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Important Lead Optimization Tools & Techniques in Drug Discovery Process

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ABSTRACT

*Lead optimization is one of the important phases of Drug Discovery Process in which researchers design, synthesize, and retest analogues of primary lead compounds. Identified lead molecules are used as the starting point for detailed chemical modifications in order to further improve their target specificity and selectivity and their pharmacokinetic and safety profiles, while maintaining the favorable properties of the lead compounds. Assessments of the pharmacological properties of Absorption, Distribution, Metabolism, and Excretion (**ADME**) of chemical leads are critical to their initial selection, and establishes benchmarks against which compounds synthesized during lead **optimization**. There are a tremendous number of tools available to discovery scientists to screen compounds for optimization of ADME properties and selection of better lead molecule. However, the use of these tools has generally been to characterize these compounds rather than to select among them. Many of the tools and technologies for lead discovery overlap with lead optimization as researchers attempt to incorporate the best drug characteristics early in the process. While the approaches taken may vary, the central theme is the same: make it better, faster, and more efficient. This review article summarized brief outline on lead optimization strategies and techniques used in drug discovery process.*

Keywords*- *Lead optimization, tools and techniques, identification, new drug.

1. INTRODUCTION

Drug discovery is a very complex and multifaceted process[1-2] which aims at identifying a compound therapeutically useful in curing and treating disease. This process involves the identification of candidates, synthesis, characterization, validation, optimization, screening and assays for therapeutic efficacy. Once a compound has shown its significance in these investigations, it will initiate the process of drug development earlier to clinical trials. New drug development process must continue through several stages in order to make a medicine that is safe, effective, and has approved all regulatory requirements. The drug discovery and development process generally follows the following path that includes a hit to lead stage as shown in Fig.1Target validation (TV) → Assay development → High-throughput screening (HTS)→ Hit to lead (H2L) → Lead optimization (LO) → Preclinical development → Clinical development this article mainly concentrates on the lead optimization phase in drug discovery process and the important strategies and techniques used to characterize the compound and establish the route.

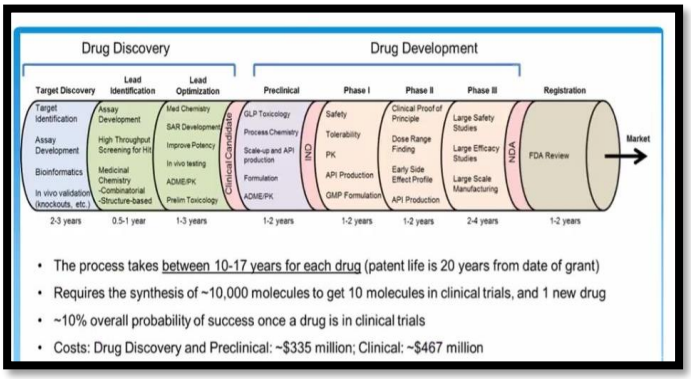

Fig. 1: The drug discovery and development process

1.1 Lead optimization

Once compounds have been identified, they need to be optimized for efficacy and safety. The design of synthetic molecules can be altered to prevent off-target binding, making them less likely to interact with molecules other than the target. Additionally, the optimal dosage and introduction route (oral, injection) is tested on two- and three-dimensional cell culture platforms. Labs must acquire data on the toxicity, efficacy, stability and bioavailability of leads, in order to accurately characterize the compound and establish the route of optimization. This means optimization[3] is accomplished through chemical modification of the hit structure, with modifications chosen by employing knowledge of the structure–activity relationship (SAR) as well as structure-based design if structural information about the target is available.

- Increase activity against the chosen target
- Alter design of molecule to prevent off-target effect
- Optimize dosage and introduction route
- Improve the drug likeness or ADME properties of the molecule.

1.2 In Vitro and In Vivo Assessment of ADME:

In classical pharmacology also called **phenotypic drug discovery (PDD)**[4], many chemical libraries of synthetic small molecules, natural products or extracts were screened in vitro or in vivo, such as intact cells, whole organisms or cell-free systems to identify substances that have a desirable therapeutic effect. But in reverse pharmacology also known as **target based drug discovery (TDD),** it has become popular to develop a hypothesis that a certain biological target is disease modifying and screen for compounds that modulate the activity of this purified target. Afterwards, these compounds are tested in animals to see if they have the desired effect.

Lead optimization is concerned with experimental testing and confirmation of the compound based on animal efficacy models and ADMET[5-7] (*in vitro* and *in situ*) tools that may be followed by target identification and target validation. Optimization of the compounds is done by medicinal chemists using advanced organic synthesis methods or by biotechnological methods for the production of biological products. If the structure of the drug target is known, computational *in silico* methods may be used for the rational design of the modifications. Once the properties of the optimized lead molecule, analyzed by all vailable *in vitro* assays and *in vivo* models, are acceptable, the lead optimization phase results in a candidate drug that may be either a small molecule or a biological product.

The preclinical drug design trial can be made of *in silico*, *in vitro*, and *in vivo* experiments. With the development of bioinformatics, big data, biology, chemistry and medicine, more and more databases are design to service for the drug discovery[8]. The databases are divided into macromolecular and small molecular databases. The macromolecular structure databases contain the crystal structures of proteins, nucleic acids, or other biopolymers. In addition, the de novo method is another computer-aided design for new drug generation on basis of 3D-structural targets and pharmacophore model[9]. To study the dynamical interaction between receptors and ligands at the atomic level, the molecular dynamics (MD) simulations supply a reliable and accurate way to explore the binding mechanism between ligands and targets[10]. The direct binding experiment *in vitro* between ligands and targets should be trial by using the methods of X-ray crystallography structural analysis, Surface Plasmon Resonance (SPR)[11], etc. Because the direct binding assay cannot guarantee the activity of screened ligands, the cell signaling pathway response experiment should be performed to check the activity of ligands in cell[12]. Due to the complex physiological environment, the active ligands *in vitro* trial may not show any response to the targeting disease *in vivo*. Hence, it need choose the suitable animal models for *invivo* experiments[13].

1.3 Isothermal titration calorimetry (ITC) –

The rapid development of science technology has prompted the emergence of several new approaches. Isothermal titration calorimetry (ITC) is one of the products of the rapid science technology development. It is most often used to investigate the binding of small molecules to larger macromolecules, such as proteins or DNA. In the measurement using ITC, several important parameters involving the binding process can be calculated, including binding affinity, enthalpy changes, and the binding stoichiometry. According to the obtained parameters, the final Gibbs energy changes and entropy changes can be specifically determined14. As ITC gives not only the binding affinity, but also the thermodynamics of the binding interaction, it is typically used as a secondary screening technique in high throughput drug discovery to eliminate false positive hits after primary screening. Characterization of the binding thermodynamics allows further hit selection and lead optimization as ITC can provide insights into the structure-activity relationship (SAR) for ligand interaction with the target. Comparing to other techniques such as fluorescence assays and NMR15 for studying the complex formation, ITC does not need any fluorescent probes or radioactive tags for data analysis. In addition, proteins used in the measurement do not require chemical modification that is ease of use and cost. In spite of various advantages, low throughput, low sensitivity, and large sample requirement are major concerns, which may hamper its application16.

Surface Plasmon Resonance (SPR) Surface Plasmon Resonance (SPR) – Surface Plasmon Resonance is the product of nano-science development. The emergence of SPR had greatly reduced the detection limit of biological analysis and it is widely used for the study of ligand binding interactions. SPR is label-free in that a label molecule is not required for detection of the analytes and capable of measuring real-time quantitative binding affinities and kinetics in sequential binding events.

Moreover, SPR is especially interesting as it can present kinetic information according to affinity data and can be used for thermodynamic studies. At the same time, SPR biosensor assays can be applied in a wide range of proteins, including membrane proteins, such as G-protein-coupled receptors (GPCRs). Another application of SPR technology is early ADME (absorption, distribution, metabolism, and excretion) profile prediction for lead compounds in drug discovery trial. During a SPR measurement17-18, the information includes data about concentration of a binding partner in a mixture as well as kinetic rate constants (association, dissociation rate constants and the equilibrium dissociation constant) for the binding interactions. Thus, SPR provides insights into the efficacy, safety, duration of action, indication, and patient tolerability of a drug.

1.4 High-throughput screening (HTS)-

High-throughput screening is a method for scientific experimentation especially used in drug discovery and relevant to the fields of biology and chemistry19-20. Through this process one can rapidly identify active compounds, antibodies, or genes that modulate a particular biomolecular pathway. The results of these experiments provide starting points for drug design and for understanding the non-interaction or role of a particular location.

High throughput DMPK (drug metabolism and pharmacokinetics) screens have become an essential part of lead optimization, facilitating the understanding and prediction of in vivo pharmacokinetics using in vitro tests. Virtual high throughput screening, where screening is done using computer-generated models and attempting to "dock" virtual libraries to a target, are also often used.

1.5 Assessment of other physicochemical properties:

Amongst the physicochemical properties associated with drug absorption include ionization (pKa), and solubility; permeability can be determined by PAMPA and Caco-2. PAMPA is attractive as an early screen due to the low consumption of drug and the low cost compared to tests such as Caco-2, gastrointestinal tract (GIT) and Blood–brain barrier (BBB) with which there is a high correlation. A range of parameters can be used to assess drug likeness such as cLogP to estimate lipophilicity, molecular weight, polar surface area and measured properties, such as potency, in-vitro measurement21-22 of enzymatic clearance etc.

1.6 De novo drug design-

Another important method for drug discovery is de novo drug design, in which a prediction is made of the sorts of chemicals that might (e.g.) fit into an active site of the target enzyme. Molecular modelling23-24 and molecular dynamics simulations can be used as a guide to improve the potency and properties of new drug leads.

HTS include fragment-based lead discovery (FBDD)25 and protein-directed dynamic combinatorial chemistry.26 The ligands in these approaches are usually much smaller, and they bind to the target protein with weaker binding affinity than hits that are identified from HTS. Further modifications into lead compounds are guided by protein X-ray crystallography of the protein-fragment complex.27

Automated screening systems are becoming an important part of pharmaceutical and biopharmaceutical drug discovery labs. Mass spectrometry is used for the detection and quantitation of metabolites. MALDI Imaging is a key technique for evaluating drug candidates and their metabolites in tissue structure rapidly and accurately. Additionally, NMR Fragment-based Screening (FBS) in the pharmaceutical industry has become a widely applied method for the discovery and optimization of lead molecules in targeted screening campaigns.

2. CONCLUSION

Generally, for drug discovery research in the laboratory, in combination with the chemical assays, cell-based and in vivo testing would perform more efficiently to obtain effective lead compounds for further drug development. Lead optimization of the identified compounds are done by altering the design of compound to prevent off-target binding, making them less likely to interact with molecules other than the target This review summarizes the various tools and techniques used for characterization and optimization of compounds and selection of better lead molecule. At last, new models are still needed to be developed for scientific researches in the future.

3. ACKNOWLEDGEMENT

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