Advances in Bioresearch
Adv. Biores., Vol 16 (3) May 2025: 262-272

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.3.262272

Advances in Bioresearch

REVIEW ARTICLE

A review on *In-Silico* methods used in Drug Design and Discovery

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ABSTRACT

Traditional drug discovery techniques have been used to effectively generate novel drugs in the past, but the entire process from lead identification and to clinical trials takes more than 12 years and costs an average of $1.8 billion USD. Additionally, the process of designing and developing new drugs is expensive, time-consuming, and complex. In this file, in-silico techniques that make use of advanced logarithms and processing power have shown to be revolutionary. In-silico methods have garnered a lot of attention lately due to their promise to expedite the time and labor-intensive process of drug discovery. The primary advantage of in-silico drug design is its cost-effectiveness in medication development and research. Grid computing, window-based physiologically based pharmacokinetic pharmacodynamic model (PBPK-PD) modeling software, Peking University Drug Design System (PKUDDS) for structure-based drug design, Java, Perl, and Python are just a few of the many software programs used in in-silico drug design. In-silico drug design can play a significant role in all phases of drug development, from preclinical discovery to late-stage clinical development. Its use in drug development aids in the selection of just strong lead molecules and may consequently prevent late-stage clinical failure, which could result in a significant reduction in cost. By combining experimental methods with in-silico methods, researchers can open up new avenues for treating complicated disorders and enhancing human health.

Keywords: *In-silico methods, drug design, CADD, molecular docking, pharmacophore modeling.*

Received 10.03.2025 Revised 06.04.2025 Accepted 22.05.2025

How to cite this article:
Srikanth Kumar K, Vishal Bharat B, Jagadeeswara Rao B, Phadtare Nisha B, Gavade Pratibha R, Gadiya Swati A, Shaikh Tabassum Bano Mehboob, Gurav Shreya A. A review on in-silico methods used in Drug Design and Discovery. Adv. Biores., Vol 16 (3) May 2025: 262-272.

INTRODUCTION

The FDA (Food and Drug Administration) defines a drug as any substance (apart from food and devices) that is intended to alter the structure or function of the body or that is used in the diagnosis, cure, alleviation, treatment, or prevention of disease. In layman's terms, a "drug" is a pharmaceutical biomolecule or a mixture of molecules that alter the body and its functions; this definition is utilized for legal purposes. [1] To predict how a particular chemical will attach to the target and how strong a bond will form is the primary goal of drug design. The strength of a tiny molecule is estimated using the molecular mechanism or dynamics. The automated process is able to forecast. [2] The scientific field of bioinformatics is the result of the integration of information science, computer science, and biology into one discipline. It makes use of cutting-edge computational methods to organize and evaluate biological data. The term "performed on computer or via computer simulation" is referred to as "*in-silico*." Pedero Miramontes, a mathematician from the National Autonomous University of Mexico, used the phrase "*in-silico*" to describe biological studies that were conducted solely on a computer. [3]

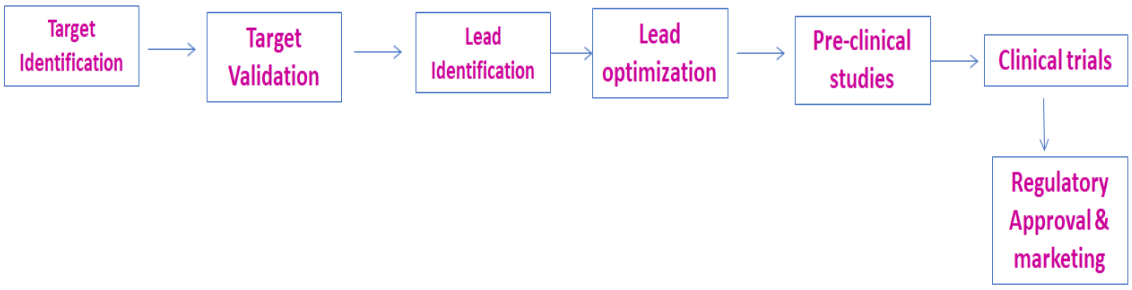

Figure1: *In-silico* drug discovery process

Drug discovery and development process

The process of finding and developing drugs; the following are crucial milestones in the drug discovery process:

Lead Identification

Lead identification in drug discovery is a critical phase where potential drug candidates (leads) are discovered, often through screening and optimization, to interact effectively with a biological target related to a disease. This process serves as a foundation for the development of a new therapeutic compound and involves several key steps, tools, and techniques.

Screening Approaches for Lead Identification

High-Throughput Screening (HTS): Large libraries of small molecules are screened against the biological target to identify potential hits that modulate the target's activity. Automated systems and robotics are typically used in this process.

Fragment-Based Drug Discovery (FBDD): In this approach, smaller molecular fragments (smaller than traditional drug-like molecules) are screened, which can later be optimized by adding or modifying chemical groups to improve binding and efficacy.

Structure-Based Drug Design (SBDD): This approach utilizes the 3D structure of the biological target, often obtained via techniques like X-ray crystallography or NMR spectroscopy, to design molecules that will fit into the target's binding site.

Computational Approaches for Lead Identification

Virtual Screening: Computational techniques are used to simulate the interaction of small molecules with the biological target. There are two types:

Ligand-based: Uses known ligands to identify new leads based on structural similarities.

Structure-based: Uses the 3D structure of the target protein to screen potential ligands.

Quantitative Structure-Activity Relationship (QSAR): This method involves building statistical models to predict the activity of new compounds based on their chemical structure.

Molecular Docking: Predicts how a molecule will bind to a target, estimating both binding affinity and orientation.

Challenges in Lead Identification

Selectivity: Ensuring that the lead molecule interacts specifically with the target and not with other off-target proteins.

Optimizing Drug-Like Properties: Leads must have favorable pharmacokinetic properties (e.g., solubility, stability) for further development.

Safety: Ensuring that the lead molecule does not exhibit toxicity during early in vitro and in vivo tests.

Lead Optimization

Lead optimization is a crucial phase in drug discovery that involves refining a lead compound to improve its properties, making it more suitable for development as a therapeutic drug. This process focuses on enhancing several characteristics of the compound, such as potency, selectivity, pharmacokinetics, and safety. Lead optimization aims to balance these factors to develop a candidate drug that can proceed to clinical trials. [4]

Key aspects of lead optimization include:

1. Improving Potency

Affinity: Enhancing the ability of the lead compound to bind more tightly to its biological target (usually a receptor or enzyme).

Activity: Increasing the biological activity of the compound, ensuring that it has a strong effect at lower concentrations.

2. Enhancing Selectivity

Target Specificity: Modifying the compound to bind more selectively to the desired biological target, minimizing off-target effects that could lead to side effects.

Minimizing Toxicity: Reducing interactions with non-target proteins or pathways that could lead to adverse effects.

3. Pharmacokinetics (ADME) Optimization

Absorption: Improving the bioavailability of the compound, ensuring that it is effectively absorbed into the bloodstream when administered.

Distribution: Enhancing how the compound is distributed throughout the body, ensuring that it reaches the target tissues.

Metabolism: Modifying the structure of the lead to improve its stability against metabolic degradation, so it has a longer half-life.

Excretion: Ensuring the compound is eliminated from the body in a way that doesn't lead to toxic accumulation.

4. Improving Drug-Likeness

Lipophilicity: Optimizing the balance between lipophilicity (ability to dissolve in fats) and hydrophilicity (ability to dissolve in water), which affects both bioavailability and toxicity.

Molecular Size and Weight: Ensuring that the compound's molecular weight remains within a range conducive to good drug-like properties, usually under 500 Da.

Rule of Five: Adhering to Lipinski's Rule of Five, which predicts good oral bioavailability if certain molecular properties are within specific ranges (e.g., molecular weight, number of hydrogen bond donors/acceptors, and lipophilicity).

Pre-Clinical Lead Development

Pre-clinical lead development is a critical stage in drug discovery, following lead optimization. During this phase, the goal is to take a well-characterized lead compound and prepare it for clinical testing in humans. This stage involves conducting a series of non-human tests to ensure that the compound is safe, effective, and has the necessary pharmacological properties to proceed to clinical trials.

Here's a detailed breakdown of the key components of pre-clinical lead development:

1. In-vitro Studies

Mechanism of Action (MoA) Studies: Confirm the biological target of the compound and how it interacts with the target (e.g., receptor binding, enzyme inhibition). These studies help ensure that the compound engages the desired pathway or target.

Toxicity Screening: Evaluate potential cytotoxicity or genotoxicity in cultured cells. These studies identify any harmful effects the compound might have on cells or DNA.

Pharmacokinetic Profiling (ADME): Study the absorption, distribution, metabolism, and excretion of the compound in cell-based systems or microsomes to predict its behavior in more complex systems.

2. In-vivo Studies

Animal Models of Disease: Test the compound's efficacy in animal models that mimic the disease being targeted (e.g., mouse models for cancer, cardiovascular disease, neurological disorders). This helps determine whether the compound can produce the desired therapeutic effect in a living organism.

Pharmacodynamics (PD): Study how the compound affects biological systems in vivo, including dose-response relationships and the duration of its effects.

Pharmacokinetics (PK): Determine how the compound is absorbed, distributed, metabolized, and excreted in animal models, giving insights into potential dosing regimens for human studies.

3. Toxicology Studies

Toxicology testing is essential for understanding the safety profile of the lead compound.

Acute Toxicity Testing: Investigates the effects of a single high dose to understand the potential for immediate toxic reactions.

Chronic Toxicity Testing: Assesses the long-term safety of repeated doses over a longer duration (weeks or months), which is important for identifying cumulative toxic effects.

Organ-Specific Toxicity: Monitors any adverse effects on key organs such as the liver, kidneys, and heart.

Genotoxicity and Carcinogenicity: Tests whether the compound has the potential to damage DNA or cause cancer. This can involve both in vitro (e.g., Ames test) and in vivo studies.

Reproductive Toxicity and Teratogenicity: Assesses the compound's potential to cause harm to reproductive organs or developmental defects in offspring.

4. Pharmacokinetic (PK) and Pharmacodynamic (PD) Studies

PK Studies: Detailed studies to measure the drug's absorption, bioavailability, half-life, and clearance in different animal species. This information helps predict how the drug might behave in humans and guides dosing regimens for clinical trials.

PD Studies: Evaluate how the drug's effects change over time in relation to its concentration in the blood. Understanding the relationship between PK and PD is key to determining the appropriate dosage that achieves therapeutic effects without toxicity.

5. Formulation Development

Stability Studies: Assess the stability of the drug in various forms (e.g., tablet, capsule, injectable) and under different storage conditions (e.g., temperature, light exposure). Ensuring that the compound remains stable during production, storage, and delivery is critical.

Bioavailability Improvement: Work on improving the bioavailability of the compound if necessary. This may include altering the formulation to enhance solubility or absorption.

6. Good Laboratory Practice (GLP) Studies

Regulatory Compliance: Pre-clinical studies must be conducted under Good Laboratory Practice (GLP) regulations to ensure data integrity and reproducibility. GLP standards are necessary for regulatory submissions to agencies like the FDA (U.S.) or EMA (Europe).

7. Toxicokinetics

Dose-Related Toxicity: Determine the relationship between dose and exposure to assess the compound's safety margins. Toxicokinetic studies help identify the maximum tolerated dose (MTD) and no observed adverse effect level (NOAEL).

Accumulation Potential: Evaluate whether the compound accumulates in tissues over time, which could lead to toxicity at higher doses or with repeated dosing.

8. Metabolite Profiling

Identification of Metabolites: Determine the primary metabolites of the compound, as they may have different pharmacological or toxicological profiles than the parent compound.

Toxicity of Metabolites: Evaluate whether any metabolites are toxic or reactive, potentially leading to off-target effects.

9. IND-Enabling Studies

Investigational New Drug (IND) Application: All data from pre-clinical studies are compiled into an IND application. This application must be submitted to regulatory authorities before human clinical trials can begin.

Regulatory Submission: Pre-clinical results are included in the IND application, along with a proposed clinical trial plan. Regulatory authorities will review the data to ensure that the compound is safe enough for human testing.

10. Animal to Human Translation

Allometric Scaling: Use data from animal PK/PD studies to predict human doses through scaling methods. This involves extrapolating the data to estimate how the compound might behave in humans, given differences in size, metabolism, and physiology.

First-In-Human Dose: Based on the toxicity, PK, and PD data from animal studies, the first-in-human (FIH) dose is determined, which represents the starting dose for clinical trials.

Preclinical lead development includes large-scale synthesis, drug formulation trials, animal in vivo research, animal safety studies, and drug metabolism studies. [5]

Clinical Lead Development

It includes the establishment of clinical study protocols using clinical investigations on patients (phase II) and comparative double blind studies on patients' studies (phase III), as well as small-scale safety and dose-ranging tests in healthy human volunteers (phase I). Numerous methods based on sequence and structure have been put forth to identify targets. By comparing the functional genomics of humans with corresponding genomics of pathogens, the sequence-based method provides functional information about the target and its positioning in biological networks to identify unique targets from disease-causing pathogens (e.g., bacteria or viruses) [6]. The general process used to identify a novel target-specific medication for a particular illness is high-throughput screening (HTS) conducted at random [7]. Large chemical libraries are examined in this procedure to see if they have the ability to alter or inhibit the target. Compounds will be tested to see if they can completely block the receptor or increase, decrease, or otherwise modify its activity, for example, if the target is a new beta lactamase enzyme [8]. In this way, new pharmacophores are finding their way to light these days quite quickly. Concurrently screening the hits against different targets is another crucial component of these screenings, as it allows for the determination of the compounds' selectivity for the target. We refer to this as cross-screening. Cross-

screening is important because, if a chemical repeatedly hits unrelated targets, it is anticipated to produce significant "toxicity" in humans (assuming it advances to the point of human clinical trials) [9]. At this point, it's important to discuss Virtual High Throughput Screening (vHTS), another screening technique. This kind of screening technique makes use of computer-generated models. These computer algorithms try to dock virtual libraries—which are made up of chemical structures in three dimensions—to a target. This approach is also economical, dependable, and fast [10–11]. Using these techniques, the researchers find "a lead molecule series." It is anticipated that the chemical structures in this series will have favorable characteristics for "drug likeness" and sufficient target specificity. One or two molecules may then be chosen for further research into potential drugs. The "lead" compound [12] is the best of these compounds.

The lead compound is further refined by randomized experiments or, more prudently, through logical drug design techniques. In order to (i) increase activity against the selected target, (ii) reduce activity against unrelated targets, and (iii) improve the "drug like" or ADME properties of the molecule, chemists and modelers first look for common features among the leads. If so, they use Structure-Activity Relationships (SARs) to improve certain structural features of the lead molecules. One of the main goals of the drug discovery process is to become more "rational." This would be an intellectually demanding strategy that would combine advanced biological assays, a restricted but highly focused chemical synthesis effort, and computer-aided molecular design (CAMD). As a result, the process of designing new drugs may become far less hazardous and resource-efficient. The logical approaches to drug design are evolving, moving away from being exclusive. A theoretical promise based on their application to creative problem-solving and the inventive creation and discovery of Novel Chemical Entities (NCEs), as well as the retrospective examination of known molecules. The literature has several effective instances of the creation and discovery of NCEs by rational design methodologies [13–14]. This process is now quicker and more efficient because to advancements in computer hardware and software.

DRUG DESIGN AND DEVELOPMENT PARAMETERS

Analysis of whole genome: The human genome project, which determines the entire human genome, serves as a model for medication design and development. It is fairly simple to create a chemical that binds to a receptor once the structure of the receptor is known. This chemical is thought to be a pharmaceutical compound.

Structure activity relationship: 3DQSAR is the tool used for this. "Quantitative Structure Activity Relationship" is what it stands for. It is the method by which a clearly defined process and chemical structure are quantitatively associated. The process starts when a chemist comes up with a theory that connects the biological activity to the chemical characteristics of a molecule or group of molecules. In the absence of a thorough comprehension of the biochemical mechanism behind activity, the hypothesis is typically improved by comparing and contrasting the structures of active and inactive molecules.

ADME: The ADME stands for absorption, distribution, metabolism, and excretion in pharmacokinetics. It is believed that when the medications travel between tissues and bodily fluids, bind with plasma or other cellular components, or undergo metabolism, they are in a dynamic state within the body. [3]

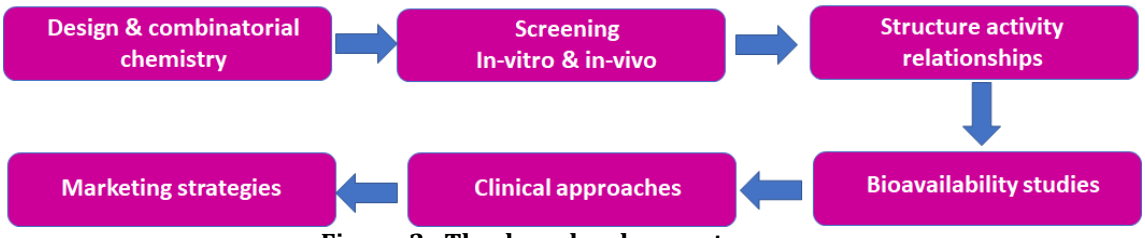

Figure-2: The drug development process

Computer-aided drug design is necessary for the creation of novel drugs:

The drug's pharmacokinetic characteristics are predicted using a computerized pre-development test prior to the drug's development. The automated pre-development test provides an estimate of the drug's toxicity prior to development. Due to CADD (computer aided drug design), which provides clarity on the medicine's potency, a significant amount of money will be needed for the creation of new drugs. The other, labor-intensive, laboratory-based approach for the pre-development examination of the new medicine is in-vivo and in-vitro analysis. To prevent financial waste, a significant amount of money is needed for the development of new drugs, which calls for the use of CADD. Compared to the laboratory procedure, the software-based CADD process took less time. The CADD technique takes less time, which

accelerates the creation of new drugs. The discovery of novel drugs is greatly aided by the CADD, and the process is rather simple. The CADD is crucial to the process of finding a medication that is safe, well-tolerated, non-toxic, and effective. All things considered, the CADD method is less complicated, time-consuming, and affordable. As a result, the CADD is an extremely practical and cost-effective method for developing novel drugs. It would prevent the loss of funds and time needed for the new drug's development. The laboratory procedure requires a lot of time and is challenging to handle. These days, a plethora of computational techniques are employed to find possible lead molecules inside enormous compound libraries. [15]

Virtual Screening

A combination of computational techniques known as virtual screening examine big databases or compound collections to find possible hits. Virtual libraries as well as corporate libraries may be searched using this method [16]. Traditionally, virtual screening techniques have been separated into two primary categories: receptor-based and ligand-based screening. In the ligand-based screening process, other compounds of interest are found in a database by using similarity sourcing techniques or by looking for a shared substructure, pharmacophore, or shape parameters within the active set. These techniques are applied to 2D or 3D chemical structures or molecular descriptors of known actives (and occasionally inactive molecules).

Drug designing Steps

The computing stage, which involves using a computer tool to make a drug molecule more exact and pharmaceutically active, is the most crucial and vital step in the drug-designing process. Some of the key characteristics of this drug design software are:

• Utilizes the free energy of the ligand/receptor complex through Auto Dock (Automated Docking of Flexible Ligands to Receptors) to naturally find the best ligand-receptor binding modes. This is known as the energy-driven technique.

• It is composed of three separate computer programs:

(i) Auto Dock, which performs ligand docking to a set of grids with the targeted protein;

(ii) (ii) Auto Grid, which computes the atomic affinities in advance;

(iii) (iii) Auto Tors, which sets up the ligand to be handled as mobile.

• Provide a scripted procedure for predicting the ligand-biomolecular target interactions, which aids in reducing the number of conformational possibilities and locating the ideal structures.

• Combines grid-based molecular affinity potentials with a Monte Carlo (MC) simulated annealing (SA) technique for the study of exceptional energy estimation.

• Described as a possible method for fitting a flexible ligand into a static protein's binding site. X-ray crystallography, protein-protein docking, combinatorial library design, virtual screening, SBDD, and biochemical mechanism investigations are among the applications of flexible docking.

• The Structure-based Drug Design (SBDD) tool was developed to facilitate combinatorial library design.

• The software's prediction is used to determine nanomolar inhibition.

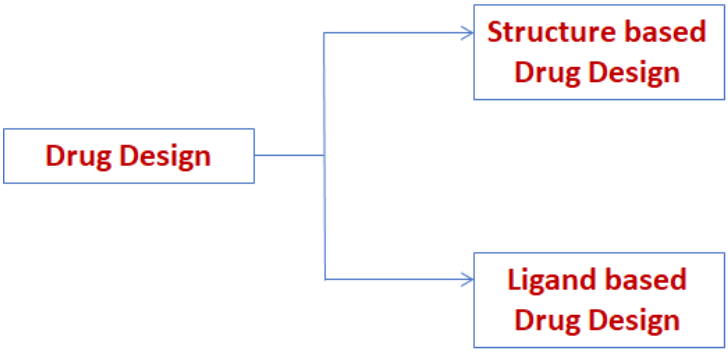

Figure 3: Drug designing methods

COMPUTER AIDED DRUG DESIGN

Computer Aided Drug Design, or CADD, is a computer-based method used in computational chemistry to find, improve, or analyze drugs and related physiologically active molecules.

Structure-Based Drug Design

Structure-based drug design (SBDD) is a drug discovery approach that creates compounds that can interact with a target protein by using its three-dimensional (3D) structure, which is usually found by methods like cryo-electron microscopy, NMR spectroscopy, or X-ray crystallography. This strategy makes

perfect sense since it enables researchers to precisely design medications to meet the binding sites of their targets, increasing the likelihood of successful treatment and minimizing unfavorable side effects. In order to compute interaction energies for each tested molecule, structure-based computer-aided drug design relies on knowledge of the target protein structure [17,18]. Target proteins that have crystallized are available in the structural database. Designing molecules that bind precisely and securely to a target while consuming the least amount of energy is known as structure-based [19,20].

Ligand-Based Drug Design

In ligand-based drug development, potency and other key qualities are enhanced by creating suitable analogs based on the understanding of structure-activity correlations (SAR). This process begins with a single compound or a group of compounds that are known to be effective against a target. The Topliss technique or basic analog design based on structural similarity or qualities can be used for designing. For design objectives, computational techniques like pharmacophore models or the structure of the molecules are frequently helpful. Quantitative Structure-Activity Relationships (QSAR) models can be tried and used if the models are strong enough for prediction purposes after a dataset of significant size and good potency becomes available. Similarly, machine-learning based models can also be used if the target is well-known and has a large number of compounds that are already known in public literature or public databases. If the machine-learning models are sufficiently resilient, they can be applied to scaffold-hopping hits, virtual screening, or filtering design concepts. Clinical candidate molecules for a number of targets, for which the target structure was unknown at the time, have been successfully delivered by Jubilant. The medicinal chemistry and computational chemistry teams work closely together to manage the projects that call for LBDD activities. Computational techniques are used in Ligand-based Virtual Screening (LBVS), sometimes referred to as ligand similarity searching, to find compounds that are anticipated to bind to therapeutic targets. In order to determine and forecast which prospective medications have the highest likelihood of binding precisely and effectively to molecules that are essential to biology, LBVS is used. Drug discovery and development procedures can be completed more quickly and effectively thanks in large part to this service. To ensure value in research and development, LBVS identifies possible ligand molecules with similar behaviors or qualities from many databases and concentrates on the most promising ones.

MOLECULAR DOCKING

A computational method for predicting ligand binding affinities to receptor proteins is called molecular docking. While it may find applications in the field of nutraceutical research, it is a powerful instrument in the drug development process. Nutraceuticals are bioactive compounds found in food sources that have the potential to treat and prevent disease. Finding their molecular targets may aid in the development of novel treatments tailored to individual diseases. This review aimed to investigate the potential applications of molecular docking in the field of dietary supplements and disease management. Firstly, an introduction to the principles of molecular docking and the several docking software tools was given. Additionally discussed are the drawbacks and challenges of applying molecular docking to nutraceutical research, such as the need for experimental validation and the dependability of scoring functions. The identification of molecular targets for nutraceuticals in several disease models—such as those for sickle cell disease, cancer, cardiovascular, gastrointestinal, reproductive, and neurological disorders—was also a focal point of the research. In order to identify new nutraceuticals' effects on disease pathogenesis, we also emphasized biochemistry pathways and models from recent studies that have revealed molecular mechanisms. Molecular docking is a valuable method in the identification of nutraceuticals' molecular targets for disease management, and this claim is persuasively supported. It might provide insights into the mechanisms of action of nutraceuticals and aid in the development of novel treatments. Consequently, molecular docking holds great promise for the development of novel medications for the treatment of illness and has a promising future in the field of nutraceutical research. The number of configurations and binding modes are determined by the experimentation approach. The Monte Carlo approach, fragment and genetic based systemic searches, are used for docking analysis. The number of configurations created and the binding modes are determined by the approach. The Monte Carlo approach, fragment and genetic based systemic searches, are used for docking analysis. This scoring system makes use of statistics from a huge collection of protein-ligand complex crystal structures to determine the observed interatomic interaction frequencies. High binding will occur in molecular interactions at the base's highest interaction frequency. Docking involving Flexible docking and Rigid docking. [21-22]

Rigid Docking:

We are looking for a way to rearrange one of the compounds in three dimensions so that, under the conditions of a scoring system, it best matches the other compounds, assuming that the compounds are rigid. It is possible for the ligand to form its shape with or without receptor binding activity. Both the

ligand and the protein are viewed as rigid entities in the simplest type of docking. This implies that during the docking procedure, no structural alterations or flexibility are permitted. Despite being less costly computationally, this approach may overlook possible binding interactions due to its failure to take into consideration the inherent flexibility of ligands and proteins.

Flexible Docking:

In computer drug design, flexible docking is a kind of molecular docking where the target protein (the protein) and the ligand (the small molecule) are both simulated as flexible entities. With the dynamic nature of both the protein and ligand taken into account, this method enables more accurate modeling of the possible binding of a drug candidate to a biological target. In flexible docking, a greater variety of binding poses can be explored by either or both of the ligand and the protein changing their shape throughout the docking process. As a result, it is possible to predict how the ligand will interact with the target and fit into the binding pocket more precisely.

Molecular Docking Steps

The intermolecular interaction between two drug molecules was investigated using the in-silico approach. The macromolecule is the protein receptor. It had an inhibiting effect. The docking process entails the following steps: [23-24]

Step–1 Protein and Ligand Preparation: The protein's 3D structure was downloaded from the Research Collaboratory Structural Bioinformatics Protein Data Bank (PDB). Pre-processing of the downloaded structure is then required. After the water molecules are eliminated, the charges stabilize, the empty residues are filled, and side chains of hydrogen atoms are added.

Step–2 Ligand Preparation: The Pub Chem Ligands molecule can be downloaded by using several databases, such as ZINC. It can be drawn in a Mol file using the Chem Sketch Tool. applied LIPINSKY'S RULE OF 5 to this ligand molecule after that. For drug-like and drug-unlike compounds, it is employed. It lowers the failure rate and raises the likelihood of success. Protein and Ligand Preparation: Based on the Protein Data Bank (PDB) structure from the Research Collaboratory Structural Bioinformatics. The downloaded structure was then processed. After the water molecules are removed from the cavity, the charges are stabilized, the missing residues are filled, and side chains of hydrogen atoms are added utilizing various methods It is possible to download the Chem Ligands molecule. It can be drawn in a Mol file using the Chem Sketch Tool. Applied LIPINSKY'S RULE OF 5 to this ligand molecule after that. For drug-like and drug-unlike compounds, it is employed. Because of the molecules' drug-like qualities, it raises the success rate and lowers the failure rate.

Step-3 Grid Generation: Constraints, excluded volumes, and rotatable groups were maintained at this location. The crucial factor in deciding is the quantity of operations (crossover, migration, and mutation) carried out. The task at hand is Binding Cavity Prediction.

Step–4 Active site prediction: the location of the protein molecule should be anticipated. Following the preparation of the protein, the water molecules and, if any, heteroatoms are extracted from the cavity.

Step-5 Docking: Analysis is done on ligand-protein interactions. The best docking score ought to be chosen.

Pharmacophore Modelling

A pharmacophore is a molecular framework that explains the essential characteristics of a molecule that give rise to its biological activity [25]. Pharmacophore models are created in order to improve knowledge regarding the interactions between ligands and proteins. They can be used to find novel compounds that meet pharmacophore criteria and are therefore anticipated to be active. If the target structure is not accessible, pharmacophore models can be constructed utilizing the structural data of the active ligands that bind to the target. The term "ligand-based pharmacophore modeling approach" refers to this. Pharmacophore models can be constructed utilizing the target's structural characteristics when the target's structure is known. This modeling approach is referred to as structure-based pharmacophore modeling [26,27]. Several pharmacophore modeling programs are available for use. Software used for pharmacophore model generation includes HipHop, Hypo Gen, Pharmer, PHASE, GASP, Pharma Gist, Pharm Mapper, MOE, Ligand Scout, and GALAHAD. Pharmacophore modeling has been used using such software at different phases of the drug discovery process. Among its widely used application fields are virtual screening, drug target fishing, ligand profiling, docking, and ADMET (absorption, distribution, metabolism, excretion, toxicity) prediction. [28,29] By overcoming the obstacles, the use of pharmacophore modeling in the drug discovery process has grown more widespread. Pharmacophore scoring systems that are utilized in molecular alignment, modeling ligand flexibility, virtual screening, and training set selection present certain difficulties. The other computational techniques play a critical role in helping us overcome these obstacles[30]. Therefore, some of these constraints are resolved by integrating pharmacophore modeling with additional computational techniques. For instance,

phamacophore modeling and molecular dynamics simulations have been combined. Better pharmacophore models have been constructed as a result of this integration. Furthermore, the advancements in pharmacophore modeling have gained impetus thanks to the contribution of the most recent computational techniques, such machine learning. This paper explains the fundamental ideas behind pharmacophore modeling as well as its main uses in the drug discovery process. Furthermore, discussed are the difficulties encountered and their likely resolutions as a result of developments in computational techniques [31-33].

PRINCIPLES OF PHARMACOPHORE MODELING

Paul Ehrlich first proposed the idea of a pharmacophore in the early 1900s. Subsequently, the term "pharmacophore" was introduced, signifying molecular characteristics that possess (phoros) the essential attributes required for a drug's biological activity (pharmacon). Pharmacophore was defined in those years as the chemical or functional groups on a molecule that give rise to biological action. Pharmacophore is defined by IUPAC (International Union of Pure and Applied Chemistry) as the total of steric and electronic properties necessary for a molecule to interact with a target and produce biological activity [34]. A compound's pharmacophore is a pattern of characteristics that determines its biological activity. This demonstrates that characteristics, rather than chemical groups, are the main focus of the pharmacophore idea. A pharmacophore pattern can be created from any atom or grouping within a molecule that exhibits characteristics linked to molecular recognition. Hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), positive and negative characteristics, aromatic rings, hydrophobic features, and their combinations can all be found in molecular pharmacophore patterns [60,61]. Multiple patterns grouped in a certain 3D (three-dimensional) arrangement make up a pharmacophore model. Every pattern is represented by a standard sphere whose radius establishes the tolerance for variation from the precise location [35,36]. There are other ways to display as well. These designs can be shown alone or in combinations. In the process of finding new drugs, two main pharmacophore modeling techniques are employed: Two methods of modeling pharmacophores are ligand-based and structure-based. A set of active ligands that are already on hand is used to design novel ligands in the ligand-based pharmacophore modeling approach [37,38]. In the event that the target structure is unavailable, this strategy is used. Similar to this, when the target protein's structure is known, the structure-based pharmacophore method is used. The first active ligands in the ligand-based pharmacophore modeling are found by searching databases or the available literature. A training set and a test set are created from the data set. Subsequently, the training set ligands undergo feature analysis. The alignment of the active ligands allows for the detection of common characteristics. The creation of pharmacophore models and the ranking of those models come next. Ultimately, pharmacophore model validation is carried out, and based on the outcomes, the optimal pharmacophore model is chosen [39,40]. The first stage in the structure-based pharmacophore modeling process is to choose and prepare the target protein structure. Predicting the binding location is the second stage. After that, a thorough analysis is done to identify the complementary chemical properties of the binding site amino acids and their layouts. The pharmacophore characteristics are then generated; these should be optimized using the modified tools in the used programs. Lastly, the activity-causing pharmacophore properties that are most important are chosen. Among the frequently used programs for structure-based pharmacophore modeling are Ligand Scout, MOE, Pocket v2, and Snooker. Similar to this, pharmacophore modeling uses a variety of servers and applications. A summary of frequently used servers and programs is provided in alphabetical order [41-44].

APPLICATIONS OF PHARMACOPHORE MODELING IN DRUG DISCOVERY

Pharmacophore modeling finds applications in docking, ligand profiling, ADMET prediction, virtual screening, and fishing drug targets. Because pharmacophore modeling is so flexible and easy to understand, new insights into its many uses are anticipated in the future. Thus, in addition to the uses described here, it might find use in side effect prediction, drug repurposing, and poly pharmacology. So many articles over the past 20 years are shown to illustrate the breadth of pharmacophore modeling's use in drug discovery. The average number of documents found in ScienceDirect, PubMed, and Scopus is represented by these numbers. These can be found by entering the keywords "pharmacophore modeling" and "drug discovery" into these search engines. The publications produced demonstrate how pharmacophore modeling is being used more and more in drug discovery [45].

CONCLUSION

From preclinical discovery to late-stage clinical development, in-silico drug design can play a major role in the drug development process. Many important measures are made to eliminate compounds that exhibit medication interactions and have adverse effects throughout the process of selecting innovative therapeutic candidates. Software for in-silico drug design was essential in the pharmaceutical industry for creating new proteins or medications. Its misuse in the process of developing new drugs aids in the selection of just one potent lead chemical and helps to avert the most recent clinical failures, which can result in a significant reduction in cost.

REFERENCES

1. Daina A, Michielin O, Zoete V. (2017). Swiss ADME: a free web tool to evaluate pharmacokinetics, drug likeness and medicinal chemistry friendliness of small molecule. Scientific Report, 3, 7(1): 1-3
2. Department of Biotechnology, R.V Collage of Engineering, Mysore Road, Bangalore- 560 059 India.
3. Green DV, Segall M (2014). Chemoinformatic in Lead Optimization. Chemoinformatic for Drug Discovery 149-178.
4. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2012) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced drug delivery reviews 64: 4-17.
5. Vacca J P, Condra J H (1997) Clinically effective HIV-1 protease inhibitors. Drug Discovery Today 2: 261-272.
6. Smith C (2003) Drug target validation: Hitting the target. Nature 422: 341-345.
7. Baig MH, Shakil S, Khan AU (2012). Homology modeling and docking study of recent SHV type β-lactamses with traditional and novel inhibitors: an in-silico approach to combat problem of multiple drug resistance in various infections. Medicinal Chemistry Research 21(9): 2229-2237.
8. Shan Y, Kim ET, Eastwood MP, Dror RO, Seeliger M A, Shaw DE (2011). How does a drug molecule find its target binding site?. Journal of the American Chemical Society 133(24): 9181-9183.
9. Doman TN, McGovern SL, Witherbee BJ, Kasten TP, Kurumbail R, et al. (2002) Molecular docking and high-throughput screening for novel inhibitors of protein tyrosine phosphatase-1B. Journal of Medicinal Chemistry 45: 2213-2221.
10. Baig MH, Danishuddin M, Khan S, Khan AU. (2012). Screening of inhibitors for S130G inhibitor resistant mutants of TEM type beta lactamase. Bioinformation 8(24): 1225.
11. Danishuddin M, Khan A, Faheem M, Kalaiarasan P, Hassan Baig M, et al. (2013). Structure-based screening of inhibitors against KPC-2: designing potential drug candidates against multidrug-resistant bacteria. Journal of Biomolecular Structure and Dynamics 32(5):741-750
12. Laskowski RA, Hutchinson EG, Michie AD, Wallace AC, Jones ML, et al. (1997) PDBsum: a Web-based database of summaries and analyses of all PDB structures. Trends Biochemical Sciences 22: 488-490.
13. Brady SF, Stauffer KJ, Lumma WC, Smith GM, Ramjit HG, et al. (1998) Discovery and development of the novel potent orally active thrombin inhibitor N-(9-hydroxy-9-fluorenecarboxy) prolyl trans-4 aminocyclohexylmethyl amide (L-372,460): coapplication of structure-based design and rapid multiple analogue synthesis on solid support. Journal of Medicinal Chemistry 41: 401-406.
14. Klebe, G.; Abraham, U.; Mietzner, T. (1994): Molecular similarity indices in a comparative analysis (CoMSIA) of drug molecules to correlate and predict their biological activity. J. Med. Chem. 37, 4130–4146.
15. Weiner, S.J.; Kollman, P.A.; Case, D.A.; Singh, U.C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. (1984): A new force field for molecular mechanical simulation of nucleic acids and proteins. J. Am. Chem. Soc. 106, 765–784
16. Ewing TJ, Makino S, Skillman AG, Kuntz ID. (2001): DOCK 4.0: Search strategies for automated molecular docking of flexible molecule databases. J Comput Aided Mol Des.;15(5):411-428.
17. Zsoldos Z, Reid D, Simon A, Sadjad BS, Johnson AP. (2006): eHiTS: An innovative approach to the docking and scoring function problems. Curr Protein Pept Sci. ;7(5):421–435.
18. Miranker A, Karplus M. (1991): Functionality maps of binding copy sites: simultaneous A multiple search method. Proteins. ;11(1):29–34.
19. Verdonk ML, Cole JC, Hartshorn MJ, Murray CW, Taylor RD. (2003): Improved protein ligand docking gold. Proteins. 2003;52(4):609–623.
20. Srinivasan J, Cheatham TE, Cieplak P, Kollman PA, Case DA. (1998): Continuum solvent studies of the stability of DNA, RNA, and phosphoramidateâ^'DNA Helices. Journal of the American Chemical Society. ;120(37):9401–9409.
21. Gabb HA, Jackson RM, Sternberg MJ. (1997): Modelling protein docking using shape complementarity, electrostatics and biochemical information. J Mol Biol. ;272(1):106–120.
22. Jain AN. (1996): Scoring noncovalent protein ligand interactions: a continuous differentiable function tuned to compute binding affinities. J Comput Aided Mol Des.;10(5):427 440.
23. Muegge I, Martin YC. (1999): A general and fast scoring function for protein-ligand interactions: A simplified potential approach. J Med Chem.;42(5):791- 804.
24. Guner O. (2005): History and Evolution of the Pharmacophore Concept in Computer-Aided Drug Design. Curr Top Med Chem.;2(12):1321–1332.

25. Sanders MPA, McGuire R, Roumen L, De Esch IJP, De Vlieg J, Klomp JPG, et al. (2012): From the protein's perspective: The benefits and challenges of protein structure-based pharmacophore modeling. Med Chem Comm. ;3(1):28–38.
26. Lin, Shu-Kun Sutter, J.M. Hoffman R. (2000): HypoGen: An automated system for generating predictive 3D pharmacophore models. In: Güner O, editor. Pharmacophore Perception, Development and Use in Drug Design. International University Line; 171–189.
27. Prachayasittikul V, Worachartcheewan A, Shoombuatong W, Songtawee N, Simeon S, Prachayasittikul V, et al. (2015): Computer-Aided Drug Design of Bioactive Natural Products. Curr Top Med Chem. ;15(18):1780–1800.
28. Gao Q, Yang L, Zhu Y. (2010): Pharmacophore Based Drug Design Approach as a Practical Process in Drug Discovery. Curr Comput Aided-Drug Des. ;6(1):37–49.
29. Yang SY. (2010): Pharmacophore modeling and applications in drug discovery: Challenges and recent advances. Drug Discov Today. ;15(11 12):444–450.
30. Vazquez J, Lopez M, Gibert E, Herrero E, Luque FJ. (2020): Merging ligand-based and structure-based methods in drug discovery : An overview of combined virtual screening approaches. Molecules. ;25:4723–4750.
31. Zeng L, Guan M, Jin H, Liu Z, Zhang L. (2015): Integrating pharmacophore into membrane molecular dynamics simulations to improve homology modeling of G protein-coupled receptors with ligand selectivity: A2A adenosine receptor as an example. Chem Biol Drug Des. ;86(6):1438–1450
32. Schaller D, Šribar D, Noonan T, Deng L, Nguyen TN, Pach S, et al. (2020): Next generation 3D pharmacophore modeling. Wiley Interdiscip Rev Comput Mol Sci.;10(4):1–20.
33. Güner OF, Bowen JP. (2014): Setting the record straight: The origin of the pharmacophore concept. J Chem Inf Model. ;54(5):1269–1283.
34. Kaserer T, Beck KR, Akram M, Odermatt A, Schuster D, Willett P. (2015): Pharmacophore models and pharmacophore-based virtual screening: Concepts and applications exemplified on hydroxysteroid dehydrogenases. Molecules.;20(12):22799-22832.
35. Bajorath J. (2015): Pharmacophore. In: Schwab M, editor. Encyclopedia of Cancer. Berlin Heidelberg: Springer; p. 2–5. ISBN: 978-3-540-47648-1.
36. McGregor MJ, Muskal SM. (1999): Pharmacophore fingerprinting. 1. Application to QSAR and focused library design. J Chem Inf Comput Sci. ;39(3):569–574.
37. Horvath D, Mao B, Gozalbes R, Barbosa F, Rogalski SL. (2005): Strengths and Limitations of Pharmacophore-Based Virtual Screening. Chemoinformatics Drug Discov. ;23: 117–140.
38. Sheridan RP, Rusinko A, Nilakantan R, Venkataraghavan R. (1989): Searching for pharmacophores in large coordinate data bases and its use in drug design. Proc Natl Acad Sci U S A. ;86(20):8165–8169.
39. Noha SM, Schuster D. (2013): Pharmacophore modeling. In: Lill MA, editor. In Silico Drug Discovery and Design. p. 80–93. ISBN: 9781909453029.
40. Horvath D. (2011): Pharmacophore-Based Virtual Screening. In: Bajorah J, editor. Chemoinformatic and Computational Chemical Biology. Springer; 261–297. ISBN: 9781493957934.
41. Leelananda SP, Lindert S. (2016): Computational methods in drug discovery. Beilstein J Org Chem. 2016; 12:2694–2718.
42. Wolber G, Langer T. (2005): LigandScout: 3-D pharmacophores derived from protein-bound ligands and their use as virtual screening filters. J Chem Inf Model. ;45(1):160–169.
43. Vilar S, Cozza G, Moro S. (2008): Medicinal chemistry and the Molecular Operating Environment (MOE): Application of QSAR and molecular docking to drug discovery. Curr Top Med Chem. ;8(18):1555.
44. Chen J, Lai L. (2006): Pocket v.2: Further developments on receptor-based pharmacophore modeling. J Chem Inf Model.;46(6):2684–2691.
45. Sanders MPA, Verhoeven S, De Graaf C, Roumen L, Vroling B, Nabuurs SB, et al. (2011): Snooker: A structure-based pharmacophore generation tool applied to class A GPCRs. J Chem Inf Model. ;51(9):2277–2292.