Original Research

The inadequacy of the reductionist approach in discovering new therapeutic agents against complex diseases

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Impact statement

The initial scope of this investigation was to build the set of human genes that are presumed to be the therapeutic intervention points of US FDA-approved drugs, in all therapeutics areas but oncology. The prerequisite for this study was the establishment of the non-redundant set of all active pharmaceutical ingredients for these disease areas. Pertaining to complex diseases, the main observation was that there is not a single instance in the history of drug discovery, where a compound, initially selected by means of a biochemical assay, achieved a significant therapeutic response. The whole field of Drug R&D faces an unacceptable lack of new treatments to address unmet medical needs. The conclusion is that complex biological assays have to be designed for the primary selection of candidate therapeutics.

Abstract

For more than 20 years, drug discovery has relied on two assumptions, i.e. (i) a therapeutic response can be triggered by modulating the activity of a single gene product, and (ii) a compound uncovered by its activity on a recombinant protein *in vitro* can perform its activity *in vivo*. Drug discovery operates accordingly by using the concepts of targets and pipelines. The target, such as a gene product, is the intended point of therapeutic intervention, and compounds that modulate its activity *in vitro* follow a series of downstream developments. This reductionist approach has developed due to advances in combinatorial chemistry, robotics, molecular biology, and genomics. The expectation of this approach is that the frequency of drug discovery will dramatically increase, while its associated cost would decrease. However, the frequency of new drug discovery has decreased, while the associated costs have surged. We performed a retrospective study that examined how successful development programs have led to marketed drugs for all indications except anti-infective and anti-neoplastic agents. We concluded that the target and pipeline paradigms are limited and are actually causing the drug development industry to collectively fail to meet the critical medical needs.

Keywords: Complex diseases, US FDA-approved drugs, physiology, *in vivo* screening, poly-pharmacology, medicinal chemistry

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Introduction

Historians and archeologists might be able to trace the exact periods when the usage of medicines began in ancient civilizations. For the purpose of this study, we speculated that the curative properties derived from plant and animal materials were recognized by early health practitioners, such as the Worshipful Society of Apothecaries of London founded during the Renaissance era.[1] In these early days of pharmacy, one can expect that there was no structured reasoning for new drug discovery; therefore, empiricism was the only way to identify the medicinal properties of plant extracts. The cause of a disease was largely unknown as was the composition of the treatment concoction. Astute botanists and apothecaries did not know the nature of the therapeutic agent or cause of the healing response, yet they mastered correlational studies and eventually succeeded in inferring the medicinal properties of crude natural products. In the triad consisting of the treatment (the input), patient (the processing system), and therapeutic response (the output), the clinical endpoint was the only variable that could be fairly reasonably assessed. These early "hit-and-miss" attempts were mostly the reason for the failure in addressing critical medical needs. However, some attempts coincidentally achieved significant medical benefits. Although very few achievements resulted from this empirical approach, there was a significant impact. In particular, these early discoveries proved that it was possible to achieve a beneficial physiological response with exogenous agents. In addition, they laid the foundation for pharmaceutical science: a xenobiotic agent can either promote a palliative response or specifically reverse the

etiology of a condition. In other words, one can alter the processing system (the patient) with the appropriate input (the medicinal treatment). Advances in organic chemistry provided the tools to determine the nature of active agents (small molecules) well before their mechanisms of action were known. For example, the ancient Greek physicians prescribed willow plant bark aqueous extracts as an antipyretic agent. However, the active ingredient in willow barks, salicylate, was not identified and purified until the mid-19th century.[2] The disease etiology and the patient remained a black box for another century, even though the nature and molecular composition of the therapeutic agent were known.[3] In many cases, life-saving medicines were discovered and developed accidentaly by biological assays. Even for the first antibiotics, which only targeted bacteria, the molecular mechanism of action (MOA) was unknown (e.g. penicillin). During this period of drug discovery, investigators identified that the active agents were small molecules; they were able to purify, characterize, and even synthesize them in the laboratory. Thus, the treatment (input) became the second variable that could be assessed after the therapeutic response (output). Nevertheless, the disease or the molecular determinants that disrupt a patient's homeostatic balance for a given indication were beyond the analytic capabilities of early investigators. Advances in molecular and cellular biology, enzymology, and histopathology enabled investigators to identify some of the biomolecular mechanisms of therapeutic xenobiotic compounds. Because the therapeutic agents were typically discovered through empirical investigation, their respective biological intervention sites were identified retrospectively (e.g. morphine and the G-protein opioid receptors,[4] aspirin and cyclo-oxygenases[5]). With these advances, the pieces of the puzzle began to fall into place; the therapeutic agent interacted with a biological target, usually a polypeptide, to trigger a therapeutic response. The perspective that a drug's MOA is mediated by a one-to-one interaction became the basis of the subsequent paradigm of drug discovery. Under this paradigm, the patient was viewed as a set of discrete molecular entities, or gene products that have one-to-one relationships with their corresponding gene loci, and each of these entities was a potential site for therapeutic intervention. Guided by this concept, the industry grew in parallel with technological advances that allowed major gains realized from the advent of recombinant DNA technology (allowing isolation of a single gene and its use as a reagent), combinatorial chemistry, protein crystallography, and structural biology. This one-to-one view signaled the advent of modern medicine's drug discovery paradigm that would be known as "rational drug design."

Based on this paradigm, every drug discovery program starts with the selection of a compound that interacts with a recombinant protein *in vitro*. This selection is achieved by screening compound libraries through biochemical assays. The recombinant protein may be the product of a human gene cloned in an expression system. Thus, the human gene is considered the "target" according to the rational drug design. Any gene with evidence implicating its association with a given indication is a common starting point for a drug discovery program, provided that its gene products are amenable to biochemical assays.

After more than 20 years, the rational drug design remains the basis of drug discovery. This approach assumes that a treatment for any disease can be achieved by acting on a single gene product and that a compound identified by its *in vitro* activity can perform similarly *in vivo*. Exploratory research laboratories in many therapeutic areas are actively searching for "targets," or providing medicinal chemists with gene products that will begin new drug discovery programs. The drug research and development (R&D) industry, for the most part, assumes that altering the activity of a single gene can provide a therapeutic response. In addition, because compounds are selected *in vitro* for only one form of a given gene, the complexity of the potential post-translational events is not considered. Furthermore, the fact that cellular and extracellular compartments are not solely composed of proteins is not represented in *in vitro* testing.

Despite legitimate expectations of increasing drug discovery through the rational drug discovery concept, this approach has not delivered.[6] Furthermore, the resources dedicated to drug R&D continually increasing, whereas the effectiveness of new small molecule pharmaceutical agents is sharply declining.[7] What factors are responsible for this reduced productivity? Many authors have argued that regulatory agencies are partly responsible. This argument would be valid only if the regulations actually had changed over the last 20 years, but this is not the case. Other explanations for the decline in productivity have included successive mergers and acquisitions (M&As), the methodology of clinical trials (in particular the lack of efficacy biomarkers), and poorly validated targets. M&A have occurred over the last 20 years. Prior to these M&As, the drug industry was divided into many entities that were typically smaller than those in other industries that also have significant R&D programs, such as the semiconductor industry. Consequently, these M&A have increased and expanded R&D operations that currently provide access to greater resources. Therefore, consolidation of the drug industry is an illogical explanation for its current lack of R&D productivity.

As far as clinical trials are concerned, there are many ongoing efforts that aim to design more cost-effective trials and focus on data collection and management issues and additional quantitative methods to assess the efficacy of candidate therapies.[8] Despite these efforts, the industry continually requires new drugs, particularly those that are intended to treat cardiovascular, metabolic, or central nervous system disorders.

Therefore, the following question still remains: what are the causes of the drug discovery shortfall? At this point, we consider the possibility that poor target selection is the root cause. To address this possibility, we conducted a study in which we investigated all approved drugs in the United States by examining the Food and Drug Administration (FDA) Orange Book database's content.

Our primary objective was to establish a set of characteristics that could identify a gene as a quality target for initial primary screening. To this end, we evaluated the entire list

of FDA-approved drugs to compile a complete list of their cognate targets. However, during the course of our efforts, we were surprised to find that the very concept of "one target" is not applicable. The vast majority of approved drugs have a polypharmacological property, implying they most likely achieve their therapeutic effect by acting on more than one gene product. The second unexpected result of our study was that the vast majority of approved drugs did not originate from an initial primary screening with *in vitro* assays, except in extremely rare cases (e.g. maraviroc, a CCR5 antagonist prescribed as an anti-HIV indication). In contrast, successful programs began with a comprehensive understanding of the underlying mechanisms of a disease that was used to design compounds that altered well-described molecular pathways.

The crucial lesson for our industry is that the drug discovery programs that begin by selecting compounds from chemical libraries, which are active against a single protein *in vitro*, will likely fail for all indications including anti-infective and anti-tumor agents. The rational drug design concept that currently guided industry efforts do not address the areas of public health that are in dire need of treatments, such as cardiovascular, metabolic, and central nervous system diseases.

Materials and methods

The Electronic Orange Book, US FDA database of Approved Drug Products content was retrieved from the following data source: http://www.fda.gov/Drugs/InformationOnDrugs/ucm129689.htm. There were 24,974 entries as of June 1, 2010. The products table has the following list of attributes: Ingredient, df; route, trade_name, applicant, strength, appl_type, appl_no, product_no, te_code, approval_date, rld, type, applicant_full_name. The "appl_type" attribute features two mutually exclusive values, new drug applications (nda or innovator), "n," and abbreviated new drug applications (anda or generic), "a." The ingredients attribute value designates the active agent. There is a one-to-many relationship between ingredient and strength (the potency of the active ingredient). Some ingredient entries feature more than one active agent (combination). A given ingredient may be approved under a distinct salt mix. The primary scope of the data mining was to assess the fraction of drugs discovered by means of high-throughput screening of libraries on *in vitro* assays. From the whole set of 24,974 elements of the FDA Orange Book, the set of actual non-redundant active agents was determined. For each non-redundant ingredient, their cognate primary indication was retrieved. Agents active on non-human biological activities (e.g. antibiotic) as well as contrasting imaging agents used for diagnostics were filtered out. Additional filters consisted of excluding biologics agents as well as anti-cancer drugs. The data filtering workflow is shown in Figure 1.

Results

US FDA Orange Book database filtering

In the face of current collective difficulties for discovering new small-molecule-based medicines, the authors believe it is time to pause, reflect, and attempt to identify the root cause. One way to address this issue is to examine the history of approved drugs and focus on successful discovery efforts. The obvious place to start such an investigation the US FDA database of approved drugs referred to as the Orange Book. This database is the prime reference for all approved drug products, which are defined by the combination of a drug's active ingredient(s), dosage, form, route of administration, strength, and the amount of active ingredient delivered by tablet or injection. In most cases, there is a one-to-many relationship between the active ingredients and drug products. In addition to multiple dosages and routes of administration, the same ingredients can appear in many drug products approved in various salt formulations or in combination with other active ingredients. For example, morphine and naltrexone can be found in different drug products either unique ingredients or in combination.

The logical stepwise series of data filtering shown in Figure 1 returned a set of non-redundant active ingredients that were used in this study. For approximately 25,000 drug products listed in the May 2010 Orange Book, we identified 742 unique active ingredients. Then, we excluded mineral salts as unique ingredients and contrast reagents for imaging (radiopharmaceuticals). We also excluded antibiotics, anti-virals, anti-fungal treatments, and anti-tumor agents because they are outside the scope of this study. The primary objective of this study was to determine how xenobiotic small-molecule-based active pharmaceutical ingredients were initially discovered; therefore, we excluded biologics and drug products that were copies of naturally occurring human molecules such as thyroid hormone. Supplemental Table 1 presents the resultant set of 394 small molecules that have been approved by the US FDA since 1982 for indications with the exception of infectious and neoplastic diseases.

Small-molecule-based drug discovery and protein targets

To achieve the initial objective of our investigation, we derived a complete set of therapeutic intervention points of approved small-molecule-based active pharmaceutical ingredients for all indications, except for cancer and infection. At the inception of this study, it was generally assumed that the concept of an active ingredient targeting either one biomolecule or a small finite set of biomolecules was the overarching principle behind the small-molecule-based marketed drugs. A series of articles was published on this principle,[9–12] but the number of therapeutic intervention points never agreed and ranged from hundreds to thousands. In addition, none of these previous studies mentioned how they determined the target for each approved drug. This justified the need to explore the paradigm and to determine the entire picture from which one could potentially infer some putative rules regarding the concept of targetability.

What are the so-called molecular interventions points of these approved active pharmaceutical ingredients (API, Supplemental Table 1)? Were these 394 active ingredients

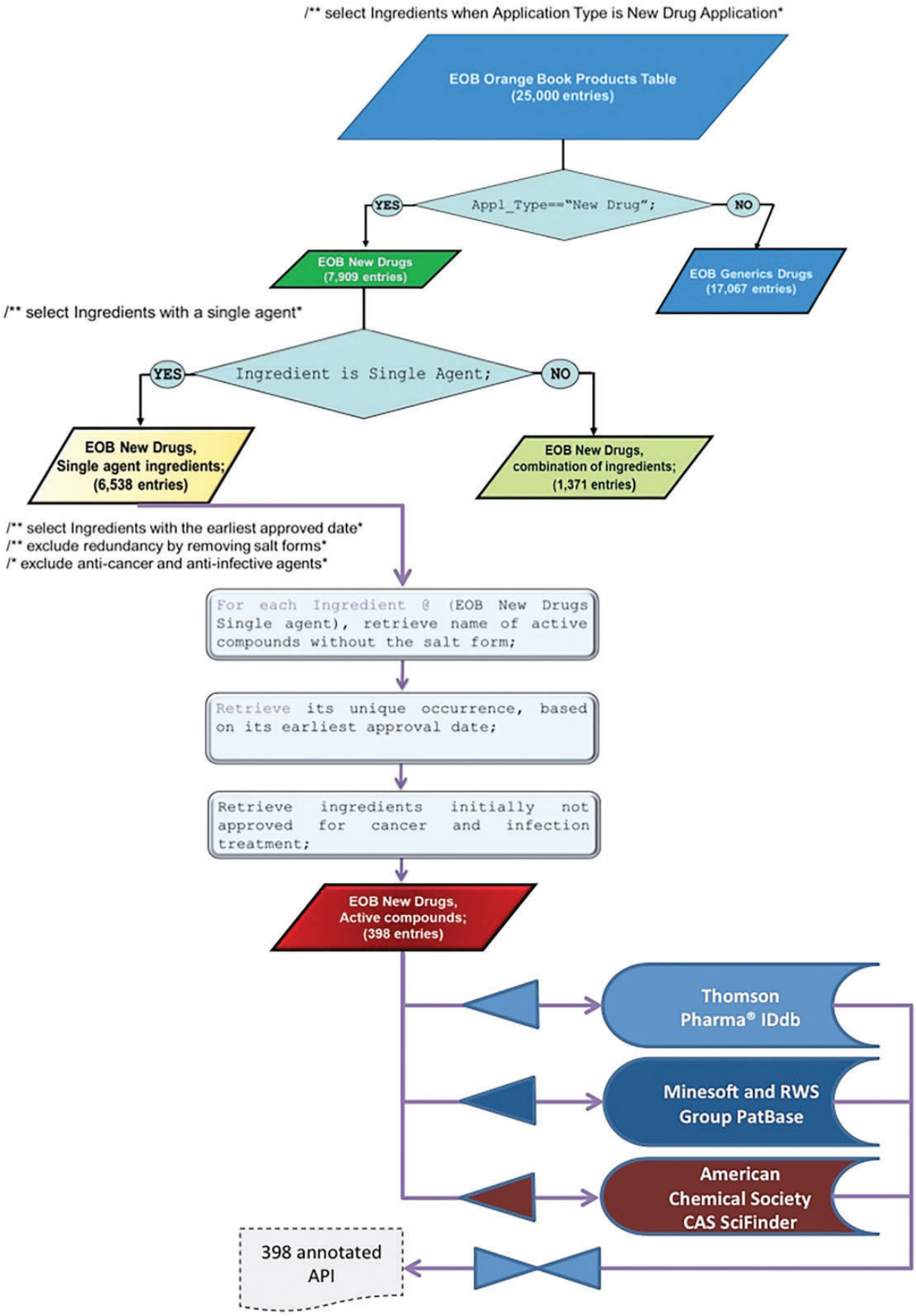

Figure 1. Workflow applied to retrieve the actual set of 398 small molecules active pharmaceutical ingredients approved by the US Food and Drug Administration and prescribed for the treatment of all indications but anti-infective and anti-cancer. (A color version of this figure is available in the online journal.)

initially discovered through *in vitro* screening performed against an isolated target protein? The answers to these questions, as well as other related questions, were not found in the US FDA Orange Book database because it does not mention targets. Hence, the next step was tracing back to the origin of these ingredients.

The study began by searching for primary events that led to the finding of the agent's therapeutic potential. This search was the most time-consuming part of the study, as this information, to our knowledge, is not recorded in any database. The following databases were queried for each ingredient: Thomson Reuters Thomson Pharma® IDdb, DrugBank, Minesoft and RWS Group PatBase, American Chemical Society CAS SciFinder (includes patents), NIH National Center for Biotechnology Information, and U.S. National Library of Medicine (PubMed).

In vitro screening vs. *ex-vivo* or *in vivo* medicinal chemistry

Of the 394 active ingredients identified, none were initially discovered by *sensu stricto* biochemical assay of small molecule libraries with an isolated protein. Although this approach is still widely applied in most drug R&D laboratories, it never delivered a marketed drug for any indications other than infections or cancer. The marketed drugs in our focus originated in one of the following ways: (i) treatment of the rare Mendelian disease, an agent replacing or emulating a missing metabolite; (ii) small molecules derived from naturally occurring metabolites of human biology, synthesized with the aim of antagonizing enzymes or receptors; (iii) natural compounds and their derivatives; (iv) early and mid-20th century organic chemistry combined with animal or complex biological assays. The relative fraction of each of these four categories is shown in Figure 2.

Mendelian diseases

Whenever a single genetic variation represents the major contributor to the inception of a disease, it is considered as a Mendelian disease. The products of these variants affect the human physiological network where there is little feedback regulation. They are the "Achilles heel" of our otherwise robust biological composition. One example is hyperammonemia, a condition in which the central metabolite ammonia is not maintained within a healthy homeostatic range. Affected individuals carry loss-of-function mutations in genes encoding for enzymes involved in the urea cycle such as the autosomal recessive N-acetylglutamate synthase 1.[13] This rare metabolic disease can be treated with carglumic acid,[14] an active ingredient derived from N-acetyl-glutamate, which is also low in these patients. Carglumic acid is a xenobiotic small molecule that causes urea metabolism to fluctuate within its normal range; therefore, administration of carglumic acid represents an etiology-based treatment. Absorption of carglumic acid corrects the deficient metabolic pathway in affected patients where it acts as a proxy allosteric activator of the carbamoyl phosphate synthetase 1, in lieu of N-acetyl-glutamate. N-acetyl-glutamate synthase deficient newborns face serious neurological complications that can lead to death in childhood if left untreated. However, children treated with carglumic acid develop normally, marking it as a truly great achievement of medicinal chemistry.

The carglumic acid treatment was conceived due to a deep knowledge of both the biochemical pathways involved in the homeostasis of ammonia and the genetic etiology of the disease. Such an accomplishment could not have occurred 20 years ago, as the medical community did not have access to the wealth of human genetic information available today. This trend will continue to evolve with the unprecedented capability of DNA sequencing that will enable the design of etiologic-based treatments for Mendelian diseases.

Semi-*in vitro* assays

There are instances where the discovery of the active ingredient could be viewed as a mixed approach of *in vivo* and *in vitro* assays, such as ambrisentan, which was approved in 2007 for the treatment of pulmonary hypertension. The initial compounds from that series were selected via a binding assay against a recombinant human endothelin-A receptor.[15] The radioligand binding assay used tested small molecules from a BASF chemical library for their ability to displace the binding of iodine 125 radiolabeled endothelin from human endothelin-A receptor expressed in transgenic Chinese hamster ovary cells. Transfected CHO cells were incubated with an excess of various tagged endothelin compounds that when below a given concentration threshold competed with the endogenous endothelin for the occupancy of the endothelin-A receptor. Endothelins are naturally occurring small paracrine mediators of vasoconstriction. Three human endothelin paralog coding genes, ET-1, ET-2, and ET-3 have been described.

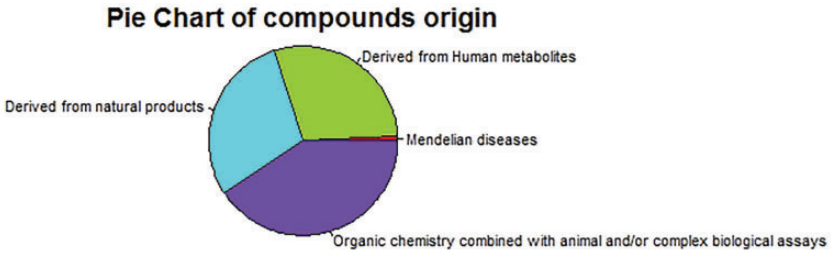

Figure 2. Repartition of the origin of discovery for the 394 active pharmaceutical ingredients approved by the US Food and Drug Administration for all indications but anti-infective and anti-neoplastic agents as of spring 2010. (A color version of this figure is available in the online journal.)

Hartmut Riechers and his colleagues at the BASF laboratory in Ludwigshafen, Germany discovered the compound series that led to the development of ambrisentan. They were able to successfully apply the paradigm of testing a chemical library in a cellular extract assay. They had a physiological intervention point, the human endothelin-A receptor, an indication, hypertension, and applied the complex assay to unravel a small molecule that could antagonize the naturally occurring peptide hormone.

Another example is aprepitant, which was approved in 2006 for the treatment of emesis, pain, and overactive bladder.[16] Aprepitant shares similarities with ambrisentan with respect to its molecular mode of action and discovery. Aprepitant is a small molecule that competes with a peptide hormone for the binding of a receptor called neurokinin 1 receptor. The primary compound selection process tested small molecules from a chemical library to find those that could displace the natural neuropeptide ligand, substance P, from the neurokinin 1 receptor. The assay was performed with bovine caudate membranes and radiolabeled substance P.

In the early 1990s, Pfizer scientists at the Groton CT USA laboratory used the same cell extract system to identify small molecules harboring peptide-mimetic activities. This type of assay was used to uncover the binding of a small molecule to a large proteinaceous entity. Although there was no biological readout in such assays, these assays were run with the target receptor, the endothelin-A receptor, and the neurokinin 1 receptor, both members of the G-protein-coupled receptor (GPCR) family. In addition, these displacement assays mimic the expected physiological situation where the xenobiotic small molecule has to compete with the naturally occurring ligand with the aim of counteracting its effect and thus delivering the expected physiological response. GPCR proteins in their respective cellular membrane environment are actually a set of various conformers. In addition, the lipid and other membrane components, as well as the natural ligand itself, have an effect on receptor activity. These cellular extracts assays can be viewed as semi-complex biological assays. They are not used to test compounds that could alter the steady state level of any given biological activity; however, they feature some level of cellular complexity with regard to the molecular environment in which a given therapeutic intervention point resides.

These cell and tissue extract assays were also used with more complex readouts. One such case leads to the development of two widely prescribed anti-epileptic and anti-neuropathic pain agents, gabapentin and its derivative pregabalin. Brain tissues from animal models were used to design assays aimed at measuring the effect of small molecules on the reuptake of neurotransmitters.[17] Gabapentin, which was designed from the naturally occurring neurotransmitter gamma-aminobutyric acid (GABA) was aimed at emulating some of GABA's physiological effects and was developed using an isolated brain slice preparation and electrographic events as endpoints.

Other examples of active ingredients that are either direct copies of naturally occurring metabolites or their analogs include acamprosate for the treatment of alcoholism, atomoxetine (derived from catecholamine and prescribed for the treatment of Alzheimer's disease, generalized anxiety disorder, and schizophrenia), and alglucerase for the treatment of Gaucher's disease.

Drugs derived from mid-20th-century organic chemistry

The vast majority of the remaining APIs originate from 19th and early 20th-century medicinal chemistry. These include opioids, sulfanilamide, adrenotropic receptors, beta-blockers, beta-agonists, benzodiazepines, and neurotransmitter uptake inhibitors.[18] What emerged from this comprehensive data mining was that medicinal chemists were developing a series of active ingredients from this small toolkit. They ran them through complex biological assays, ranging from cell extracts and tissues (e.g. smooth muscle assays) to animal models, as part of an effort to amend the pharmacokinetics and potential therapeutic properties of these compounds. Therefore, currently marketed drugs were discovered by creating variations on a theme. Previous generations of medicinal chemists acknowledged the fact that the rate-limiting step for developing new medicines is the primary observation that a given compound has biological activity. The whole discipline of drug discovery actually inherited centuries of empirical discoveries in botany and apothecary, and once the molecular structure of the alkaloids that could trigger some physiological effects were identified, the medicinal chemists' laboratories designed a series of molecules.

A prototypical case is the expansion of phenothiazines (also known as tricyclic agents) that are widely prescribed for a range of psychiatric indications. Neurologists designed complex biological assays with clinically relevant endpoints, such as the uptake of monoamine neurotransmitters. In the late 1950s, reserpine, a plant alkaloid, was extracted from the root of a plant growing in India that was known by locals to have neurological effects.[19] Subsections of the complex molecular structure of reserpine were chemically synthesized in laboratories involved in fine chemistry for dyes and non-therapeutic-related objectives.

Medicinal chemists had possessed a series of complex alkaloids from plants that became the source of their future discoveries. These plant-derived compounds usually have either a narrow therapeutic index or poor pharmacodynamic properties. One notable effort of these multiple contributions to psychiatric drug discovery originated in the Janssen Laboratories in Beerse, Belgium.[20] New series were derived that departed from the usual tricyclic backbone of traditional phenothiazines.[18] From these new series, haloperidol emerged and performed better with respect to some physiological endpoints than the standard of care at the time, the prototypical tricyclic agent chlorpromazine. Preclinical assays and preliminary clinical results showed that haloperidol would benefit patients with psychiatric and neurological disorders. Pimozide was derived from the original haloperidol compound and was approved for the treatment of schizophrenia in 1984.[21] The derivation of Pimozide was followed by a series of structural analogs: risperidone, iloperidone, paliperidone, ziprasidone, and

aripiprazole, all of which were subsequently approved for the same indication.

Another example of this mode of discovery is the development of sumatriptan and all its derivatives: rizatriptan, naratriptan, frovatriptan, zolmitriptan, almotriptan, and eletriptan.[22] The objective was to design compounds that would limit vasoconstriction given that vasoconstriction of brain vessels is believed to cause migraines. It is well known from traditional medicinal chemistry that ergot alkaloids produced by a certain fungus affect the tonicity of vascular smooth muscle.[23] Equipped with *in vivo* assays testing vasoconstriction and using as positive controls the naturally occurring ergots alkaloids, the derivatives were successfully synthesized and displayed improved pharmacokinetics and physiological effects.

In the critically important indication of anti-anginal, ranolazine is an example of a series derived from the piperazine scaffold tested on canine models of myocardial conduction.[24] In the case of memantine, approved in 2005 for the treatment of neuropathic pain and schizophrenia, the history of this agent started in the early 1960s in Eli Lilly's laboratory. The development program started with the synthesis of sulfonylurea derivatives with the aim of developing hypoglycemic agents. Preclinical results suggested that this class of agents has significant central nervous system effects, notably acting on N-methyl-D-aspartate receptor channels.[25]

The following final paragraph of this section briefly reports the initial event that led to the discovery of five major modern medicines:

- The immune depressant sirolimus (also known as rapamycin) originated as an antibiotic agent extracted from soil bacteria extract from Easter Islands.[26]
- Sildenafil was developed to mimic cGMP, which acts on smooth muscle tonicity. Various "cGMP-like" molecules were assayed in physiological tests and lead to the development of Viagra.[27]
- Statins were originally derived from evaluating anti-cholesterogenesis activities of compounds produced by microorganisms.[28]
- Clopidogrel (Plavix), approved in 2007 for the treatment of thromboembolism, originated from thienopyridines that had been described by Nakanishi *et al.*[29] for having anti-inflammatory properties.
- Varenicline prescribed for nicotine dependence is a derivative of a plant alkaloid, namely cytisine. Cytisine natural compound was extracted from *Laburnum anagyroides* 40 years ago and was described to have neurological effects.[30] The genealogy of this aforementioned list of six agents (memantine, sirolimus sildenafil, statins, clopidogrel, and varenicline) provides prototypical cases of how these 394 agents were originally discovered. The striking conclusion is that none of them were derived from the drug discovery method widely used in both the industry and academics institutions for the last 20 years.

Two outliers

There are two instances of drugs derived from a compound selected *in vitro* in a biochemical assay with an isolated form of a gene product. These compounds are maraviroc and imatinib, an anti-retrovirus[31] and anti-cancer agent,[32] respectively. These instances are not considered because maraviroc and imatinib are for anti-viral and anti-cancer indications.

Discussion

Based on the precedence of empirical approaches, it is possible to modulate the steady state level of cellular and even physiological quantities with the proper use of xenobiotic compounds (e.g. metformin for the treatment of diabetes mellitus).[33] Therefore, it is possible to "control" living systems at the biochemical level, as opposed to genetic engineering that alters the system's genetic constitution. In addition to antibiotics and anti-neoplastic agents, small-molecule-based therapeutics represents invaluable therapeutic tools among medical options to treat or mitigate either life-threatening or debilitating conditions. Conceptually, these active ingredients interfere with human physiology in such a way that they alter its course. Drug R&D could theoretically adopt an engineering approach provided that the whole set of components that comprise a human system, including all macromolecules and metabolites, would be known along with the interactions between these components. Despite continuous progress in our understanding of biological systems, the life sciences and medical research may not anytime soon identify even a modest fraction of the molecular events occurring in a single prokaryotic cell. This precludes the application of an engineering approach where one needs to be able to quantitate an entire set of variables for a given system to plan which action needs to be adopted to modify it. Hence, Drug R&D is left with the discovery approach where a collection of molecular agents are assayed with a test that aims to predict their potential therapeutic properties. The pioneering work of Paul Ehrlich and his successors[12] showed that xenobiotic agents interact with proteinaceous components, and some of these interactions contributed to the physiological outcome. Whenever DNA recombinant technology and biochemical assays became available, it became tempting for the drug R&D organizations to set up chemical library screening against isolated proteins. This approach looked promising because it allows screening of a very large amount of small molecule libraries in a cost-effective and high-throughput manner. Since the 1990s, drug discovery applies a well-defined recipe, which primarily consists of identifying a target or molecular intervention point. Once a given gene has been deemed qualified as a target based on a series of direct or indirect evidence, the next steps include cloning the gene, producing its cognate polypeptide in mass in a protein expression system, and design a biochemical assay where one activity of that protein can be tested. Chemical libraries are assayed: small molecules that, in the conditions of the test, reproducibly and at low concentration affect the activity of the "target" protein follow a series of chemical development

primarily aiming at optimizing their biochemical property. As shown in this study, this approach never delivered clinically valuable agents for indications other than anti-neoplastic and anti-viral areas. In addition, small molecules discovered by means of the *in-vitro* approach paradigm are usually unsafe based on their so-called off-target multiple molecular interactions. Not a single drug on the market today, with the exception of maraviroc and imatinib, originated from an *in vitro* screening on isolated targets. This approach has been conducted for more than 20 years, and there is no reason to believe it will ever deliver a valuable medicinal agent. Retrospectively, there are many reasons why this approach did not and will not deliver.

Reason #1

There are more proteins than genes. There is not a one-to-one relationship between the human genome and its proteome. There is a one-to-many. Beyond splice variation, a newly synthesized polypeptide undergoes several post-translational modifications during its history within the cell. Proteins are highly dynamic entities existing as a set of multiple conformers. Basic proteomic measurements showed that there are at least three logs of additional complexity between the set of genes and its cognate set of proteins.[34] This level of complexity is completely ignored in *in vitro* assays. It is very likely that in the course of all high-throughput screenings run in our industry over the last 20 years, some were run against protein forms that do not occur naturally in human physiology.

Reason #2

Living organisms including humans do not exclusively constitute proteins. A set of complex macromolecules, such as lipids, carbohydrates, nucleic acids, and even complex metabolites, are ignored by the rational drug discovery approach. The industry focuses on what can be manipulated *in vitro*, hence missing a range of biologically active molecules that could interact with xenobiotic agents and deliver a therapeutic response.

Reason #3

Evidence that a small molecule can trigger a change in the activity of a given protein *in vitro* is not evidence that the same agent will cause the same event *in vivo*. The small molecule could simply be unable to reach its targets in the cell for several reasons: (i) unstable within the biological media, and in the cell if the target is intra-cellular; (ii) interact with many other macromolecules including other proteins such that its actual free soluble form is actually significantly lower than measured.

Reason #4

Complex diseases, as opposed to rare Mendelian genetic diseases, are multifactorial by definition. They occur as a result of a series of environmental insults occurring within the context of a given genetic makeup. They are not caused by the variation of a single effector variable. These syndromes, such as diabetes mellitus, neurodegenerative diseases, and inflammatory diseases, are multi-causative events and determined by a change in more than one genetic or environmental effector. Therefore, the main and irredeemable flaw of the target concept for the discovery of therapies that treat complex diseases is to contemplate the possibility of altering a multifactorial system by acting on one of its components. The present comprehensive study of physiologically active ingredients confirms this later point in that most active ingredients deliver their therapeutic effect by acting on multiple nodes of the human physiological system. Current standards of care such as metformin, pregabalin, and paliperidone for the treatment of diabetes type II, chronic pain, and schizophrenia, respectively, deliver their clinical effect by a partially unknown molecular mechanism.

Our human physiological system regulates its homeostasis with short-term and long-term adjustments. Short-term adjustments occur in response to sudden changes in the value of external variables, such as during food intake or during stress. Long-term adjustments set the level of multiple components in a complex physiological system that is applicable to the current environment. For example, if at the end of a long winter a light-skinned individual exposes their skin to bright sunlight for long, the individual will trigger an inflammatory reaction aimed at protecting the damaged skin. After a series of moderate exposures to sunlight, melanin pigmentation increases as its synthesis has been gradually up-regulated and will provide a long-term protection for the skin cells and underlying tissue. Short-term reactions to acute stress or sudden changes are controlled primarily by simple hormonal and paracrine systems. This acute regulatory system of the human physiological systems is the best known of the two physiological regulations level and has been widely used for designing drugs. The concept is fairly straightforward, once the ligand and receptors involved in a given acute physiological event have been described, the objective is to design a mimetic compound that will either agonize or antagonize the hormonal signal. With regard to the aforementioned example of the short-term and long-term adjustments of the skin cells to a change in an environmental condition (UV light exposure), the short-term fix would be to antagonize the effect of inflammatory cytokines, knowing that excessive inflammatory reaction is actually more harmful. The design of a treatment, which would prevent skin injury, would consist of promoting the production of melanin prior to the inflammatory event. In that regard, it is fair to claim that most drugs currently prescribed for the management of cardiovascular, inflammatory, and psychological disorders are palliative solutions: they act on the acute short-term physiological components anytime the combination of internal and environmental conditions perturb the system and affect its homeostasis. These treatments do not address the etiology of the condition; they leverage the short-term hormonal and neurotransmission systems in the human body to maintain the system within its range. Whenever the root cause of these disorders remains, these palliative treatments eventually become ineffective. In the sunlight exposure example, the skin injury could be prevented by a combination of lowering the exposure to the

environmental insult and/or the administration of a xenobiotic agent that will promote a greater production of melanin.

The history of drug discovery has shown that complex and usually (but not exclusively) naturally occurring compounds are small molecules with etiological curative properties.[35] These molecules do not interact with the acute short-term physiological systems. They perturb the cellular network of the tissue they target in such a way that they promote a long-term regulation. Recent reports suggest that this new approach combining cell biology, genomics, and medicinal chemistry would allow the discovery of biologically active and clinically valuable compounds.[36–39] These cases prove that a systems biology approach, combining complex biological assays (e.g. cell-based assays) featuring multiple endpoints (i.e. multivariate assays as opposed to the *in vitro* univariate approach) represents a reasonable alternative for rebooting the drug discovery paradigm.

DECLARATION OF CONFLICTING INTERESTS

The author does not have any conflict of interest in connexion to the manuscript. The author has full access to all study data, takes full responsibility for the accuracy of the data analysis, and has authority over manuscript preparation and decisions to submit the manuscript for publication and control its publication.

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