



Overview of the Advancement in the Drug Discovery and Contribution in the Drug Development Process

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The drug discovery and development of a new chemical entity from original idea to the regulatory approval for launch into the market is a very long and complex journey. The process is complex and can take between 10-15 years, with a significant financial implication of cost of about \$1 billion and more. The bioactive molecules as lead compounds may be derived from several sources such as plant, animal sources or as synthetic compounds. The odyssey of drug discovery/development may take several years elucidate valid evidence-based information from data mining, before a target selection for the progression of the drug discovery platform. The successful selection of identified drug target is an important objective for many Pharmaceutical industries before laying down a platform for research and development on hit target identification of compounds with promising biological and pharmacological activities with the potential to produce a good drug. The objective of this review paper is to develop a comprehensive review on the odyssey of drug discovery and development from the research and development standpoint with special bias within the frame of

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the preclinical level, hit and lead compound identification, optimization and validation, using different high throughput assay development platform. The consideration of a potential active pharmaceutical product necessitates a good test battery for hit identification, lead optimization a good process for candidate selection of a candidate compound for drug development and putting into consideration the regulatory compliance guideline for new drug approval before the attain the market and subsequent post marketing surveillance process.

Keywords: Drug discovery/development; hit; lead target identification; new chemical entity; high throughput screening; assay development.

1. INTRODUCTION

The drug discovery process take place once the five-challenging question for drug discovery and development has been answered. These questions are; Is there a medical need to develop new drugs? Do we have information on the prevalence of the disease? What is the market potential or pharmacoeconomic potential of producing the drug? Do we have a biochemical target as shown by receptor pharmacology studies? Can the bioactive molecule be successfully synthesized that have potential for target selectivity, are potent *in vivo*, and good bioavailability? [1] Are the potential compounds have therapeutic potential/ efficacious in disease models, can show dose response relationship, and are less toxic in test models? Is there a disease or any clinical condition with no known drugs or the drugs do not meet the treatment conditions [1-3]? Any drug product or new chemical entity (NCE) is a bioactive compound, other than food, that is used for treatment, prevention and diagnosis or symptoms relief of a disease or abnormal condition [1,3]. A drug can also be considered as a substance with the potential to alter the mood or body function, or can be habit-forming addictive in nature particularly the narcotics [1-4].

Several research and development reported by the Pharma industries and research institutions have pool together data from developing a hypothesis which indicates that the inhibition or activation of a protein or pathway can lead to a therapeutic effect under a disease condition [1,2]. The therapeutic endpoint of this activity may lead to a selection of different drug targets which may need more validation before advancement is made into the lead compound discovery phase which serves as justification for the drug discovery effort [1,2]. During the lead discovery phase, there is an interest in an intensive data mining leading to the identification of a drug-like small molecule also known as bio-active therapeutic agent, or the candidate alert notice (CAN), that has the potential to advance into the

preclinical phase of discovery, and if successful, can progress into the clinical trial phase, followed by regulatory authority approval, patenting and marketed medicine [1,5].

2. TYPES OF PHARMACEUTICAL COMPANIES

There are different types of pharma companies involved in drug discovery and development. They include:

- ✓ **Pharmaceutical Drug Discovery /Development:** They take drug discovery from hit, lead optimization to the drug development phase and marketed product. This category of company includes examples like Pfizer, GlaxoSmithKline, Merck Sharp, Novartis, AstraZeneca [5].
- ✓ **The Pharmaceutical Drug Delivery companies:** They take the lead compound from different sources to development and market. Most generic drugs are produced by these companies like Élan Corporation, Alza Corporation.
- ✓ **The Biotech-Pharmaceuticals:** They develop biotechnology tools, methods, devices and gene targets for the drug development integration. They usually collaborate with other pharma companies or go into merger. Examples include Genentech, Amgen.
- ✓ **Contract Research Organization (CRO):** They are not directly involved in the drug development process, but are in charge of running and conducting clinical trials for the pharma companies [5].

2.1 Major Challenges of Pharmaceutical Companies

The major challenges faced by Pharma companies are many as elaborated as follows;

Time and money-The drug development is a long and challenging process that can take between 10-15 years [6] taking into consideration developing a new compound and getting regulatory approval for launching into the market [2]. Other challenges are competition, research and development spending, patent life of approved drugs, price controls and pharmacoeconomic potentials, government legislation, regulatory compliance requirements, managed health care, Cost of new enabling technology, Management of alliances and biotech venture [5-8]. "The discovery of new drugs became a science since the Neanderthals era when the people of Mesopotamia, Egypt, Greece and China practice started using herbal products to treat different illnesses" [2,5]. During the mediaeval periods the administration of *elixir* was popular and well developed by alchemists. The scientists in the past 100-150 years have made Significant progress in drug discovery thanks to a more structured and well-designed laboratory-based drug discoveries of new medicines that have been used for the treatment and survival of millions of populations [5,6]. "The fall of the German stock exchange in 1873 gave rise to a recovery period that was accompanied with an economic boom, leading to the birth of industrial revolution, and subsequently, an expansion of chemical and electrical industries" [2,3,7]. "The stimulated interest of huge investment of German industries in the manufacture of synthetic dyes was what gave Germany the top position ahead of all its competitors in modern pharmaceutical technology. The German chemists developed not only to become very powerful in the field of organic chemistry, but also promoted an increase in the growth of the German pharmaceutical industry" [8].

"The leading and emerging German industries were the manufacturers F. Bayer & Company and Farbenfabriken Hoechst who developed a shift in paradigm when their chemists discovering and developing dyes had the potential to produce new medicines" [2,9]. "One of the Germans' greatest early scientists was Paul Ehrlich, who was the initiator of the research on colorful dyes and their capacity to interact with histological and cellular structures" [2,4,10]. Ehrlich through his long research over many decades, later received grants from many chemical companies who were interested in funding new dyes research. Through his discovering that dyes were biologically active, a number of compounds were isolated and

evaluated through high-throughput screening (HTS), currently still in use today in research institutions/academia and chemical industries [11]. "Ehrlich discovered that, the biological potential of a chemical compound such as a dye depended on its chemical composition and the cell on which it acts" [12]. Ehrlich successfully demonstrated the relationship between chemistry, biology and medicine He also postulated that chemical dyes were the catalyst contributing to the great pharmaceutical revolutionary advancement. [13]. "Ehrlich was also inspired by other scientists like Louis Pasteur, Robert Koch, Emil von Behring and Shibasaburo Kitasato active researchers in the field of microbiology, chemistry and immunology" [2]. "In the last part of the 20th century, Ehrlich discovered the receptor theory, which became an important key instrument leading to the understanding of the mechanism of drugs receptors binding, based on the structural differences in chemical compositions" [14,31]. "Ehrlich's research in therapeutic areas for the treatment of infectious diseases with drugs derived from the German dye industry motivated the development of different ways of using organic chemistry to modify certain starting dyes use in finding new chemical structures with promising biological activity" [13,15]. "Ehrlich is considered as the founder of chemotherapy and the theory of his '*magic bullet*' is applicable in modern science often used by scientists when developing small molecules that attack pathogens but remain avirulent to healthy tissues" [9]. "During the two World Wars, essential medicines that was supplied by Germany became very scarce and led to gradual shift towards the exploitation of synthetic drugs" [2,9].

3. SYNTHETIC ORGANIC CHEMISTRY

"Synthetic organic chemistry is an exceptionally important discipline and play a key role in drug discovery" [16]. "It is very adaptable and integrate innovative techniques used in drug discovery. Much early synthetic drug discovery was targets for cancer drug development and was derived from an observation that mustard gas, used in chemical warfare during World Wars I and II, destroyed lymphatic tissue and the formation of bone marrow. The reports of Dr. Gilman, Goodman and collaborator was the pioneer study and led to laying the foundation for conducting the first clinical trials using nitrogen mustards (β chloroethylamines) in 1942 at Yale-New Haven Hospital, USA. The outcome of the

clinical trials was only reported four years later, due to the secrecy and cold war between superpowers during the World War II" [17,18]. A series of DNA alkylating agents were developed which led to an increased understanding of DNA and recombinant technology in the 1950s.

"Many bioactive metabolites have been discovered, such as the *Vinca* alkaloids and purine/pyrimidine synthesis inhibitors [2,19], mainly sponsored by stakeholders like the National Cancer Institute (NCI)". "Such studies have led to the evaluation and understanding of primarily cytotoxic compounds. In the early 1970s, the significance of natural product-based early drug discovery has understood and achievement gave rise to the elucidation of many phytochemicals" [20]. "The *de novo* synthesis of many of promising novel agents was a lead to compounds that was initially too complex and too expensive to allow progression into early stages of clinical trials. New synthesis improvement has caused a paradigm shift from enhancing natural product screening through the stage of discovery initiatives, providing an opportunity for the identification of natural products as potential lead compounds" [17,21]. These lead compounds were subsequently tested for pharmacological activity and safety.

"The recent advancement in organic chemistry have led to the complete synthesis of many complex natural products, that has significantly improved the methods with which chemists can now deal with the complexity of many of these naturally-derived metabolites" [2,22]. "Synthetic chemistry has also contributed in the development of drug delivery and prodrug strategies, by focusing on the development of selective therapeutics with reduced side-effect" [23]. Although research in cancer has been the focus of many synthetic drug discovery, this was conducted in collaboration with research in other therapeutic disease areas as has been shown in Fig. 1, indicating an elaborate chronology of the drug innovation processes.

"With the onset of the genomics era and the study and understanding of events at the molecular level there has been a change in the landscape of drug discovery [35]. Data mining have revealed that the generation of many data has caused not only to speculate on an expanding druggable genome, but also to give new opportunities for moving drug discovery to a higher level" [2,23]. "The number of potential genes/receptors that are targets for existing drugs has been a topic for discussion and that

also depends on the analysis performed. However, a valid estimate for number of gene products as potential drug target is in the region of 300-500" [5,7,24]. "The number of drug targets is likely to increase as the human genome is estimated to encode 20,000-25,000 human gene products, although it will take quite some time to validate drug targets at the protein level, with an added level of complexity. Both gene and protein expression profiling methodologies have been developed although with major challenges in the last two decades with these methods used to monitor and catalogue changes in the expression of genes and their respective protein products" [25]. "More priority has been put towards the interest of understanding of the human disease at the molecular level than to the elucidation of changes in biochemical processes associated with disease phenotypes" [2].

The drug discovery objective is focused on the generation of identifiable therapeutic targets that can reduce the drug development attrition [6]. "The mapping of the human genome was a great breakthrough for the scientists working at the interface of chemistry and biology in drug discovery, exploiting the use of the data available for the discovery of new blockbuster drugs. The geometric increase in the cost of drug discovery poses a major hindrance for Research and development, especially during period of great recession. The question always asked is can research and development (R&D) in the emerging markets creates opportunities on how to progress successful research to the level of developing good and blockbuster drugs" [2,7].

4. WHY DO WE DISCOVER NEW DRUGS?

Scientists are in constant research for potential new drugs due to the following reasons; There has been an increased discovery in the past half a century of large therapeutic bioactive molecules from natural products, and the increase of many pharmaceutical companies. Many academic and research institutions are on the increase with much progress in the understanding of disease aetiology and pathophysiology. The mechanism of drug actions and pharmacokinetics is well understood for many therapeutic areas [3,15]. Most current disease treatment algorithms only lead to symptom relief with reported drug adverse effects in some cases. The problem of increase in drug resistance and tolerance, low efficacious drugs against pathogenic invasions in cases of (Tuberculosis, HIV malaria are well documented.

The changing lifestyle and increase in life span have created life style disease, and changing social attitudes have created more market for “lifestyle drugs”. There is the need to discover new treatment for old and evolving diseases as the old molecules are not meeting the therapeutic requirements like in the case of current medicines for HIV, Diabetes, Cancer and other metabolic diseases. The elucidation of the complete human genome, significant progress in molecular biology, global knowledge in the domain of ‘OMICs’ & protein engineering has given rise to better understanding of disease mechanism, biochemical pathways, pharmacogenomics and pharmacokinetics in the understanding of new drugs [15,25-27].

5. THE EVOLUTION OF MODERN DRUG DISCOVERY

“At the beginning of the 20th century, drug discovery was championed mostly by a few outstanding scientists such as Paul Ehrlich and his collaborators. The strategy in the new era necessitate a multidisciplinary collaboration of various disciplines such as chemistry, computational modelling, structural biology and pharmacology” [2,13]. The information from data base and accessible literature on specific disease or drug target is now used by researchers to make decision on what intervention are suitable and efficacious for therapeutic benefit. The exact nature of how discovery research can progress depends on the resources available: for example. Small research institution consisting of academic team may not be financially demanding as a large pharmaceutical company in terms of how to manage the problems of validating a novel target or developing ‘hit’ and ‘lead’ compounds specific to modulate a drug target [28]. The drug discovery process is illustrated in Fig. 2, which is an approximate model which is used by pharmaceutical companies. However, small biotech company or university research can also engage through multiple collaborations to source funding and grant for research. The drug discovery process can be initiated at various stages and capable of bringing about the results necessary to advance a project to a higher level.

“As far back as the mid-80s, drug discovery was geared towards the isolation of natural products and medicinal chemistry was the main area for a research team to find more potent and selective compounds superior to the natural product or synthetic compound themselves. After isolation and characterization of the natural products,

structure-activity relationship (SAR) studies were and still are very important tool in optimizing a pharmacophore” [29]. “Initially, a drug design process was an important course of action between the synthesis of new chemical entities by a synthetic/medicinal chemist and the screening of the compound for biological activity by a pharmacologist” [2]. “The drug discovery process was chemistry-oriented rather than target driven. The discovery process of a drug now involves a multidisciplinary effort that is synergistic, which requires a high throughput screening (HTS) procedures. This research is also based on empirical findings from clinical investigations such as Lipinski’s rule of 5 [9]. Compound selected as hits are progressed into a ‘lead’ compound, which can undergo thorough pharmacological and toxicological testing. The results from Lead testing enable a research team to make decision whether it is cost effective to continue with the progression of a specific project” [6,15].

“Medicinal chemists most often screen virtual or commercial compound to identify hit molecules. These compounds undergo a second stage in order to prepare the compound libraries of small molecules, measure their activity and correlate the results and determine the chemical structure with optimum activity” [12]. “This analysis may make use of the structure activity relationship (SARs), computational chemistry, combinatorial chemistry and enzymatic and cellular assays, to help unravel biological activity derived from unique mechanism of action of a small molecule. The selection of a lead compound and the development of a synthetic pathway for its preparation on a large scale for preclinical and clinical investigations must be considered at an early stage in the discovery process. If the lead compound cannot be synthesized on a large scale, progression to clinical evaluation will not be possible” [2,15]. “Similarly, researchers must also devise suitable *in vitro* and *in vivo* tests to assess the activity and toxicity of the compounds produced. If there is no suitable way of testing a hit or lead molecule *in vivo* the project may come to a halt unless it is decided to spend resources on developing appropriate models” [3,28-30].

“Currently hit and lead compounds with known bio-activity are evaluated for potential testing for phase I and II studies in the early stages of the discovery process. For example, many high throughput screening (HTS) assays are used to detect cytochrome P450 (CYP) substrates or inhibitors, which can reduce the number of drug

attritions of novel drugs from the market due to poor affinity for major CYP metabolizing isozymes” [2,16]. “HTS CYP data can be exploited by medicinal chemist to understand drug-drug interactions at an early stage of the discovery process and in some cases could resolve the issue through a targeted modification of the CYP interacting functionality” [32].

HTS assays have been developed and have enhanced research collaborations by generating large numbers of bioactive molecules with different types of pharmacophore.

5.1 Combinatorial Chemistry in Drug Discovery

“Combinatorial chemistry (combiChem) pioneer application was for the generation of peptide arrays in 1984 and then evolved into a new discipline have now known to have revolutionized drug discovery” [2,33]. “The early generations of combiChem scientists created an impact in the industry by modification of the common use of a number of terminologies, and abbreviations that became widespread in the medicinal chemistry literature such as, deconvolution, diversomer, split-and-mix, multipin, solid phase organic chemistry or synthesis (SPOC or SPOS), submonomer synthesis, Teflon bag (T-bag) etc” [2,17,34]. “The early scientists in the domain of combiChem research required different management solutions than the classical synthetic chemists. For instance, the chemists involved in planning a traditional synthesis for developing target compounds or a natural product usually conducted a retrosynthetic analysis for determination of the best, and

probably the cheapest approach to obtain the drug target. On the other hand, combinatorial chemists generally used forward synthesis strategies in which the building blocks are commercially available and easy to synthesize” [2]. The chemical information systems were also important that can be quickly accessed through the updated databases of inventory and commercially available reagents as useful tools for reagent procurement by the combinatorial chemists.

“As combiChem progressed from solid-phase synthesis to solid-supported synthesis, new synthetic strategies and techniques have also evolved. Some of these synthesis strategies are now well integrated into the drug design process such as the microwave synthesis [2,5,11,36], fluorous synthesis [21], click chemistry [37] and flow reactors” [19]. “Concerning traditional drug design, combiChem relies on organic synthesis methodologies and exploits automation and miniaturization for the synthesis of large libraries of compounds, which can accelerate the drug discovery process. The combinatorial approach is sometimes systematic and repetitive, and uses sets of commercially available chemical reagents to produce a diverse set of molecular entities” [2,6]. The combinatorial approach is very important in the early stage of drug discovery and allows HTS to be used, combining rapid synthesis of chemical compounds to be screened by using both enzymatic and cellular assays for evaluation. The rapid turnaround time of data allows good information flow, which guarantees second and third generation of bioactive compounds to be rapidly generated. CombiChem is involved with both “parallel” synthesis and “split and-mix” synthesis as illustrated in (Fig. 3).

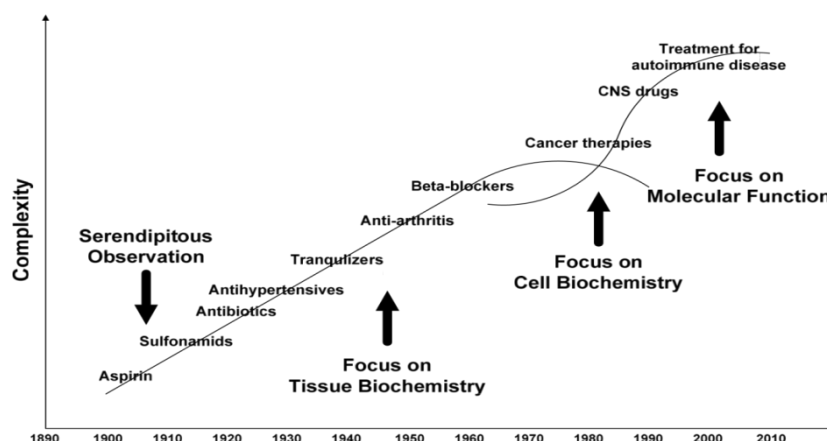


Fig. 1. Chronology of drug innovation process [2]

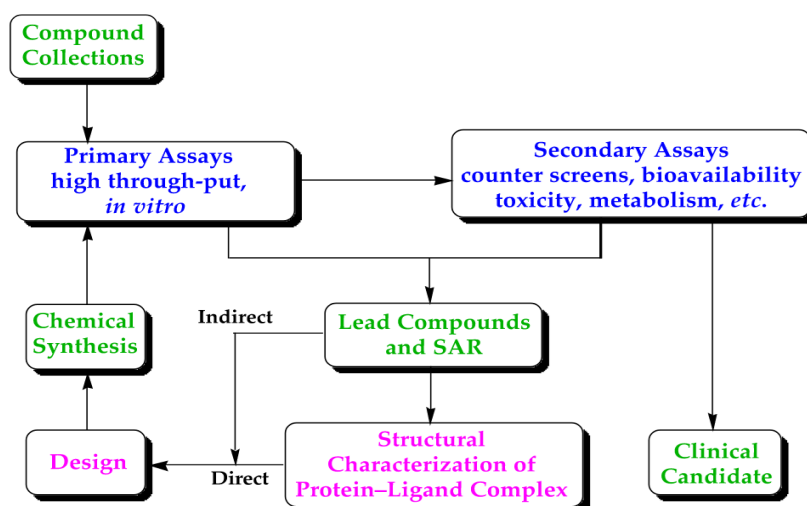


Fig. 2. A flow chat of the drug discovery process in the 21st century [2,5]

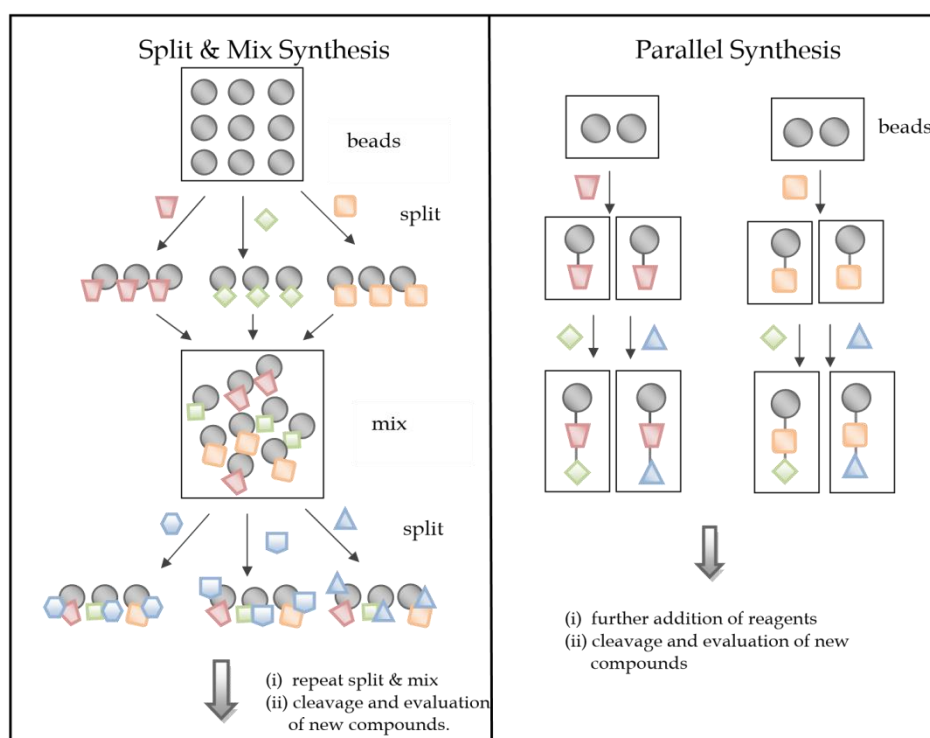


Fig. 3. Combinatorial chemistry approaches. The parallel synthesis is mainly applied in the generation of larger quantities of a small bioactive compound and the split and mix synthesis used in generating smaller quantities of a larger number of bioactive molecules [2]

5.2 Discovery of Small Molecules for Testing of Biological Pathways and Potential New Drug Targets

“The human genome mapping developed for a better and advanced understanding of both the pathophysiology causes and function of biological targets and the development of HTS assays had an objective to produce a higher

number of new chemical entities (NCEs or for medicinal use. Unfortunately, for some reason this has not been the case for many reasons. Computational molecular modelling has provided scientists with an understanding of biochemical activities at the molecular level” [2,37]. “An understanding of the receptor and drug binding process of small molecules to many macromolecules such as DNA is now well

understood. However, the same cannot be said about smaller molecules with other non-receptor drug targets. Many studies are still to be conducted probably due to the lack of interest or the understanding that the so-called “undruggable” proteins can be successfully targeted” [2].

“It has been estimated that only 10–14% of the proteins encoded in the human genome are ‘druggable’ by using the existing ‘drug-like’ molecules [6,38]. However, given the *chemical space*, and depending on the parameters used, the complete set of all possible small molecules, has been calculated to contain 10^{30} – 10^{200} structures [39], there are large number of yet uncovered chemical structures”. “Taking into consideration the limitations of chemical libraries in addressing challenging targets, it is important to recognize that the vast majority of accessible libraries of small molecules are based on existing drugs” [40]. Drugging targets that are recognized as targets by applying the principles such as the Lipinski’s “rule of five”, that have yielded success in the past is a safe territory and that research can be continued effectively.

5.3 Chemical Genetics

“Modern genetics began with the theoretical framework of the nature of inheritance in plants developed by the German-Czech scientist Gregor Mendel in the mid-19th century” [2]. “The science in chemical genetics when compared with genetics is only a couple of decades old, but has gained popularity in recent years. Chemical genetics has very much its origin in classical genetics and has adapted most of the methods and terminology already established” [2,41]. Genetic knockouts have been the principal concept to illustrate biological pathways and causal agents of pathological diseases. Currently, in addition to chemical genetics, the fields of chemical biology and related modern fields have advanced the discovery and development of small molecules and their use as chemical ‘knockdowns’ [12,42]. Chemical genetic principle interphase with most of the experimental sciences and contributes in the understanding of the biological systems through the availability of tools that can be used to disrupt them [43]. “The success to close the genotype-phenotype gap, biological research has evolved beyond genomics, proteomics, and dissection of biological systems into their different constituents” [5,44].

“Protein function is regulated by complex networks of other biomacromolecules, small molecules and supramolecular structures like membranes while genetic manipulation can lead to a permanent alteration of the native structure of the network. Chemical inhibitions can occur with small molecule modulators of protein function providing temporal control using dose-response explorations without necessarily transforming the biological network” [9,45]. “It is very common to use small molecules to inhibit a biological system due to their dynamic nature, which offers many advantages such as: (i) the ability to target a single domain of a multidomain protein, (ii) can allow precise temporal control that is crucial for rapidly activating processes, (iii) can target orthologous or paralogous proteins, and enable comparisons between species or redundant functions, (iv) do does not directly alter the concentrations of a targeted protein, thus avoiding indirect effects on multiprotein complexes” [3,34,46]

“Combination chemical genetics (CCG) is the systematic testing of multiple adverse effects involving chemical probes, which include either chemical combinations or mixed chemical and genetic disturbances Combination chemical genetics can be used to increase the complexity of the test system to show the diseased state of a cell” [47]. “Classical and chemical genetics (Fig. 4) are mainly separated into forward screens, in which uncharacterized perturbors are tested against a selected phenotype for the detection of genes associated with that phenotype, and *reverse* screens, in which a specific gene or protein is modulated for monitoring of multiple phenotypes to determine the effects of that specific drug target” [15,42,48]. “Studies involving combined perturbations can also be classified with the mechanistic focus shifted from individual targets to interactions between targets” [49].

5.4 Chemical Biology

“Significant contribution has been made through chemical biology in drug discovery and has offered new technologies that can improve the understanding of human health” [2]. “By considering the temporal control made by small molecules and the ability to use combinations of small molecule modulators, chemical biology attempts to complement the use of pure biological analysis in studying a wide range of biological systems” [49]. “Chemical biology also attempts to respond to questions in complex test

systems and may provide commercial chemical probes that can be used to probe pathways and elucidate more biological targets" [2,13]. "The discovery of the potency and selective deacetylase inhibitors tubacin and histacin are examples of how important chemical genetics contribute in combination with computational methods such as principal component analysis (PCA)" [50]. "However, good chemical probes for *in vitro* and especially *in vivo* perturbation are not easy obtain due to the fact small molecules are generally pleiotropic and they have multiple dose-dependent molecular targets that are often not fully characterized, and may lead to unexpected activities" [1,20].

"Obstacles and challenges are the same to those in drug discovery and development and small molecules often have inherent problems such as *in vitro* aggregation, poor solubility, difficulty in crossing biological membranes and reactive or toxic effects. Currently, the development of chemical probes for *in vivo* testing may be too ambitious a goal. As a result, evaluation of the effect of chemical 'knockdowns' in clinically relevant tissue should in the near future be in more complex assays that mimic for example malignant tissue" [41].

5.5 Target Identification

"Drugs attrition occurs in the clinical development process for two main reasons; the first is that they are not efficacious and the second is that they are not safe (toxicity). Therefore, one of the most important steps in developing a new drug is target identification and validation" [1,7]. "A target is a broad term which can be applied to a range of biological activities which may include for example proteins, genes and RNA" [51]. "A good target needs to be efficacious, safe, meet clinical and commercial needs and, most especially be 'druggable'. A 'druggable' target is accessible to the identified drug molecule, be it a small molecule or larger biologicals, and upon binding, elicit a biological response which may be measured both *in vitro* and *in vivo*" [7,52]. "It has been shown that some target classes are more responsive to small molecule drug discovery, such as for example, G-protein-coupled receptors (GPCRs), whereas antibodies are good at blocking protein/protein interactions. There are also examples of phenotypes in humans where mutations can nullify or over activate the receptor, for example, the voltage gated sodium channel NaV1.7, both mutations incur a pain phenotype, insensitivity or

oversensitivity respectively" [53]. An alternative approach is to use phenotypic screening to identify disease relevant target. Fig. 5 illustrate the discovery process from target identification and validation up to submission of a compound and the approximate timeline for these processes. Food and Drug Administration (FDA), Investigational New Drug (IND) and New Drug Application (NDA). The drug discovery time line is shown in Fig. 6.

"Extensive literature search for available biomedical data has led to a significant increase in drug target identification. Data mining deals with the use of a bioinformatics approach not only to help in identifying potential disease target, but also in selecting and prioritizing". [54]. "The data can be generated from a variety of sources but can also include publications and patent information, gene expression data, proteomics data, transgenic phenotyping and compound profiling data" [1,12]. "Target identification approaches include examining mRNA/protein levels to determine whether they are expressed in disease and if there are any correlation with disease exacerbation or progression" [1,2]. "Another major approach in target identification is to consider genetic associations, for example, is investigating the link between genetic polymorphism and the risk of disease or disease progression or the polymorphism functional. Clones are individually screened by immunostaining techniques and those that preferentially and strongly stained the malignant cells are chosen. The antigens recognized by those clones were isolated by immunoprecipitation and identified by mass spectroscopy" [55].

5.6 Target Validation

"Once drug targets are identified, the target can be fully exploited for study. Validation techniques can range from *in vitro* tools through the use of whole animal models, to modulation of a desired target in disease model" [1,9]. Fig. 6 shows target identification and validation of multifunctional process.

"Antisense technology is a potentially powerful assay which utilizes RNA-like chemically modified oligonucleotides which are designed to be complimentary to a region of a target mRNA molecule" [4,56]. "Binding of the antisense oligonucleotide to the target mRNA prevents binding of the translational machinery thereby blocking synthesis of the encoded protein. An

example of the power of antisense technology has been demonstrated by researchers at Abbott Laboratories who developed antisense probes for the rat P2X3 receptor” [14]. “When administered by intrathecal minipump, to avoid toxicities associated with bolus injection, the phosphorothioate antisense P2X3 oligonucleotides had marked anti-hyper-analgesic activity in the Complete Freund’s Adjuvant model, an indication of an unambiguous role for this receptor in chronic inflammatory states” [4]. “Interestingly, after the administration of the antisense oligo-nucleotides was discontinued,

receptor function and analgesic responses returned. In contrast to the gene knockout approach, antisense oligonucleotide effects are reversible and the continuous presence of the antisense is required for target protein inhibition” [57]. “The chemistry associated with creating oligonucleotides has resulted to cases of molecules with low bioavailability and observed toxicity, making their *in vivo* testing problematic” [58]. “This has been exacerbated by non-specific actions, problems with controls for these tools and a lack of diversity and variety in selecting suitable nucleotide probes” [57].

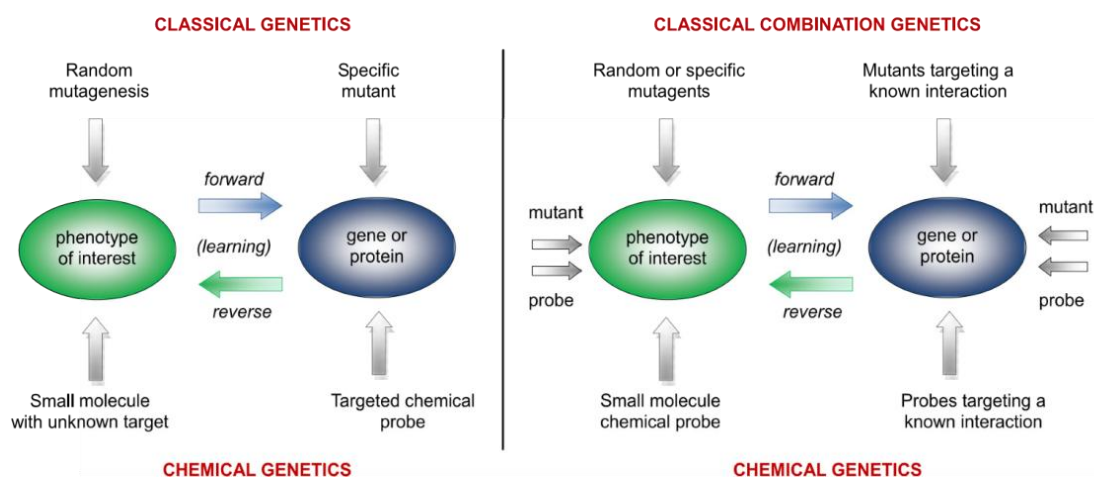


Fig. 4. Combined perturber studied in the context of forward and reverse genetics. The focus of classical and chemical genetics is to explore the functionality of individual genes or proteins. In combination chemical genetics, focuses on the investigations on the interactions between targets or conditional target dependencies, and the perturbations are applied as combinations [2]

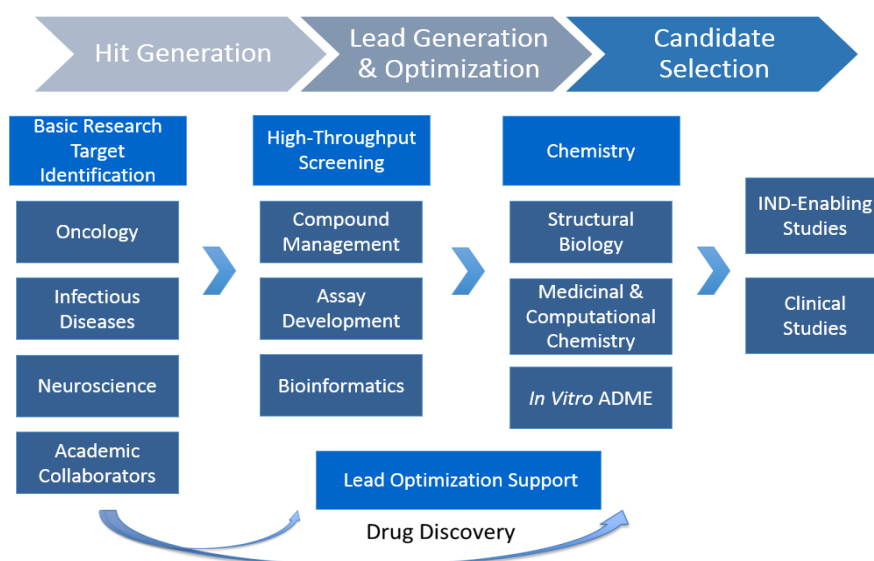


Fig. 5. The drug discovery process from hit generation to lead optimization [55]

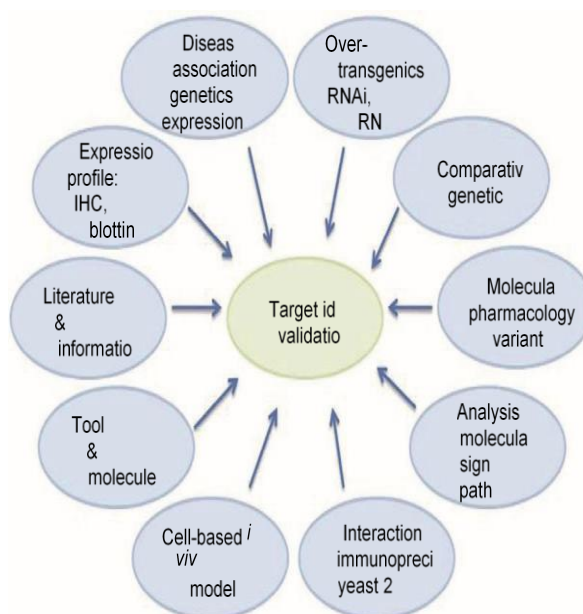


Fig. 6. Target identification and validation of a multifunctional process [2,23]

An alternative to gene knockouts assay is the use of gene knock-ins, where a non-enzymatically functioning protein is used in replacing the endogenous protein [1]. “These animals can have a different phenotype to a knockout, for example when the protein has structural as well as enzymatic functions [58], and these mice should predominantly mimic more closely what happens during treatment with drugs, that is, the protein is there but functionally inhibited” [3,45]. “Currently, the desire to make tissue restricted and/or inducible knockouts has grown. Although these approaches are technically challenging, the most obvious reason for this is the need to overcome embryonic lethality of the homozygous null animals. Other reasons include avoidance of compensatory mechanisms due to chronic absence of a gene-encoded function and avoidance of developmental phenotypes” [1]. However, the use of transgenic animals is generally expensive and time-consuming and therefore in order to circumvent some of these issues, the use of small interfering RNA (siRNA) has become increasingly popular for target validation.

“Double-stranded RNA (dsRNA) specific to the gene to be silenced is introduced into a cell or an organism, where it is recognized as exogenous genetic material and can activate the RNAi pathway. The ribonuclease protein Dicer is activated which binds and cleaves dsRNAs to produce double-stranded fragments of 21–25 base pairs, with a few unpaired overhang bases

on each end” [59]. “These short double-stranded fragments are called small interfering RNA (siRNA). These siRNAs can be separated into single strands and integrated into an active RNA-induced silencing complex (RISC). After integration into the RISC, siRNAs base pair to their target mRNA induce cleavage of the mRNA, thereby preventing it for use as a translation template [60]. The RNAi technology still has the major problem of delivery to the target cell, but many viral and non-viral delivery systems are currently under investigation” [61].

“Monoclonal antibodies are also an excellent target validation tool as they interact with a larger region of the target molecular surface, allowing for a better discrimination between even closely related targets and often providing higher affinity. In contrast, small molecules are disadvantaged by the need to interact with the often more conserved active site of a target, while antibodies can be selected to bind to unique epitopes” [62]. “This particular specificity is the basis for their lack of non-mechanistic (or ‘off-target’) toxicity, which is a major advantage over small-molecule drugs. However, antibodies cannot cross cell membranes restricting the target class mainly to cell surface and secreted proteins. One impressive example of the efficacy of a mAb *in vivo* is that of the function neutralizing anti-TrkA antibody MNAC13, which has been shown to reduce both neuropathic pain and inflammatory hypersensitivity [63], thereby implicating the nerve growth factor (NGF) in the initiation and

maintenance of chronic pain. Finally, the classic target validation tool is the small bioactive molecule that interacts with and functionally modulates effector proteins”.

5.7 The Hit Discovery Process

“Following the target validation process, it is during the hit identification and lead discovery phase of the drug discovery process that compound screening assays are developed” [1]. A ‘hit’ molecule can have different meaning for different researchers but a hit is a compound which has the desired activity in a compound screen and whose activity is confirmed upon retesting [3,64]. “A variety of screening paradigms has been developed to identify hit molecules as shown in Fig. 7. High throughput screening (HTS) involves the screening of the entire compound library directly against the drug target or using a more complex assay system, such as a cell-based assay, whose activity depend on the target but which may require secondary assays to confirm the site of action of compounds” [17,65]. This screening paradigm involves the use of complex laboratory automation but does not assume any prior knowledge of the nature of the chemotype likely to have activity at the target protein.

“Focused or knowledge-based screening concerns the selection from the chemical library smaller subsets of molecules that have potential activity at the target protein, based on knowledge of the target protein and literature or patent precedents for the chemical classes likely to have activity at the drug target” [66]. “This type of knowledge has led to early discovery paradigms using pharmacophores and molecular modelling to conduct virtual screens of compound databases” [67]. “Fragment screening involves the generation of very small molecular weight compound libraries which are screened at high concentrations and which typically lead to the generation of protein structures to enable compound progression” [68].

5.7.1 Physiological screening.

“This is a tissue-based approach which is based on a method to identify molecules of interest. The output of a compound screen is typically termed a hit molecule, which has been demonstrated to have specific activity at the target protein” [3, 69]. “Screening of hits form the basis of a lead optimization chemistry programme to increase potency of the chemical series at the primary drug target protein. During the lead discovery

phase molecules are also screened in cell-based assays predictive of the disease state and in animal models of disease to characterize both the efficacy of the compound and its likely safety profile” [3].

5.8 Assay Development

“In the recombinant technology era the most assays in the industry depend on the creation of stable mammalian cell lines over-expressing the target of interest or upon the overexpression and purification of recombinant protein to establish the biochemical assays” [10]. “However, in recent years there has been an increase in the number of reports describing the use of primary cell systems for compound screening” [70]. “Generally, cell-based assays have been applied to target classes such as membrane receptors, ion channels and nuclear receptors and typically generate a functional read-out as a consequence of compound activity” [71]. “On the contrary, biochemical assays, which have been applied to both receptor and enzyme targets, often simply measure the affinity of the test compound for the target protein” [11]. “Many assay models have been used to support compound screening and the choice of the assay model is dependent upon the biology of the drug target protein, the equipment infrastructure in the host laboratory, the experience of the scientists in that laboratory, whether an inhibitor or activator molecule is sought and the scale of the compound screened” [72].

High through put screening in drug discovery needs to demonstrate the following characteristics:

1. Pharmacological relevance of the assay. If available, studies should be performed using known ligands with activity at the target under study, to determine if the assay pharmacology is predictive of the disease state and to demonstrate that the assay is capable of identifying compounds with the desired potency and mechanism of action.
2. Reproducibility of the assay. Within a compound screening environment, it is a requirement that the assay is reproducible across assay plates, across screen days and, within a programme that may run for several years, across the duration of the entire drug discovery process.
3. “Assay costs. Compound screening assays are mostly performed in micro-titre plates.

In the academia screening of relatively small numbers of compounds assays typically use 96-well or 384-well microtitre plates, whereas in industry or in HTS applications assays the 384-well or 1536-well microtitre plates format in assay volumes as small as a few microlitres are used" [73]. In each case assay reagents and assay volumes are selected to minimize the costs of the assay.

4. Assay quality. Assay quality is usually determined based on the Z' factor [74]. "This is a statistical parameter that in addition to considering the signal window in the assay also considers the variance around both the high and low signals in the assay. The Z factor in the gold standard for industry means of measuring assay quality on a plate basis. The Z factor has a range of 0 to 1; an assay with a Z factor of greater than 0.4 is considered appropriately robust for compound screening although many groups prefer to work with assays with a Z factor of greater than 0.6" [3]. "In addition to the Z factor, assay quality is also monitored through the inclusion of pharmacological controls within each assay. Assays are deemed acceptable if the pharmacology of the standard compound(s) falls within predefined limits" [8,75]. "Assay quality is characterized by many factors and generally, high-quality assays are created through implementation of simple assay protocols with few steps, minimizing wash steps or plate to plate reagent transfers within the assay. The use of stable reagents and biologicals ensures that all the instrumentation used to perform the assay is under optimum performance" [3, 27]. "This is typically achieved by putting in place good quality control practices for all items of laboratory" [76].
5. "Effects of compounds in the assay. Chemical libraries are mainly stored in organic solvents such as ethanol or dimethyl sulphoxide (DMSO). Thus, assays need to be configured in a way that are not sensitive to the concentrations of solvents used in the assay. Typically, cell-based assays are intolerant to solvent concentrations of greater than 1% DMSO, whereas biochemical assays can be performed in solvent concentrations of up to 10% DMSO" [77]. Studies have also been performed to establish the false negative and false positive hit rates in the

assay. When developing any HTS assay, for the screening of several million molecules over several weeks, it is best practice to screen training sets of compounds to verify that the assay is performing under optimum condition.

"Compound libraries have been assembled have small molecular weight molecules that obey chemical parameters such as the Lipinski Rule of Five [77], and especially to have molecular weights of less than 400 and clogP (a measure of lipophilicity which affects absorption into the body) of less than 4". "Molecules with these characteristics have been termed 'drug-like', in consideration of the fact that the majority of clinically marketed drugs have a molecular weight of less than 350 and a cLogP of less than 3. It is very important to initiate a drug discovery process with a small simple molecule as lead optimization, to improve potency and selectivity, that typically involves an increase in molecular weight which in turn can lead to safety and tolerability issues" [78]. "Once a number of hits have been obtained from virtual screening or HTS, the first role for the drug discovery team is to select and define which compounds are the best to work on. This selection process is essential as, from a large library, a team will likely be left with many possible hits which they will need to reduce, confirm and cluster into series through several steps to achieving this. First, although this is less of a problem as the quality of libraries have improved, compounds that are known by the library curators to be frequent hitters in HTS assays need to be removed from further consideration" [79]. "Secondly, a number of computational chemistry algorithms have been developed to group hits based on structural similarity, to ensure that a broad spectrum of chemical classes is represented on the list of compounds taken forward. Analysis of the compound hit list using these algorithms allows the selection of hits for progression based on chemical cluster, potency and factors such as ligand efficiency which gives an idea of how well a compound binds for its size (log potency divided by number of 'heavy atoms' i.e. non-hydrogen atoms, in a molecule)" [80,35].

"The next phase in the initial refinement process is to generate dose-response curves using fresh sample of the compound in the primary hit discovery assay and the demonstration of normal competitive behavior in hits is important. Bioactive compounds that show an all or nothing response are not acting in a reversible manner

and may actually not be binding at all to the target protein, with the activity at high concentrations resulting from an interaction between the sample and other components of the assay system” [81]. “Reversible active metabolites are preferable due to the fact that their effects can be more easily ‘washed-out’ following drug attrition and withdrawal, which is an important consideration during clinical trials in patients. A compound dose–response curve facilitates the generation of a half maximal inhibitory concentration which can be used to compare the potencies of candidate compounds” [45,82]. “Almost all HTS compound libraries are stored as frozen DMSO solutions with the condition to prevent that, after some time, the compound can become degraded or modified. Studies have shown that compound libraries without protection in storage loses its potency when the compound was resynthesized and used in re-testing” [1,9].

“With reliable dose–response curves generated in the primary assay for the hit target, the stage is set to examine the surviving hits in a secondary assay, based on its availability, for the target of choice. This may not necessary be an assay in a high throughput format but can involve evaluating the effect of the compounds in a functional response, for example in a second messenger assay or in a tissue-or cell-based bioassay” [2]. “Activity in this setting will give reassurance that compounds are able to modulate more intact systems rather than simply interacting with the isolated and often engineered protein commonly applicable in the primary assay” [83]. “Throughout the confirmation process, medicinal

chemists attempt to examine cluster compounds into groups which could form the basis of lead series. As part of this process, consideration can be given to the properties of each cluster such as the identification of structure–activity relationship (SAR) with a number of compounds, the identification of a group of compounds which have some section or chemical motif in common and the addition of different chemical groups to this core structure that can lead to different potencies. Chemical synthesis can also be examined at this stage, the ease of preparation, potential amenability to parallel synthesis and the ability to generate diversity from late-stage intermediates can also be assessed” [84].

5.9 Hit-to-Lead Phase in Discovery

The aim of this stage of the drug discovery is to refine each hit series to produce more potent and selective bioactive compounds which shows pharmacokinetic properties necessary for examination of their efficacy in any available *in vivo* models [85]. Typically, the work now consists of intensive SAR investigations around each core compound structure, with measurements being made to establish the magnitude of activity and selectivity of each compound. This needs to be carried out systematically and, where structural information about the target is known, structure-based drug design techniques using molecular modelling and methodologies such as X-ray crystallography and NMR can be applied to develop the SAR faster and in a more focused way. This type of activity will also often give rise to the discovery of new binding sites on the target proteins [13,86].

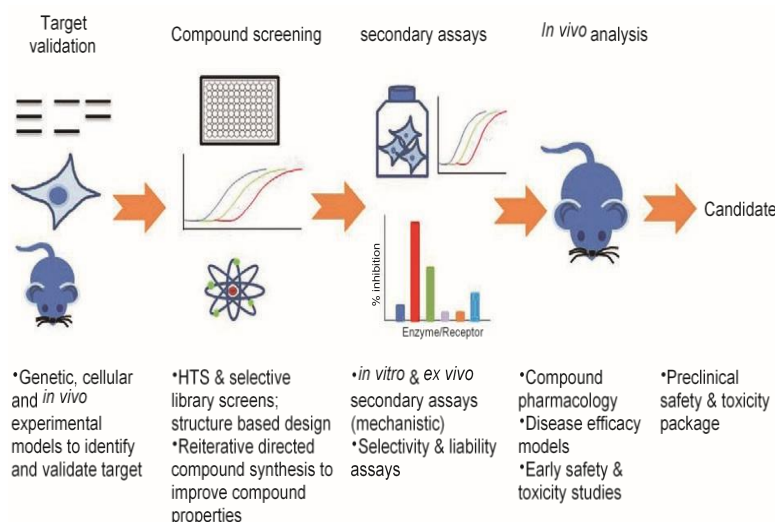


Fig. 7. Summary overview of drug discovery High Throughput Screening (HTS) Assays [28]

A series of screening consist of a relatively high throughput assay to establish the activity of each molecule on the molecular target, together with assays in the same format for sites with known selectivity or where selectivity can be a problem. Some compounds meeting basic criterion at this stage can be moved to other assays, which should include higher order functional investigations against the molecular target and also whether the compounds are active in primary assays in different species [87]. The key *in vitro* assays in the early drug discovery process is illustrated in Table 1.

6. IC₅₀, HALF MAXIMAL INHIBITORY CONCENTRATION

Solubility and permeability evaluations are important to determine the rejecting or accepting of potential of a compound to be a drug, that is, drug substance often needs access to a patient's circulation and therefore they may be injected or adsorbed in the digestive system [88]. Deficiency in one more parameter in a molecule may in some cases be adjusted. For example, in the case where formulation strategies can be used to design a tablet in such a way that it is able to dissolve in a particular region of the gut on a pH where the compound is more soluble [96-98]. A bioactive metabolite that lacks both these properties is very unlikely to become a drug irrespective of how potent it is in the primary screening assay [33,89]. Microsomal stability is a useful tool of the ability of *in vivo* metabolizing enzymes to modify and then remove a compound. Hepatocytes are sometimes used in this type of study instead and these may lead to more extensive results but may not use routinely as there is the need for fresh preparation on a regular basis. CYP450 inhibition is also evaluated as it is an important predictor of whether a new compound might affect the metabolism of an existing drug during co-administration [90]. If one or more of these properties is less than ideal, then it is important to screen many more compounds specifically for those properties.

Major compounds that can meet the target potency and selectivity, as well as most of the physicochemical and ADME targets, may be assessed for PK in rat models. The parameters for consideration are normally the half-life of >60 min when the compound is administered intravenously and a fraction in excess of 20% absorbed following oral dosing even though, different targets may require very different PK

profiles [16,80,91]. In large pharma company with in-house drug metabolism pharmacokinetics (DMPK) departments, numerous compounds may be profiled while in academic environments when there may be funds for only a predefined number of these expensive investigations [81,92]. As the receptor antagonist programme advances through the hit-to-lead phase, a number of compounds can be prepared which have potency in the nanomolar range with a benign selectivity profile except for some potency at the human ether-a-go-go related gene (hERG channel) which encodes the pore-forming subunit of the rapidly activating delayed rectifier potassium channel (I_{Kr}), which is important for cardiac repolarization. Dysfunction of hERG causes long QT syndrome and sudden death, which occur in patients with cardiac ischemia. A potassium voltage-gated ion channel important for cardiac function of which an inhibition can cause cardiac liability. Ideally the hERG study aim is to have an activity over 30 μ M or at least a 1000-fold selectivity for the target [83,93]. A number of hit compounds were examined in PK studies and can be found to have a reasonable half-life following intravenous dosing but poor plasma levels can be noted when the compound are given orally to rats [94,95]. It can be assumed that some of these compounds, represents the end of the hit-to lead phase of the project although they may not likely themselves to be progressed, they are capable of responding to tests in disease models [2,13].

6.1 Where do Potential Lead Come from

From various HTS assay leads can be selected. However, there are many sources of lead compounds as illustrated in Fig. 8, from acquisition compound, natural sources, newly synthesized or endogenous ligand [84].

6.2 Pre-Screening of Lead Compounds Using DEREK Software

Early prescreening of lead compound to the detection of early compound attrition can be made through the use of Deductive Estimation of Risk based on Existing Knowledge (DEREK) software. DEREK software is an *In-Silico* screen which signals the occurrence of a specific toxic response of an unknown compound when compared with a known compound in the data, although it does not provide a quantitative estimation of the prediction [42,85]. DEREK operate on several basic rules, consisting of the descriptions of molecular substructures (structural alerts) that is associated with toxic end

points like mutagenicity, carcinogenicity, skin irritation etc. Since substructures can exist in many molecular forms, the rules are not chemical specific but can serve as broad generalizations vis-a-vis the chemical structure (acid or halogen containing molecule, alkylating agent, and

chemical substructure associated with some toxic effect (Pharmacophore). For example, substructures like epoxides, cyanohydril known to be mutagenic are linked to some scientific justification to derive a knowledge base extracted from experimental data.

Table 1. Key *in vitro* assays in early drug discovery process [9]

Assays	Target value	Outcomes
CYP450-inhibition	➤ 10 mM	Main drug metabolic enzyme whose inhibition can cause toxicity
Caco-2 permeability P_{app}	➤ $1. \times 10^6 \text{ cm}^{-1}$ (asymmetry <2)	Caco-2 colon carcinoma cell line-applicable permeability estimation across intestinal epithelium. Important for drug absorbed from the gut
Aqueous solubility	➤ 100 mM	Applicable for <i>in vitro</i> assays and <i>in vivo</i> drug delivery testing
Hep G2 hepatotoxicity	No effect at 50 μ g IC_{50} or EC_{50}	Human HepG2 cells often act as a surrogate for the study of toxicity effects on human liver, an important cause of drug failure in the clinic
Log D $_{7.4}$	0-3 (for BBB penetration ca.2)	A measure of lipophilicity and movements across membranes
Microsomal stability Cl_{int}	< 30 mLmin ⁻¹ mg ⁻¹ protein	Liver microsomes contain membrane-bound drug metabolizing enzyme. This assay measures compound clearance and give an idea of how fast it will be cleared out <i>in vivo</i>
Cytotoxicity in suitable cell line	No effect at 50 μ g IC_{50} or EC_{50}	Reduce the likelihood of cellular toxicity <i>in vivo</i>
MDR1-MDCK permeability P_{app}	> $1 \times 10^6 \text{ cm}^{-1}$ (as	The Madin-Dary Canine Kidney (MDCK) cell lines transfected with the multiple drug resistant protein 1 (MDR1) gene, and Breast cancer resistant protein (BCRP), which encodes the efflux protein P glycoprotein (P-gp). They are important efflux transporters in most tissues such as the intestine, kidney and brain. P-gp are mostly used in predicting intestinal and brain permeability.

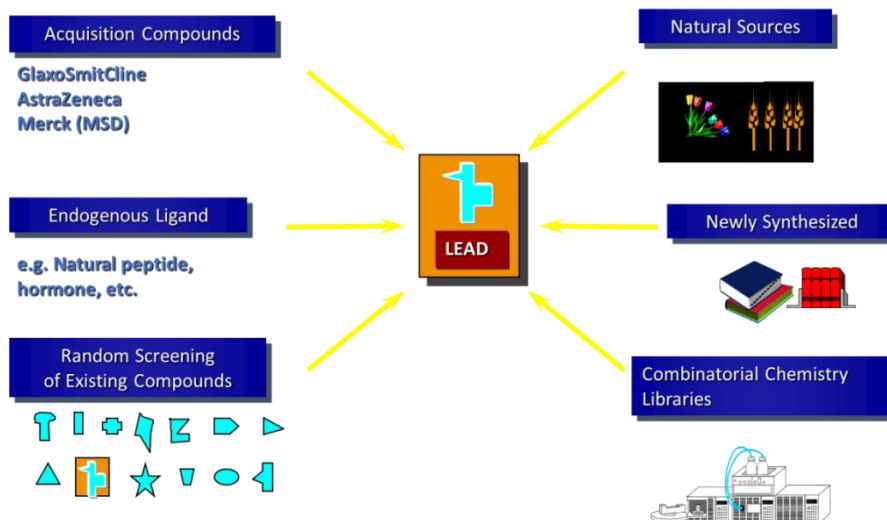


Fig. 8. Sources of potential lead compounds [14]

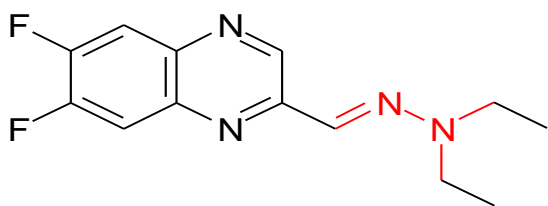


Fig. 9. Chemical substructure implicated in some toxic effect- PHARMACOPHORE

6.3 Lead Optimization Phase

The focus on the lead optimization phase is to maintain good physico-chemical properties in lead compounds in order to improve the quality in the lead structure. Lead optimization programme also modify the structure of compounds to minimize hERG liability and to improve the absorption of the compound [2]. Thus, more regular the compounds are checked on hERG affinity and CACO2 permeation are done for compound to show the availability which maintained their potency and selectivity at the principal target but with much reduced hERG affinity, and probably a better apparent permeation than initial lead compounds [86]. When lead compounds are examined for PK properties in rat, one of such compounds, with 8 nM affinity at the receptor of interest, had an oral bioavailability of over 40% in rats and about 80% in dogs [2,9].

Compounds at the lead optimization stage are considered to have met the initial goals of the lead optimization phase and could be considered ready for final characterization before its consideration as a preclinical candidate for the continuation of the drug discovery process [99-102]. There is a need after lead optimization for chemist team to continue to explore avenue to produce synthetic compounds in order to produce potential back up molecules, in case where the compound undergoing further preclinical or clinical characterization fails and, more strategically, to look for follow-up series [89].

The stage at which the various elements that constitute further characterization are carried out may vary from company to company, and parts of this process may be incorporated into the lead optimization phase. However, compounds generally require evaluations in genotoxicity models such as the Ames test and *in vivo* models of general behavior such as the Irwin's test [103-105]. High-dose pharmacology, PK/PD studies, dose linearity and repeat dosing PK

looking for drug-induced metabolism and metabolic profiling all need to be carried out by the end of this stage [90,91]. Consideration also needs to be given to chemical stability issues and salt selection for the putative drug substance. All the information generated about lead compound at this stage could allow for the preparation of a target candidate profile which together with toxicological and chemical manufacture and quality control considerations may form the basis of a regulatory submission to allow clinical trials in human to begin [2,11].

The generation of hit compounds to preclinical candidate selection is not considered a routine activity as it usually takes a long time., with rarely any short cuts and significant intellectual input required from scientists from a multidisciplinary team [94-98]. The quality of the hit-to-lead compound starting point and the technical platform expertise of the necessary team put in place are the key determinants of a successful outcome of this phase of work. Generally, in the pharma industry for each project about 200 000 to >10⁶ compounds may be screened initially following hit-to-lead and lead optimization programmes. About hundreds of compounds are screened to scale down to one or two candidate molecules, usually from different chemical series [25,52]. In academic research institutions, screens are more likely to be of a focused nature due to the high cost of an extensive HTS or compounds can be derived from a structure-based approach. Only 10% of small molecule projects within the Pharma industry may make it to candidate selection, as attrition of many compounds occur for multiple reasons at different stages [16,92]. The reasons for compound attrition can include (i) poor configuration of a suitable and regulatory compliant (ii) lack of hit development obtained from a HTS assay; (iii) development of compounds that do not behave as desired in secondary or *in vitro* and *in vivo* tissue assays; (iv) compounds that are toxic *in vitro* or *in vivo*; (v) compounds that have undesirable side effects which cannot be easily screened out or separated from the mode of action of the drug target; (vi) having compounds that cannot produce a good PK or PD profile in line with the dosing regimen required in man. For example, if the compound require administration a once a day as a tablet then the compound will need to have a half-life *in vivo* suitable to achieve this; and (vii) inability for the compound to cross the blood brain barrier especially for compounds whose target lies within the central nervous system [91-93]. The attrition rate for protein

therapeutics, once the target has been identified, is much lower due to less off target selectivity and prior experience of PK of some proteins, such as antibodies [15,93].

7. CONCLUSION

Preclinical activity is of high risk and with less financial return which make funding by stakeholder very challenging. However preclinical phase of drug discovery is still relatively less costly in terms of pharmacoeconomic point of view than many processes carried out later on in the drug development and clinical phases. An understanding and establishment of transparency in the cost of each stage of development within large pharma company may help reduce some of their costs and there are many initiatives moves as companies advocate for biotech innovations mentality and accountability for research costs.

Once a drug candidate has been selected, the attrition rate of compounds advancing into the clinical phase is also high, as only one in 10 candidates has the possibility of ever reaching the market. As the lead compound progresses to late stage of clinical development the financial consequences of drug attrition are much higher. There has been global debate in the pharmaceutical industry on how to improve the success rate of drug candidates to get approval. Most drug candidates that reaches the clinical stage, become increasingly difficult to kill the project, as at this stage the project has become public knowledge and thus termination can influence confidence in the company and shareholder value [106-109]. Carrying out more studies prior to clinical development such as improved toxicology screens (using failed drugs), establishing predictive translational models based on a better understanding of the pathophysiology of the disease understanding and identifying biomarkers may help to increase success rate of potential drug candidates. The collaboration between the academia, research institution in particular in drug development can add value in bringing more effective drugs to the market for patients' consumption.

CONSENT AND ETHICAL APPROVAL

It's not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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