and was invariant over the 9-year study period.[10] Since significantly more TDD efforts were conducted during this period,[6] the overall launch rate for first in class drugs underestimates the intrinsic probability of technical success (pTS) of classic PDD.[10]

A hybrid of classic phenotypic and target-directed strategies, which blends the use of physiologically relevant biological systems with the high throughput and statistical robustness of modern assay technologies, may have a higher pTS for launching first in class drugs than either classic PDD or TDD. Academia has utilized modern phenotypic approaches to study cell cycle, stem cell renewal, cell migration, metastasis, and induction of pluripotent stem cells.[11−15] However, such "neoclassic" PDD approaches are not widely used in Pharma because of concerns about assay performance, statistical robustness, perceived difficulties in establishing compound structure−activity relationships (SARs), anticipated limited applicability of chemoinformatics tools, and the difficulty/requirement for elucidating a molecular drug target.

The results outlined in this presentation address commonly perceived issues related to the use of complex biological systems for modern lead generation. Our data, using an angiogenesis assay incorporating a coculture of primary human endothelial and stromal progenitor cells, indicate that phenotypic assays can be statistically robust, can be used to identify novel compound scaffolds by chemoinformatics enabled hit expansion, and can provide evidence of compound structure−activity relationships. Identification of novel molecular targets important to angiogenesis, acetyl Co-A carboxylase (ACC) and a protein related to cellular β-secretase (β-sec) activity, demonstrates that PDD provides a means to test multiple biologically relevant pathways in a target agnostic fashion. These attributes of PDD enabled the discovery of chemical scaffolds that were readily differentiated, structurally and mechanistically, from antiangiogenic agents that constitute the current standard of care (SOC) and demonstrated in vivo activity. We conclude that PDD complements gene-specific target-directed strategies, may mitigate TV risks, and has the potential to enhance innovation in drug discovery.

RESULTS AND DISCUSSION

Phenotypic Assay Systems. Modern phenotypic assays should capture key aspects of the physiological process such as relevant cell types, cell−cell interactions, growth factors, signal transduction pathways, and molecular targets while utilizing in vitro systems amenable to rigorous statistical validation and high throughput operations. Angiogenesis is a complex biological process that requires the migration, differentiation, physical interaction, and coordinated cellular signaling of precursor and mature forms of endothelial cells with stromal cells. Although VEGF is recognized as the dominant proangiogenic stimulus, other protein factors modulate angiogenesis.[16,17] Previously we described and statistically validated an in vitro

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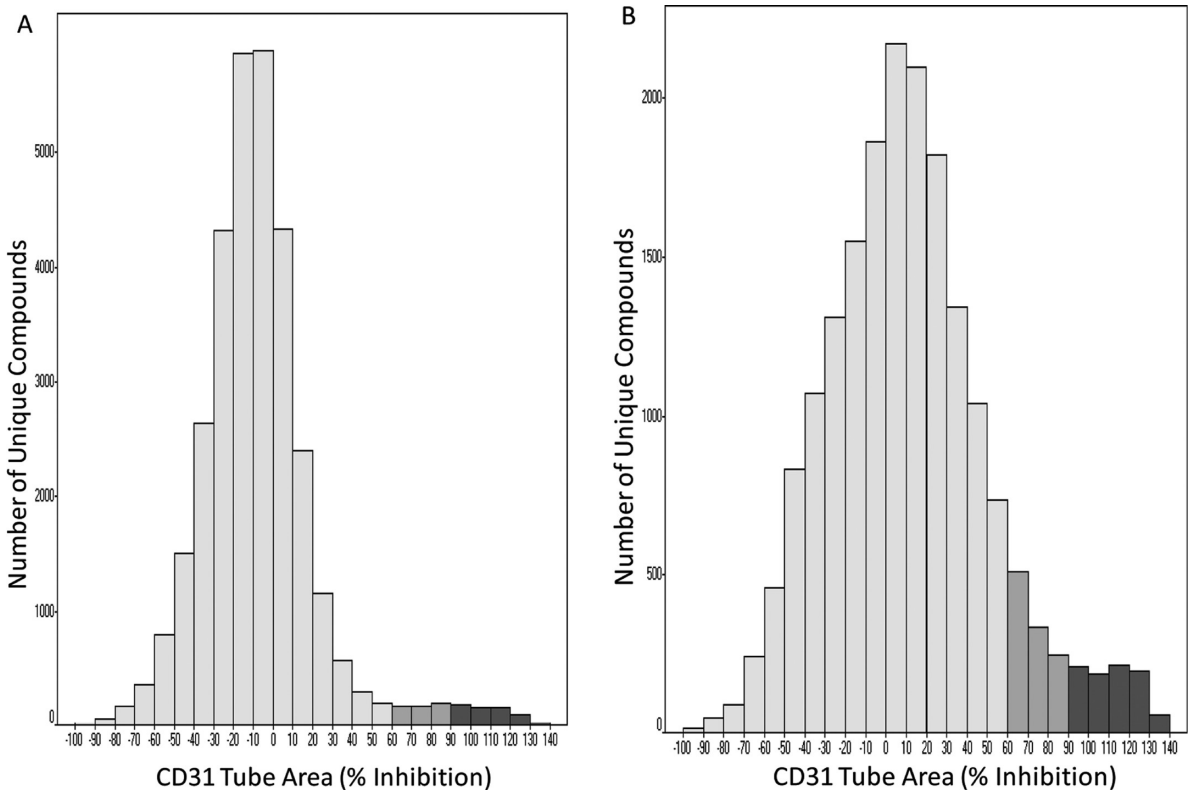

Figure 1. Screening results of medium throughput in vitro angiogenesis assay. (A) Phenotypic primary screening: activity distribution of single point testing data of 32 000 compounds. Compounds were tested at 2 μM as described.[18] The mean percent inhibition was −8.2%. 3.7% and 1.9% of tested compounds were ≥2STD and >3STD above the mean (shaded) corresponding to >60% and >90% endothelial cord inhibition, respectively. (B) Phenotypic hit expansions. Compounds with confirmed endothelial cord inhibition activity, no overt cytotoxicity, and no predicted/known kinase activity were used as seeds for virtual screening of the Lilly compound collection as described in Experimental Section. 19 000 compounds relating to similar structures and novel scaffolds were identified and tested as described for the primary screen. The observed mean of the single point data distribution is significantly shifted toward higher cord area inhibitory activity (10%) with 10.6% and 4.8% of tested compounds exhibiting >60% and >90% inhibition, respectively (shaded).

model of neovascularization utilizing a coculture of endothelial (ECFC) and stromal (ADSC) cell precursors.[18] ADSCs have pericyte-like qualities,[19] and collagen-fibrin matrix cocultures of ECFCs/ADSCs form functional blood vessels in vivo.[20] Therefore, the in vitro ECFC/ADSC assay captures multiple aspects of the biological complexity related to neovascularization with minimal assumptions on the relevance of various signaling pathways and molecular targets. In contrast, use of cellular assays systems that overexpress or monitor specific signaling components relies on the accuracy of previous TV studies and provides focused readouts that may not fully capture the biological complexity of the system.

Phenotypic Screening and Hit Expansion. Marketed drugs that inhibit VEGFR and related receptor tyrosine kinases (RTKs) currently constitute antiangiogenic (AA) SOC.[21] In order to differentiate from AA SOC, we sought to identify compounds that inhibit endothelial cord formation (ECF) in vitro via non-kinase mechanisms and do not exhibit undesirable cellular mechanisms such as overt cytotoxicity or cell cycle inhibition.[18] Approximately 32 000 internal compounds originating from target biased screening libraries and unrelated chemical diversity were tested in the ECFC/ADSC coculture system. The distribution of single point (SP) activity (Figure 1A) from the medium throughput screen (MTS) is enriched in compounds with high ECF inhibitory activity with 4% of tested compounds showing activity of >2 STD above the mean, corresponding to >60% inhibition (Figure 1A, shaded). Compounds active in SP screening were retested in dose response; approximately 65% of single point actives were confirmed. Elimination of actives that were overtly cytotoxic, inhibited cell cycle,[18] associated with kinase activity, or had unpromising structures resulted in 194 compounds that inhibited ECF activity with IC_{50} < 6 uM. These compounds were subsequently used to search for additional compounds within the Lilly collection using structure based chemoinformatics approaches and traditional medicinal chemistry principles. This hit expansion (HE) process and subsequent compound clustering identified 19 000 compounds that were tested in SP mode. The SP activity distribution (Figure 1B) from the first two rounds of HE exhibits a significant enrichment in active compounds relative to the primary screen (Figure 1) with nearly 11% and 5% of compounds exhibiting >60% and >90% ECF inhibition, respectively.

Figure 2 summarizes the discovery of unique chemical clusters of compounds showing >60% SP ECF inhibition. The MTS identified a total of 654 active compound clusters, 178 of which were represented in subsequent HE testing. Significantly, HE identified 1017 additional active chemical clusters that were distinct from compounds within the original screening libraries. Analysis of the compounds that had confirmed ECF activity without overt cytotoxicity indicates that virtual screening and chemoinformatics methods such as similarity searching, 2D scaffold hopping, 3D-ROCS, and machine learning methods identified active, noncytotoxic compounds. These observations

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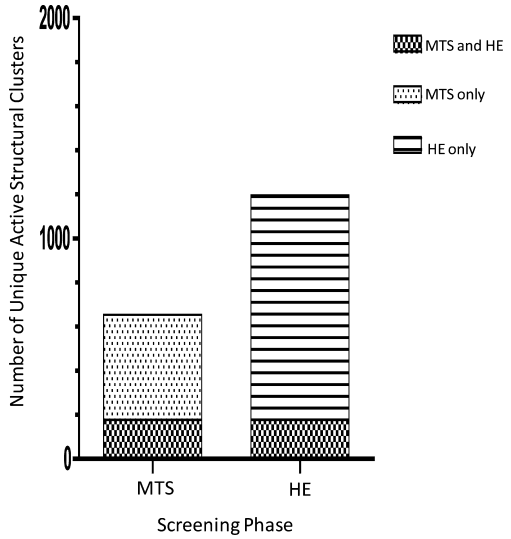

Figure 2. Phenotypic actives are structurally diverse. Unique compound clusters observed during the medium throughput screen and hit expansion phases are indicated. Screening actives were confirmed and triaged as described in the text; 194 compounds were used as "seeds" for similarity searching and scaffold hopping by chemoinformatics interrogation of the Lilly compound collection for the first round of hit expansion. The number of unique clusters corresponding to active compounds (single point activity >60%) from the 32 000 compound primary screen and the collective 19 000 compounds from two hit expansions are indicated. Of the 1195 active compound clusters identified after hit expansion, 178 clusters (~4700 compounds) were observed in the medium throughput screen and 1017 clusters (~8800 compounds) were novel active chemical scaffolds. 476 chemical scaffolds (~4300 compounds) active in the screen were either deprioritized using criteria given in the text or were inactive upon retest.

indicate that structure–activity relationships can be successfully modeled with phenotypic end points to identify active novel chemical diversity even when MOA and exact molecular target(s) are unknown.

Biological Diversity of Phenotypic Actives. Mining of biological activity databases in conjunction with results visualization using heat maps composed of target class phylogenetic trees provides a means to visualize known and predicted biochemical activity and selectivity of compounds.[22] Figure 3A summarizes the activity profiles of four confirmed actives from the ECF screen; three compounds show predominant kinase, GPCR, or NHR activity with multiple activities observed within and across target families (Figure 3A). Follow-up on SP actives from hit expansion 1 (Figure 1B) identified 451 compounds that inhibited angiogenesis in a dose dependent manner with $IC_{50} \leq 3.5$ uM and exhibited desired cell cycle and cytotoxicity phenotypes.[18] Figure 3B summarizes the ECF potency and predominant molecular target class known to be modulated by these compounds. Potent ECF inhibition is observed for compounds with known kinase activity (31.9%), an expected result given the known importance of VEGF and RTKs to angiogenesis.[17,23] Interestingly, potent ECF inhibition is observed with compounds with known activities on GPCRs (21.5%), NHRs (4.9%), and PDEs (2.4%), target families not commonly associated with angiogenesis (Figure 3B). Significantly, 39.2% of ECF inhibitors and their structural analogues did not have activity in any biochemical assay in which they were tested (Figure 3). Although this analysis suffers from the caveat that compounds are not systematically tested in all available biochemical assays, an issue that is somewhat mitigated by interrogation of databases with compounds that are structurally similar to query molecules, the approach provides an overall view of the biochemical activities associated with ECF inhibitors derived from phenotypic screening. These results are consistent with the notion that phenotypic lead generation broadly interrogates multiple signaling pathways and molecular targets in an unbiased fashion. Although speculative, the high percentage of confirmed inhibitors with unknown biochemical activity (Figure 3B) suggests that PDD readily identifies compounds that utilize novel mechanisms and/or molecular targets.

Differentiation of Phenotypic Actives from Standard of Care. Pharma seeks to identify novel therapeutics that are clearly differentiated from and are therapeutically superior to competitor's compounds. The first approved SOC small molecule inhibitors of angiogenesis were Sutent and Nexavar, which are multitargeted RTK inhibitors that potently act on VEGFR2 and PDGFRβ[24] and are approved for use in metastatic renal cell carcinoma. The broad biological and chemical diversity of the confirmed ECF inhibitors from the phenotypic screen (Figures 2 and 3) increases the likelihood of identifying AA compounds with novel mechanisms of action (MOA). In order to maximize differentiation from SOC AA, only compounds that did not exhibit biochemical kinase activity were considered further. Similarly, compounds that exhibited overt cytotoxicity or cell cycle inhibition, undesirable cellular AA mechanisms,[18] were eliminated. Finally only compound scaffolds that had promising structures, were amenable to synthetic chemistry, and possess favorable druglike/physical properties compounds were advanced. Use of multiple and rigorous exclusion criteria enhances the pTS of identifying safe and efficacious druglike molecules, an exercise more relevant to pharmaceutical research than academia where investigating basic biology and identifying novel molecular targets and MOAs are valued.

Multiple compound scaffolds fulfilling many of these filter criteria were identified through the phenotypic screen (not shown). The structures of two exemplary ECF inhibitors are shown in Figure 4A. Figure 4B summarizes the in vitro activities of compounds **A** and **B**, their phenotypic profiles, and a comparison to SOC AA drugs Sutent and Nexavar. Compounds **A** and **B** inhibit endothelial cord formation with potencies ranging from 21 to 41 nM without overt cytotoxicity in the coculture system, results that are comparable to those of SOC (Figure 4B). Significantly, the PDD approach readily identified compounds that inhibit ECF through non-kinase mechanisms (Figure 4B), an important point of differentiation from SOC and potentially useful for the development of therapeutics for clinical combination with marketed drugs. Significantly, compound **A** and Sutent both exhibited dramatic in vivo inhibition of angiogenesis in mice bearing U87MG xenograft tumors (Figure 5).

Compound Structure–Activity Relationships and Phenotypic Assays. In order to optimize screening actives, medicinal chemists require in vitro assays that detect compound SAR. It is commonly believed that once active molecules have been identified by phenotypic screens, they are best optimized using gene-specific biochemical assays,[24−26] suggesting that assays utilizing complex biological systems lack statistical robustness which limits their SAR utility. Although cell based assays are generally more difficult to enable and validate than biochemical assays, the collective Lilly experience with assays monitoring various cell cycle related end points,[27] secretion of ApoE and insulin, endothelial cord formation, osteoblast-like differentiation,[18] and

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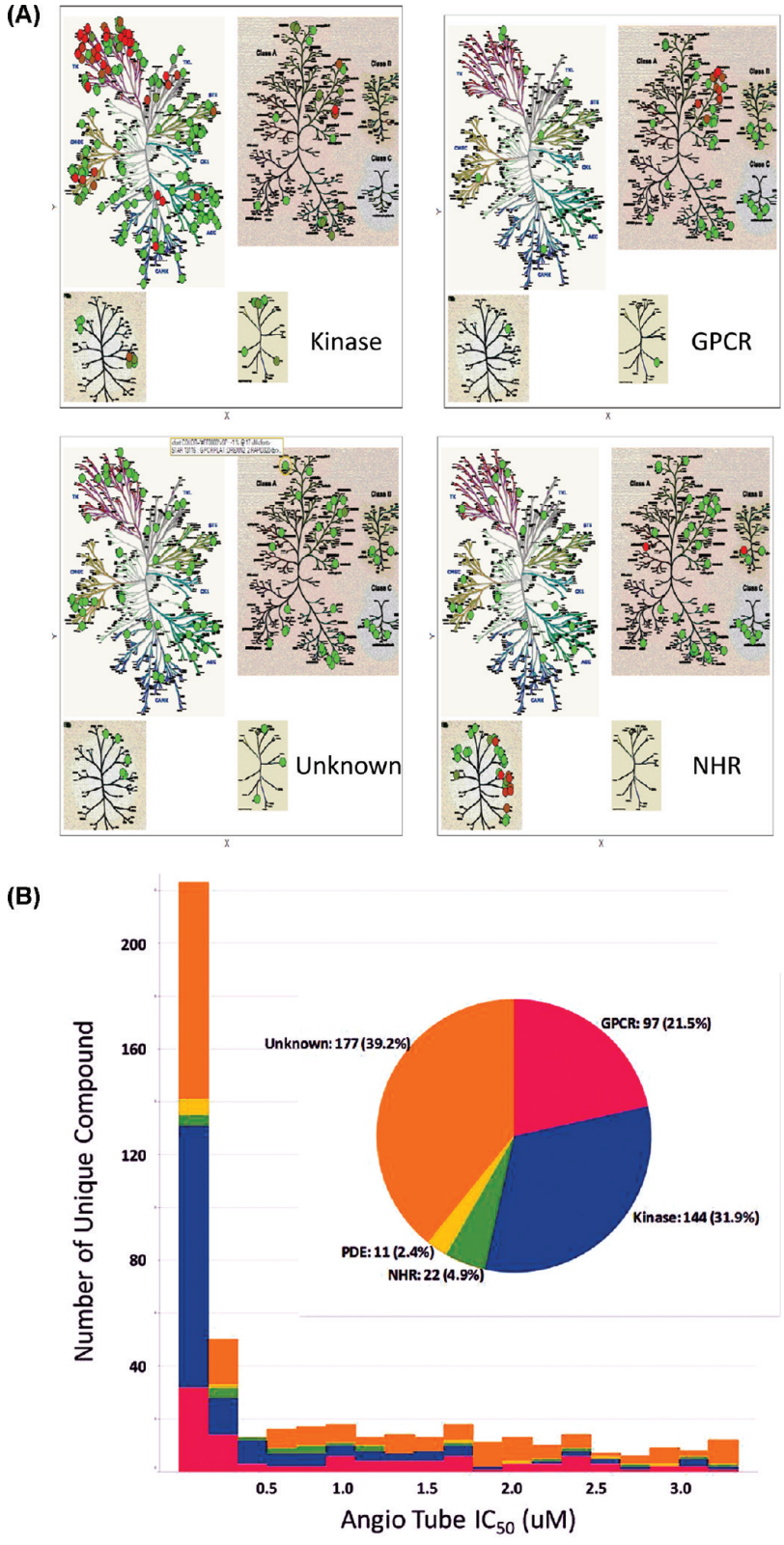

Figure 3. Phenotypic actives utilize diverse molecular mechanisms. 451 confirmed inhibitors of endothelial cord formation (IC_{50} < 3.5 μM) which did not display overt cytotoxicity or cell cycle activity[18] were identified following hit expansion 1. (A) Visualization of known and predicted biochemical activities of four endothelial cord formation inhibitors using a heat map display based on the phylogenetic diagrams of the kinase, GPCR, NHR, and PDE target families.[22] Phenotypic actives with predominant biochemical activities in kinase, GPCR, or NHR target families are indicated; a compound without identified activity is designated "unknown". Color coding is as follows. Red indicates potent activity (EC_{50} or IC_{50} of ≤10 nM). Green indicates poor activity EC_{50} or IC_{50} of >10 uM). (B) Distribution of endothelial cord inhibition potencies for confirmed phenotypic actives. Results of database mining were visualized using the phylogenetic-activity display, and compounds were classified on the basis of their predominant biochemical activities or designated "unknown" if no tested or predicted activity was associated with the compound. The predominant target class of phenotypic actives is indicated with the total number of molecules and percentage of molecules found in each target class (inset).

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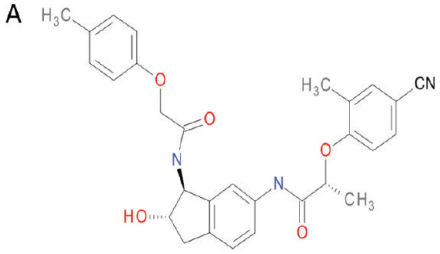
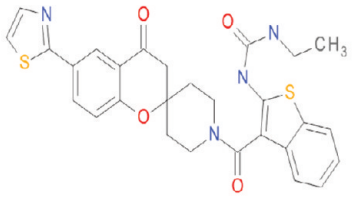
A



Compound A



Compound B

B

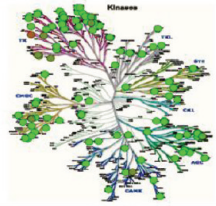
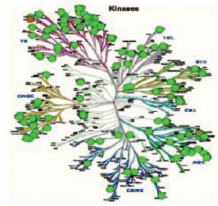
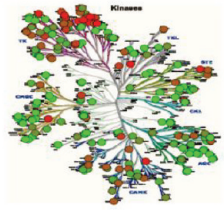
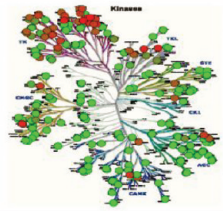
	Compound A	Compound B
Cord Area Inhib (EC50)	0.021 uM	0.041 uM
ECFC-ADSC Cytotoxicity (EC50)	> 10 uM	> 10 uM
ECFC Migration (IC50)	>40 uM	13 uM
G2M (4N EC50)	> 10 uM	> 10 uM
Kinome		
	Sutent	**Nexavar**
Cord Area Inhib (IC50)	0.0096 uM	0.00421 uM
ECFC-ADSC Cytotoxicity (EC50)	> 10 uM	5.46 uM
ECFC Migration (IC50)	13.6 uM	16.7 uM
G2M (4N EC50)	> 10 uM	> 10 uM
Kinome		

Figure 4. Comparison of phenotypic actives with antiangiogenesis standards of care. (A) Structures of two compounds identified by the phenotypic endothelial cord assay. (B) Functional comparison of phenotypic actives and antiangiogenesis standard of care, Sutent and Nexavar. The biochemical kinase activity for each molecule is summarized in the kinase phylogenetic-activity display. Color coding is as follows. Red indicates potent activity (EC_{50} or IC_{50} of $\leq$10 nM). Green indicates poor activity EC_{50} or IC_{50} of >10 uM). Phenotypic characterization and determination of EC_{50} and IC_{50} values used statistically validated assays as summarized in Experimental Section.

cell migration[28] indicates that complex cell based assays can support SAR if effort is taken in the design, statistical validation,[29] and operation of the assay.[18,22,27,28]

By use of statistically validated biochemical, cell based substrate phosphorylation and phenotypic end points to systematically compare the SAR of ~1000 cell cycle kinase inhibitors, Low et al.[22] demonstrated that phenotypic readouts respond to compound structural modifications in an equivalent manner as biochemical and cell based phosphorylation assays when linkage between molecular target and phenotype exists. Similarly, the differential ECF activity of the four diastereomers of compound **A** (Table 1) suggests that the angiogenesis coculture assay is able to detect specific interactions between the compounds and their respective molecular target(s). The similar potency shifts observed between two phenotypic assays, the ECFC/ADSC angiogenesis and the functional β-secretase assays (Table 1), suggest that the diastereomers work through a specific, although unknown, ligand−protein interaction. Although investigators have expressed concerns about the ability of phenotypic assays to support SAR[24−26] and the optimization of molecular properties,[10] our experience (Figure 6, Table 1)[18,22,27,28] and the results of others[30] indicate that this is not a general issue.

Phenotypic Actives and Mechanism of Action. Questions related to the identity of putative molecular drug targets inevitably arise during discussions of PDD. Mining of biochemical activity databases is one method to identify molecular targets associated with a phenotypic activity. Data mining of 1100 ECF inhibitors (IC_{50} < 2.5 μM) that were *not* filtered for kinase or cell cycle inhibition identified clusters of biochemical activities associated with kinases involved with

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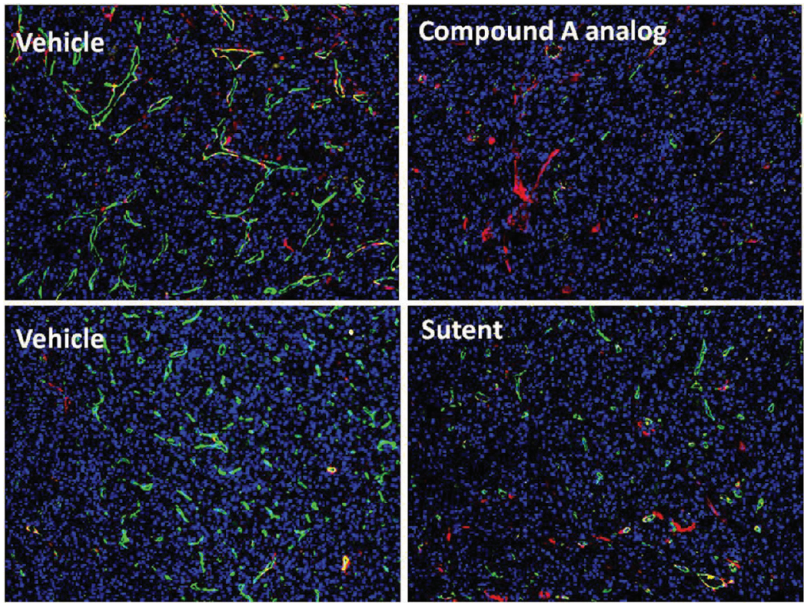
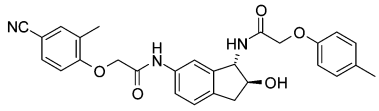
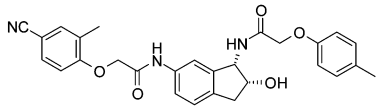
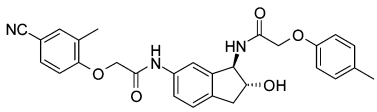
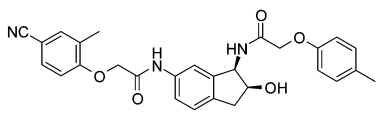


Figure 5. Inhibition of angiogenesis in vivo. Mice bearing U87MG xenograft tumors were treated with vehicles, compound **A** (30 mg/kg) or Sutent (10 mg/kg), administered twice a day for 3 days as described in Experimental Section. Tumors were removed, fixed, sectioned, stained, and examined by fluorescence microscopy as described in Experimental Section. Total cell number (Hoechst 33342) and the extent of blood vessel network formation, pericyte coverge, and tumor hypoxia were monitored with antibodies to CD31, smooth muscle actin, and Glut-1 respectively. Color coding is as follows: green = CD31 (endothelial tubes); blue = total nuclei; red = smooth muscle actin; yellow = Glut-1.

Table 1. Structure–Phenotypic Activity Relationships[a]

Structure	Stereochemistry	Cord Area IC50 (uM)	Nuclear Count IC50 (uM)	Functional β-secretase IC50 (uM)	γ-secretase IC50 (uM)
	1S, 2S	0.022	> 10 uM	0.142	>5
	1S, 2R	1.45 (66X)	> 10 uM	4 (28X)	>5
	1R, 2R	2.5 (114X)	> 10 uM	13.6 (96X)	>5
	1R, 2S	9.1 (414X)	> 10 uM	45.6 (321X)	>5

[a]The potencies of compound **A** diastereomers were determined in cellular assays measuring inhibition of endothelial cord formation (cord area), ECFC/ADSC cytotoxicity (nuclear count), functional *β*-secretase, and *γ*-secretase activities as summarized in Experimental Section. The decrease in compound potency relative to the 1*S*,2*S* diastereomer is indicated in parentheses.

angiogenesis (Abl, CSF1R, EphBs, FGFRs, Flts, KDR, NTRKs, PDGFRs, and Src) and cell cycle (Aurora, CDKs, and CHEK) as expected (not shown). ECF inhibitors with known activities associated with adrenergic *α*-1B and *α*-2C and serotonin 2A and 2C receptors were observed; however, follow-up testing with additional compounds targeting these GPCRs did not result in activity correlations between GPCR and ECF activity (data not shown) even though these receptors have been implicated in angiogenesis in other experimental systems.[31−33] These results highlight the importance of signaling and cellular

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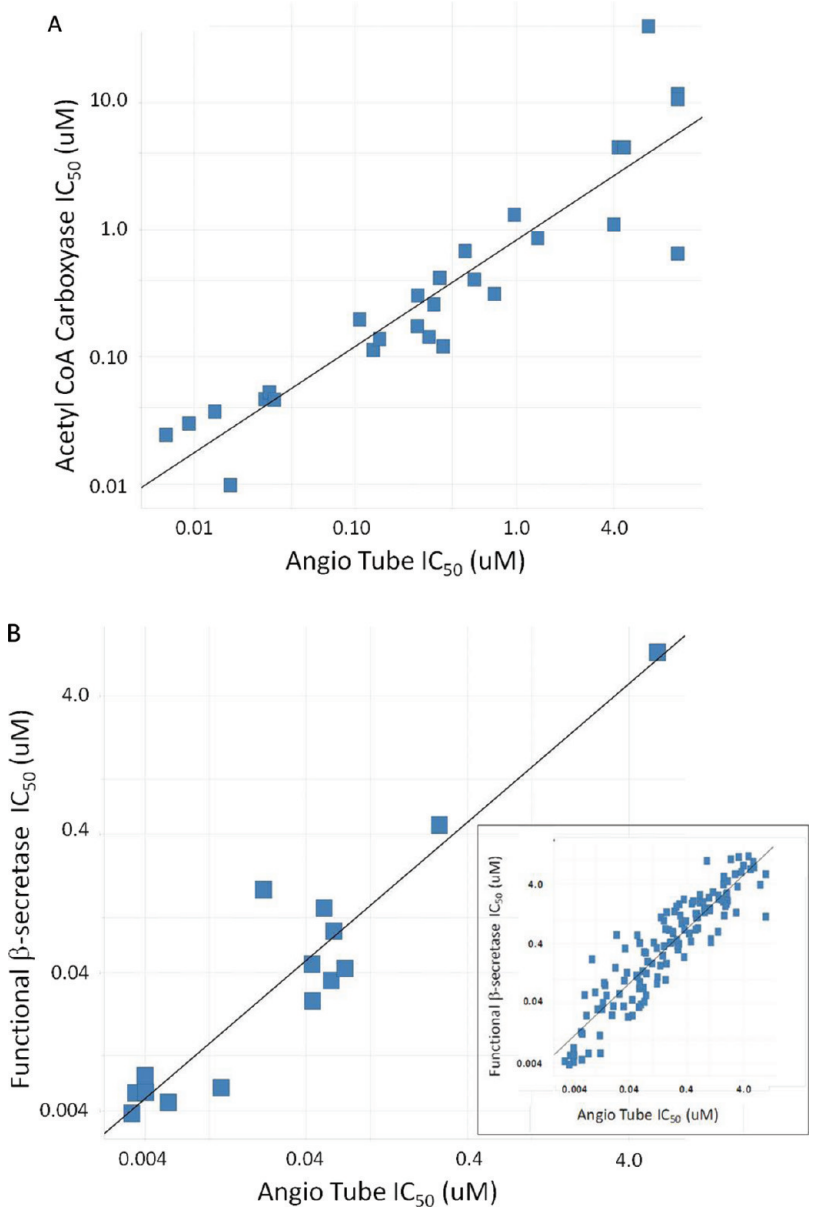

Figure 6. Molecular targets of phenotypic actives identified by compound structure−activity correlations. (A) Cell based activity correlation between inhibition of acetyl-CoA carboxylase and endothelial cord formation activities for 29 analogues of compound **B**. Linear regression line is indicated, R^2 = 0.81 with $p < 0.001$. (B) Cell based activity correlation between inhibition of cellular β-secretase and endothelial cord formation activities for 306 analogues of compound **A**. Linear regression line is indicated, R^2 = 0.65 with $p < 0.001$(inset). A subset of 15 compound **A** analogues were further tested for activity in biochemical BACE and cellular γ-secretase assays as summarized in Experimental Section. Linear regression line is indicated, R^2 = 0.95 with $p < 0.001$.

context in the linkage of a molecular target to a biological phenotype.

Mining of biochemical activity data also identified ACC and functional β-sec as potential molecular targets associated with AA activity (not shown). Cellular inhibition of ACC by various compound **B** analogues demonstrated good potency correlations with inhibition of ECF in the ECFC/ADSC coculture system (Figure 6A). In addition, inhibition of ECF was observed with a series of structurally distinct, nonspiro ACC inhibitors and soraphen A, a natural product that is a potent ACC inhibitor[34,35] (data not shown). Taken together, the activity correlations between inhibition of ECF and cellular ACC activity with three structurally distinct compound series suggest that ACC is a likely molecular target for ECF in the ECFC/ADSC coculture system. A role of ACC in angiogenesis has not been previously documented; moreover, existing studies predict that inhibition of ACC via phosphorylation of Ser 77 would lead to increased angiogenesis[36,37] rather than inhibition of angiogenesis as experimentally observed (Figure 6A).

Functional correlation between inhibition of cellular β-sec and ECF activity was observed with over 300 analogues of compound **A** (Figure 6B, inset). In order to better characterize the MOA of this series, a subset of these analogues was tested in a proteolysis assay using recombinant IgG construct of the BACE-1 ectodomain[38] and a cellular assay that monitors the γ-secretase mediated cleavage of a recombinant Notch-1 construct[39] (Figure 6B). Although γ-secretase has multiple roles in angiogenesis (reviewed in ref 40), each of the 15

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compound **A** analogues had undetectable inhibition of Notch-1 cleavage up to 5 μM (not shown) whereas ECF inhibition and functional
β-sec activity was potent and highly correlated over a 1000-fold concentration range (Figure 6B). These functional β-sec inhibitors also had no discernible effect on proteolysis catalyzed by purified BACE-1-IgG (not shown), suggesting that inhibition of the functional β-sec activity by these analogues may involve (1) additional cellular BACE-1 accessory proteins,[41,42] (2) BACE-1 dimer interactions that occur in a cellular context but not in BACE-1-IgG,[43,44] or (3) a protease, distinct from BACE-1, but that has somewhat overlapping cellular activity. In contrast to the well-known roles of γ-secretase in angiogenesis,[40] the potential function of BACE-1 or cellular β-sec activity in angiogenesis has been described, to our knowledge, in one publication.[45]

Taken together, these results illustrate how PDD approaches agnostically and simultaneously interrogate multiple molecular targets and signaling pathways of direct biological relevance in a manner that is independent of the veracity of prior target validation studies.[46]

Phenotypic Deconvolution and Molecular Targets of Approved Drugs. The identification of novel molecular targets related to angiogenesis suggests that thorough activity profiling may facilitate the deconvolution of phenotypic actives (Figure 6). This approach is limited by the lack of biochemical profiling data due to the operational costs and limited availability of assays needed for comprehensive compound characterization. As a result, 39% of the confirmed ECF inhibitors and their structural analogues did have known biochemical activity (Figure 3). The difficulty and uncertainty related to the identification of molecular targets for phenotypic actives have been commonly considered a weakness of the PDD approach. In this regard it is important to better understand the scientific, drug discovery, and regulatory rationales that underlie this viewpoint.

Life scientists currently enjoy ready access to information on the composition and expression patterns of genes at a genomic scale and possess an unprecedented ability to manipulate and measure biology at the molecular level. Such reaching capabilities have contributed to a molecular-centric view of contemporary biology that may contribute to misconceptions regarding the level of molecular target detail that is required for regulatory submission of an NME. Guidance from the Center for Drug Evaluation and Research and the Center for Biologics Evaluation and Research at the Food and Drug Administration indicates that identification of the molecular target for a drug is *not* required for initiation of clinical trials (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm071597.pdf).[47] Accordingly, 29% of the small molecule NMEs approved by the FDA from 2001 to 2004 (excluding natural products or imaging agents) did not have known molecular targets.[48]

The "one drug, one target paradigm", a hallmark of gene-specific TDD strategies, sought to minimize off-target side effects.[49] However, analysis of known drug−protein interactions has put this "magic bullet paradigm" into question; target specificity is rare for approved drugs with "known" molecular targets.[3,50] Analyses of 890 approved drugs indicate that 788 share molecular targets with at least one other drug.[3] Extensive mining of seven databases by Mestres et al.[50] indicate that on average each drug interacts with six known molecular targets. Given the overall poor target selectivity of known drugs[3,50] and that identification of molecular targets is not required for FDA approval, we feel that in vivo efficacy and safety should be the principle criteria for advancing phenotypic therapeutics.

Pharma Reality, Target Validation, and Phenotypic Drug Discovery. Productivity issues in the pharmaceutical industry are multifaceted and have been the subject of many editorial and review articles.[1,4,51,52] Analysis indicates that late stage clinical failures remain the primary reason for poor Pharma productivity,[4] which has been attributed to poor target validation and the lack of predictive biomarkers that translate to clinical studies.[4,53] Clinically relevant target validation goes beyond mRNA expression profiling and is a difficult, time-consuming process that requires successful integration of biology, chemistry, and pharmacology.[24,46,54−58] Notable failures in preclinical TV that were invalidated only after compounds advanced to clinical trials include farnesyl transferase,[59] matrix metalloproteases,[60] and neurokinin receptor.[61] Edwards et al. highlighted the difficulties and high risk associated with TV and suggested that these activities may be best suited for an open access Pharma consortium[56] where precompetitive TV data are shared.

TV difficulties have also lessened the impact of the genomics revolution on drug discovery.[51,57] Analysis of the molecular targets for launched drugs from the mid-1980s to mid-2000s reveals that the appearance of new targets has been nearly constant (5 per year) for the past 20 years.[3,62] Moreover, the appearance rate of novel drug targets was not significantly increased by the availability of novel molecular targets identified by deep EST sequencing.[7] In addition, difficulties in TV[46,58] and the widespread use of gene-specific TDD approaches in Pharma may have focused drug discovery efforts onto a relatively small set of highly validated molecular targets, a scenario that is conceivably related to decreased Pharma innovation and increased numbers of "me too" drugs.[3]

In contrast to gene-specific TDD approaches, PDD strategies directly interrogate complex biological systems in a molecular agnostic manner. If care is taken to develop in vitro biological systems that capture key aspects of the relevant in vivo biology, PDD expands the molecular playing field from a single molecular target and function to multiple processes, signaling pathways, and molecular functions/targets. It follows that PDD approaches enable the direct chemical interrogation of physiologically relevant biology in a manner that is independent of the target validation status of specific molecular components. This notion that PDD strategies may mitigate TV risk is consistent with Swinney's observation that PDD approaches led to a higher discovery rate of first in class NMEs approved by the FDA between 1999 and 2008.[10]

Given the views that late stage clinical failures may be due, in large part, to inadequate TV,[4] it may be useful to reevaluate how the choice of a lead generation strategy, one of the earliest decision points in the drug development process, may impact overall pTS. It is noteworthy that the majority of phenotypic assays that identified the majority of first in class NMEs utilized low throughput, physiology based animal or tissue systems and/or tested selected compounds based on prior knowledge.[10] These classic PDD approaches are more difficult and lower throughput than target-based approaches which readily incorporate advances in lab automation and high throughput chemical diversity testing. As a result, TDD approaches have become the predominant lead generation strategy used by Pharma.[6] We have explored a "neoclassic" hybrid of traditional phenotypic and target directed drug discovery approaches that

blend the use of physiologically relevant biological systems with the high throughput and statistical robustness offered by modern technologies. We find that common concerns related to PDD in regard to assay performance, statistical robustness, difficulties in establishing compound structure−activity relationships, and applicability of chemoinformatics tools are not necessarily realized (Figures 1−6, Table 1).[18,27,28] Identification of phenotypic actives that inhibit ACC and cellular β-sec activity, novel biochemical targets involved in angiogenesis (Figure 6), demonstrates that PDD provides a means to agnostically interrogate multiple, biologically relevant pathways and molecular targets in a manner that is independent of their validation status in the scientific literature.[46,58]

Estimates of the "druggable genome" indicate that roughly 1100 molecular targets have significant primary sequence homology to known drug targets,[3,62] suggesting that greater than 95% of the predicted proteins encoded by the human genome[8] are not modulated by current therapeutics. Results from phenotypic testing of compounds accepted to the Lilly PD[2] initiative demonstrate that small molecules with chemical diversity significantly different from internal collections and known drugs and are therefore likely to have distinct molecular mechanisms[63] had high confirmed hit rates (2−10%) and excellent selectivity between five phenotypic assays.[18] This observation suggests that high throughput, modern PDD approaches in conjunction with novel chemical diversity provides a means to access novel molecular targets and cellular mechanisms[18] and emphasizes the importance of academic−industrial collaborations like the Open Innovation Drug Discovery initiative (https://openinnovation.lilly.com/dd/).

Chemical diversity is large, and biology is complex. New approaches are needed to enhance our ability to find novel and efficacious therapeutics. Our results indicate that novel chemical diversity in conjunction with a modern, neoclassic PDD strategy may provide a means to identify molecules that modulate physiologically relevant signaling pathways and novel molecular targets, decrease TV risk, and increase the pTS of translating preclinical to clinical results.

EXPERIMENTAL SECTION

Assays. All cell growth and cell based assay incubations were conducted at 37 °C in a humidified incubator at 5% CO_2 unless otherwise noted. The in vitro angiogenesis model was operated in a 384-well format utilizing a coculture of endothelial and stromal precursor cells, ECFC (Endgenitor)[64] and ADSC (Zen Bio),[20] respectively, as described.[18] The CD31 positive cord area channel was used for single point screening at 2 μM compound; screen actives were confirmed using a 10-point dose response curve utilizing CD31 cord area and total nuclei count as a measure of ECF activity and overt cytotoxicity, respectively.[18] ECFC migration was quantitatively measured using the Oris cell migration plates (Platypus Technologies, LLC, Madison, WI) as described.[28] Cell cycle arrest in G2 or M phase was measured in HeLa (ATCC CCL-2) cells as described.[18,27] Enzymatic activity of soluble human BACE was measured by hydrolysis of a labeled peptide substrate by a purified BACE-IgG construct.[38] Functional γ-secretase activity was measured in HEK293 cells (ATCC CRL-1573) expressing recombinant human APP by monitoring the production of soluble of APPsβ as described[38] but using an ELISA format and a Lilly propriety mouse monoclonal antibody to the β-secretase neoepitope of hAPP. Functional γ-secretase activity was measured in HEK293 cells expressing a human Notch-1 construct, analogous to the constitutively active mouse NotchΔE[39] lacking an extracellular ligand binding domain. Cellular γ-secretase activity was measured with a Lilly propriety mouse monoclonal antibody to the γ-secretase neoepitope of human Notch-1. Cellular acetyl CoA carboxlyase activity was measured by incorporation of [^{14}C]acetate into triacylglycerol (TAG) using modifications of published procedures.[65,66] In short, HEK293 cells were switched from growth medium, DMEM medium supplemented with 10% fetal bovine serum and 1% antibiotic−antimycotic (Gibco), to Opti-MEM medium containing the designated compound concentration in 0.2% DMSO. After 1 h, fatty acid labeling was initiated by addition of 30 μM [^{14}C]acetate (Amersham, CFA13). After 4 h, the medium was removed and samples were washed with Hank's balanced salt solution and aspirated dry. Cells were dissolved with Triton X-100 (0.175%) containing basic ethanol and isopropanol at room temperature. Labeled TAG was extracted into a heptane phase and measured by liquid scintillation counting. In vitro assays were statistically validated for run to run plate uniformity by determining the Z' score[67] between plates of minimum and maximum assay signals. Compound potency reproducibility was validated for day to day variation by using the minimum significant ratio (MSR)[29] determined for at least 20 compounds of varying potency. Assays passed Eli Lilly−NIH Chemical Genomics Center guidelines for assay enablement and statistical validation; $Z' > 0.4$ and MSR ≤ 3 (http://spotlite.nih.gov/assay/index.php/Table_of_Contents).

In Vivo Xenograft Studies. U87MG glioblastoma tumor cells were mixed 1:1 with Matrigel (BD Biosciences) and implanted subcutaneously in the right rear flank of athymic nude female mice (Harlan) at 5.0×10^6 cells/injection. Xenografts were grown to an average tumor volume of 250 mm^3, and the mice were randomized at baseline according to tumor volume and body weight (10/group). Compound **A** was formulated in 100% peanut oil as the vehicle and administered as subcutaneous injections twice daily for 3 days. Sutent was formulated in 10% acacia and admistered by oral gavage twice daily for 3 days. Three days after treatment, xenografts were excised and placed into zinc-Tris fixative (BD Pharmingen). Tumors were blocked in paraffin and sectioned as 4 μm slices. Slides were baked at 60 °F for 1 h and then deparaffinized in xylene (4 × 10 min); rehydrated with ethanol/water immersions with final washes in TBST; blocked with protein block (Dako) for 30 min; stained with a combination of Hoechst 33324, rat anti-mouse CD31 (Pharmingen)/anti-rat Alexa-488 (Invitrogen), rabbit anti-GLUT1 (Dako)/anti-rabbit Alexa 647 (Invitrogen), and mouse anti-smooth muscle actin/Cy3 (Sigma); imaged using a Marianas digital imaging workstation configured with a Zeiss Axiovert 200M inverted fluorescence microscope (Intelligent Imaging Innovations).

Hit Expansion. Several rounds of HE were executed using a variety of virtual screening technologies. Substructure and similarity searches utilizing structural fingerprints, Tanimoto similarity, and internal measures were used to fill existing active clusters with structurally similar analogues to explore structure−activity relationships. In order to investigate chemical space not represented in the screening libraries, 2D scaffold-hopping (Lilly tools using 2D pharmacophore descriptors), 2D SVM models,[68] and 3D ROCS (http://www.eyesopen.com/rocs) searches were used. Structural clusters were defined by an internal program that utilizes a variant of leader clustering and Taylor−Butina cluster algorithm[68] and several internal chemoinformatics similarity measures.

Data Mining and Visualization. Pharmacology and biochemical database mining was enabled by a Lilly program that takes a list of query compounds and comprehensively searches internal and external in vitro data associated with the compounds and their structural analogues. The resulting data are visualized either as a heat map of compound activity verses molecular target or as a phylogenetic diagram of GPCR, kinase, NHR, and PDE target families (human kinome provided courtesy of Cell Signaling Technology, Inc., www.cellsignal.com) with color coded biochemical activity. In both visualization methods, red indicates high activity (EC_{50} or IC_{50} of $\leq$10 nM) and green indicates low activity (EC_{50} or IC_{50} of >10 uM).

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ABBREVIATIONS USED

AA, antiangiogenic; ACC, acetyl CoA carboxylase; ADSC, adipose derived stem cell; β-sec, β-secretase; ECF, endothelial cord formation; EST, expressed sequence tag; ECFC, endothelial colony forming cell; GPCR, G-protein-coupled receptor; IgG, immunoglobulin G; MOA, mechanism of action; MTS, medium throughput screen; NHR, nuclear hormone receptor; NME, new molecular entity; PDD, phenotypic drug discovery; Pharma, pharmaceutical industry; pTS, probability of technical success; PDE, phosphodiesterase; RTK, receptor tyrosine kinase; SAR, structure–activity relationship; SOC, standard of care; SP, single point; TDD, targeted drug discovery; TV, target validation; VEGF, vascular endothelial growth factor

REFERENCES

(1) Munos, B. Lessons from 60 years of pharmaceutical innovation. *Nat. Rev. Drug Discovery* **2009**, *8*, 959–968.

(2) Booth, B.; Zemmel, R. Prospects for productivity. *Nat. Rev. Drug Discovery* **2004**, *3*, 451–456.

(3) Yildirim, M. A.; Goh, K. I.; Cusick, M. E.; Barabasi, A. L.; Vidal, M. Drug–target network. *Nat. Biotechnol.* **2007**, *25*, 1119–1126.

(4) Paul, S. M.; Mytelka, D. S.; Dunwiddie, C. T.; Persinger, C. C.; Munos, B. H.; Lindborg, S. R.; Schacht, A. L. How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat. Rev. Drug Discovery* **2010**, *9*, 203–214.

(5) Lechleiter, J. C. Lilly COE Lechleiter Calls for Repair of the "Engine of Biopharma Innovation"; 2009

(6) Macarron, R.; Banks, M. N.; Bojanic, D.; Burns, D. J.; Cirovic, D. A.; Garyantes, T.; Green, D. V.; Hertzberg, R. P.; Janzen, W. P.; Paslay, J. W.; Schopfer, U.; Sittampalam, G. S. Impact of high-throughput screening in biomedical research. *Nat. Rev. Drug Discovery* **2011**, *10*, 188–195.

(7) Adams, M. D.; Kelley, J. M.; Gocayne, J. D.; Dubnick, M.; Polymeropoulos, M. H.; Xiao, H.; Merril, C. R.; Wu, A.; Olde, B.; Moreno, R. F.; et al. Complementary DNA sequencing: expressed sequence tags and human genome project. *Science* **1991**, *252*, 1651–1656.

(8) International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. *Nature* **2004**, *431*, 931–945.

(9) Stancato, L.; Uhlik, M. Enabling phenotypic lead generation through high content imaging. *Eur. Biopharm. Rev. Digital* **2008**, *1*, 38–43.

(10) Swinney, D. C.; Anthony, J. How were new medicines discovered? *Nat. Rev. Drug Discovery* **2011**, *10*, 507–519.

(11) Quintavalle, M.; Elia, L.; Price, J. H.; Heynen-Genel, S.; Courtneidge, S. A. A cell-based high-content screening assay reveals activators and inhibitors of cancer cell invasion. *Sci Signaling* **2011**, *4*, ra49.

(12) Mayer, T. U.; Kapoor, T. M.; Haggarty, S. J.; King, R. W.; Schreiber, S. L.; Mitchison, T. J. Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen. *Science* **1999**, *286*, 971−974.
(13) Yarrow, J. C.; Totsukawa, G.; Charras, G. T.; Mitchison, T. J. Screening for cell migration inhibitors via automated microscopy reveals a Rho-kinase inhibitor. *Chem. Biol.* **2005**, *12*, 385−395.
(14) Chen, S.; Do, J. T.; Zhang, Q.; Yao, S.; Yan, F.; Peters, E. C.; Scholer, H. R.; Schultz, P. G.; Ding, S. Self-renewal of embryonic stem cells by a small molecule. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 17266−17271.
(15) Chen, S.; Zhang, Q.; Wu, X.; Schultz, P. G.; Ding, S. Dedifferentiation of lineage-committed cells by a small molecule. *J. Am. Chem. Soc.* **2004**, *126*, 410−411.
(16) Carmeliet, P.; Jain, R. K. Molecular mechanisms and clinical applications of angiogenesis. *Nature* **2011**, *473*, 298−307.
(17) Folkman, J. Angiogenesis: an organizing principle for drug discovery? *Nat. Rev. Drug Discovery* **2007**, *6*, 273−286.
(18) Lee, J. A.; Chu, S.; Willard, F. S.; Cox, K. L.; Sells Galvin, R. J.; Peery, R. B.; Oliver, S. E.; Oler, J.; Meredith, T. D.; Heidler, S. A.; Gough, W. H.; Husain, S.; Palkowitz, A. D.; Moxham, C. M. Open innovation for phenotypic drug discovery: the PD2 assay panel. *J. Biomol. Screening* **2011**, *16*, 588−602.
(19) Traktuev, D. O.; Merfeld-Clauss, S.; Li, J.; Kolonin, M.; Arap, W.; Pasqualini, R.; Johnstone, B. H.; March, K. L.; Traktuev, D. O.; Merfeld-Clauss, S.; Li, J.; Kolonin, M.; Arap, W.; Pasqualini, R.; Johnstone, B. H.; March, K. L. A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. *Circ. Res.* **2008**, *102*, 77−85.
(20) Traktuev, D. O.; Prater, D. N.; Merfeld-Clauss, S.; Sanjeevaiah, A. R.; Saadatzadeh, M. R.; Murphy, M.; Johnstone, B. H.; Ingram, D. A.; March, K. L. Robust functional vascular network formation in vivo by cooperation of adipose progenitor and endothelial cells. *Circ. Res.* **2009**, *104*, 1410−1420.
(21) Crawford, Y.; Ferrara, N. VEGF inhibition: insights from preclinical and clinical studies. *Cell Tissue Res.* **2009**, *335*, 261−269.
(22) Low, J.; Stancato, L.; Lee, J.; Sutherland, J. J. Prioritizing hits from phenotypic high-content screens. *Curr. Opin. Drug Discovery Dev.* **2008**, *11*, 338−345.
(23) Adams, R. H.; Alitalo, K. Molecular regulation of angiogenesis and lymphangiogenesis. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 464−78.
(24) Terstappen, G. C.; Schlupen, C.; Raggiaschi, R.; Gaviraghi, G. Target deconvolution strategies in drug discovery. *Nat. Rev. Drug Discovery* **2007**, *6*, 891−903.
(25) Hart, C. P. Finding the target after screening the phenotype. *Drug Discovery Today* **2005**, *10*, 513−519.
(26) Knight, Z. A.; Lin, H.; Shokat, K. M. Targeting the cancer kinome through polypharmacology. *Nat. Rev. Cancer* **2010**, *10*, 130−137.
(27) Lee, J. A.; Cox, K.; Kriauciunas, A.; Chu, S. The Roles of High Content Cellular Imaging in Lead Optimization. In *High Content Screening: Science, Techniques, and Applications*; Haney, S., Ed.; Wiley-Interscience: Hoboken, NJ, 2008; pp 249−268.
(28) Gough, W.; Hulkower, K. I.; Lynch, R.; McGlynn, P.; Uhlik, M.; Yan, L.; Lee, J. A. A quantitative, facile, and high-throughput image-based cell migration method is a robust alternative to the scratch assay. *J. Biomol. Screening* **2011**, *16*, 155−163.
(29) Eastwood, B. J.; Farmen, M. W.; Iversen, P. W.; Craft, T. J.; Smallwood, J. K.; Garbison, K. E.; Delapp, N. W.; Smith, G. F. The minimum significant ratio: a statistical parameter to characterize the reproducibility of potency estimates from concentration-response assays and estimation by replicate-experiment studies. *J. Biomol. Screening* **2006**, *11*, 253−261.
(30) Chung, C. W.; Coste, H.; White, J. H.; Mirguet, O.; Wilde, J.; Gosmini, R. L.; Delves, C.; Magny, S. M.; Woodward, R.; Hughes, S. A.; Boursier, E. V.; Flynn, H.; Bouillot, A. M.; Bamborough, P.; Brusq, J. M.; Gellibert, F. J.; Jones, E. J.; Riou, A. M.; Homes, P.; Martin, S. L.; Uings, I. J.; Toum, J.; Clement, C. A.; Boullay, A. B.; Grimley, R. L.; Blandel, F. M.; Prinjha, R. K.; Lee, K.; Kirilovsky, J.; Nicodeme, E. Discovery and characterization of small molecule inhibitors of the BET family bromodomains. *J. Med. Chem.* **2011**, *54*, 3827−3838.
(31) Park, S. Y.; Kang, J. H.; Jeong, K. J.; Lee, J.; Han, J. W.; Choi, W. S.; Kim, Y. K.; Kang, J.; Park, C. G.; Lee, H. Y. Norepinephrine induces VEGF expression and angiogenesis by a hypoxia-inducible factor-1alpha protein-dependent mechanism. *Int. J. Cancer* **2011**, *128*, 2306−2316.
(32) Asada, M.; Ebihara, S.; Yamanda, S.; Niu, K.; Okazaki, T.; Sora, I.; Arai, H. Depletion of serotonin and selective inhibition of 2B receptor suppressed tumor angiogenesis by inhibiting endothelial nitric oxide synthase and extracellular signal-regulated kinase 1/2 phosphorylation. *Neoplasia* **2009**, *11*, 408−417.
(33) Muthig, V.; Gilsbach, R.; Haubold, M.; Philipp, M.; Ivacevic, T.; Gessler, M.; Hein, L. Upregulation of soluble vascular endothelial growth factor receptor 1 contributes to angiogenesis defects in the placenta of alpha 2B-adrenoceptor deficient mice. *Circ. Res.* **2007**, *101*, 682−691.
(34) Vahlensieck, H. F.; Pridzun, L.; Reichenbach, H.; Hinnen, A. Identification of the yeast ACC1 gene product (acetyl-CoA carboxylase) as the target of the polyketide fungicide soraphen A. *Curr. Genet.* **1994**, *25*, 95−100.
(35) Jump, D. B.; Torres-Gonzalez, M.; Olson, L. K. Soraphen A, an inhibitor of acetyl CoA carboxylase activity, interferes with fatty acid elongation. *Biochem. Pharmacol.* **2011**, *81*, 649−660.
(36) Izumi, Y.; Shiota, M.; Kusakabe, H.; Hikita, Y.; Nakao, T.; Nakamura, Y.; Muro, T.; Miura, K.; Yoshiyama, M.; Iwao, H. Pravastatin accelerates ischemia-induced angiogenesis through AMP-activated protein kinase. *Hypertens. Res.* **2009**, *32*, 675−679.
(37) Stahmann, N.; Woods, A.; Spengler, K.; Heslegrave, A.; Bauer, R.; Krause, S.; Viollet, B.; Carling, D.; Heller, R. Activation of AMP-activated protein kinase by vascular endothelial growth factor mediates endothelial angiogenesis independently of nitric-oxide synthase. *J. Biol. Chem.* **2010**, *285*, 10638−10652.
(38) Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, J.; Edenson, S.; Lile, J.; Jarosinski, M. A.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J. C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **1999**, *286*, 735−741.
(39) Kopan, R.; Schroeter, E. H.; Weintraub, H.; Nye, J. S. Signal transduction by activated mNotch: importance of proteolytic processing and its regulation by the extracellular domain. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 1683−1688.
(40) Boulton, M. E.; Cai, J.; Grant, M. B. gamma-Secretase: a multifaceted regulator of angiogenesis. *J. Cell. Mol. Med.* **2008**, *12*, 781−795.
(41) Majercak, J.; Ray, W. J.; Espeseth, A.; Simon, A.; Shi, X. P.; Wolffe, C.; Getty, K.; Marine, S.; Stec, E.; Ferrer, M.; Strulovici, B.; Bartz, S.; Gates, A.; Xu, M.; Huang, Q.; Ma, L.; Shughrue, P.; Burchard, J.; Colussi, D.; Pietrak, B.; Kahana, J.; Beher, D.; Rosahl, T.; Shearman, M.; Hazuda, D.; Sachs, A. B.; Koblan, K. S.; Seabrook, G. R.; Stone, D. J. LRRTM3 promotes processing of amyloid-precursor protein by BACE1 and is a positional candidate gene for late-onset Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 17967−17972.
(42) Zhang, C.; Browne, A.; Child, D.; Divito, J. R.; Stevenson, J. A.; Tanzi, R. E. Loss of function of ATXN1 increases amyloid beta-protein levels by potentiating beta-secretase processing of beta-amyloid precursor protein. *J. Biol. Chem.* **2010**, *285*, 8515−8526.
(43) Westmeyer, G. G.; Willem, M.; Lichtenthaler, S. F.; Lurman, G.; Multhaup, G.; Assfalg-Machleidt, I.; Reiss, K.; Saftig, P.; Haass, C. Dimerization of beta-site beta-amyloid precursor protein-cleaving enzyme. *J. Biol. Chem.* **2004**, *279*, 53205−53212.
(44) Multhaup, G. Amyloid precursor protein and BACE function as oligomers. *Neurodegener. Dis.* **2006**, *3*, 270−274.
(45) Paris, D.; Quadros, A.; Patel, N.; DelleDonne, A.; Humphrey, J.; Mullan, M. Inhibition of angiogenesis and tumor growth by beta and gamma-secretase inhibitors. *Eur. J. Pharmacol.* **2005**, *514*, 1−15.

(46) Prinz, F.; Schlange, T.; Asadullah, K. Believe it or not: how much can we rely on published data on potential drug targets? *Nat. Rev. Drug Discovery* **2011**, *10*, 712.
(47) Lumpkin, M.; Woodcock, J.; Zoon, K. *Guidance for Industry: Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well Characterized, Therapeutic, Biotechnology-Derived Products*; Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research: Rockville, MD, 1995; p 10.
(48) Swinney, D. C. Biochemical mechanisms of new molecular entities (NMEs) approved by United States FDA during 2001−2004: mechanisms leading to optimal efficacy and safety. *Curr. Top. Med. Chem.* **2006**, *6*, 461−478.
(49) Hopkins, A. L.; Mason, J. S.; Overington, J. P. Can we rationally design promiscuous drugs? *Curr. Opin. Struct. Biol.* **2006**, *16*, 127−136.
(50) Mestres, J.; Gregori-Puigjane, E.; Valverde, S.; Sole, R. V. The topology of drug−target interaction networks: implicit dependence on drug properties and target families. *Mol. BioSyst.* **2009**, *5*, 1051−1057.
(51) Garnier, J. P. Rebuilding the R&D engine in big pharma. *Harv. Bus. Rev.* **2008**, *86*, 68−70 72−6, 128.
(52) Munos, B. Can open-source drug R&D repower pharmaceutical innovation? *Clin. Pharmacol. Ther.* **2010**, *87*, 534−536.
(53) Ma, P.; Zemmel, R. Value of novelty? *Nat. Rev. Drug Discovery* **2002**, *1*, 571−572.
(54) Flordellis, C. S.; Manolis, A. S.; Paris, H.; Karabinis, A. Rethinking target discovery in polygenic diseases. *Curr. Top. Med. Chem.* **2006**, *6*, 1791−1798.
(55) Lindsay, M. A. Target discovery. *Nat. Rev. Drug Discovery* **2003**, *2*, 831−8.
(56) Edwards, A. M.; Bountra, C.; Kerr, D. J.; Willson, T. M. Open access chemical and clinical probes to support drug discovery. *Nat. Chem. Biol.* **2009**, *5*, 436−40.
(57) Fishman, M. C.; Porter, J. A. Pharmaceuticals: a new grammar for drug discovery. *Nature* **2005**, *437*, 491−493.
(58) Mullard, A. Reliability of "new drug target" claims called into question. *Nat. Rev. Drug Discovery* **2011**, *10*, 643−644.
(59) Appels, N. M.; Beijnen, J. H.; Schellens, J. H. Development of farnesyl transferase inhibitors: a review. *Oncologist* **2005**, *10*, 565−578.
(60) Fingleton, B. MMPs as therapeutic targets—still a viable option? *Semin. Cell Dev. Biol.* **2008**, *19*, 61−68.
(61) Boyce, S.; Hill, R. *Proceedings of the 9th World Congress on Pain*; Devor, M., Ed.; International Association for the Study of Pain Press: Vienna, Austria, 2000; pp 313−324.
(62) Overington, J. P.; Al-Lazikani, B.; Hopkins, A. L. How many drug targets are there? *Nat. Rev. Drug Discovery* **2006**, *5*, 993−996.
(63) Martin, Y. C.; Kofron, J. L.; Traphagen, L. M. Do Structurally Similar Molecules Have Similar Biological Activity? *J. Med. Chem.* **2002**, *45*, 4350−4358.
(64) Yoder, M. C. Is endothelium the origin of endothelial progenitor cells? *Arterioscler., Thromb., Vasc. Biol.* **2010**, *30*, 1094−1103.
(65) Alberts, A. W.; Ferguson, K.; Hennessy, S.; Vagelos, P. R. Regulation of lipid synthesis in cultured animal cells. *J. Biol. Chem.* **1974**, *249*, 5241−5249.
(66) Bijleveld, C.; Geelen, M. J. Measurement of acetyl-CoA carboxylase activity in isolated hepatocytes. *Biochim. Biophys. Acta* **1987**, *918*, 274−283.
(67) Zhang, J. H.; Chung, T. D.; Oldenburg, K. R. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screening* **1999**, *4*, 67−73.
(68) Butina, D. Unsupervised data base clustering based on Daylight's fingerprint and Tanimoto similarity: a fast and automated way to cluster small and large data sets. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 747−750.