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Computer–Aided Drug Discovery and Design (CADDD) - A New Approach for the Development of Novel Drugs

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ABSTRACT

The process of drug discovery and development is a highly complex, daunting and time-consuming. It has been estimated that the traditional method of drug development takes billions of USD and about 15 years. However, with the advent of modern computational (or say in-silico) techniques, now scientists are capable of speeding up the developmental process with high efficiency and at a lower cost. The process of designing novel medicinal drugs using in-silico methods is collectively called as- Computer-aided drug discovery and design (CADDD). Majority of the research across the globe are now conducted using in-silico techniques such as structure- and ligand-based drug designing, virtual screening, quantitative structure-activity relationship (QSAR), molecular modeling etc. These computational methods play an important role right from the conceptualization of a promising drug candidate to its experimental development. In this review article, our focus is to discuss some of the major in-silico techniques extensively being used. Furthermore, we also aim to explain the discovery and design of the some of the drugs developed through computational methods such as captopril.

INTRODUCTION

X-ray diffraction and nuclear magnetic resonance (NMR) spectroscopy techniques have been immensely helpful in unraveling chemical composition and three-dimensional (3-D) geometry of a small organic molecule, particularly proteins.[1] Such 3-D structures can be accessed at open access protein databases (*http://www.rcsb.org*).

These 3-D structures of proteins significantly reveal the information about various physiological processes based on interactions between proteins or between proteins and ligands. In 1962, Max Perutz and John Kendrew received Nobel award for the work on the structure of myoglobin. Since then, several scientists have made a considerable contribution to the determination of protein structure until the recent Nobel Prize in Chemistry 2012, which was awarded jointly to Brian Kobilka and Robert Lefkowitz for their work on structural and functional studies of G-protein-coupled receptors (GPCRs).[2]

Currently, many technologies have been developed and employed to boost the drug discovery process. The process of designing novel medicinal drugs using *in-silico* methods is collectively called as Computer-aided drug discovery and design (CADDD).[3] Since its emergence, CADDD has highly emerged to harness various sources of information to facilitate the development of new drugs that interact with the therapeutically interesting protein targets, thus, providing a conducive environment to expedite the drug discovery process by enabling vast libraries of compounds to be screened and synthesized in relatively less time and moreover, at a very low cost. Today, CADDD is a widely used term to represent the process of drug discovery and development, from lead discovery, optimization, target identification and validation, with the aid of numerous computational tools and techniques.[4, 5] Most widely employed computational tools for computer-assisted drug design include, Discovery Studio, MOE, the Schrödinger package and SYBYL which supplies comprehensive drug-design packages/programs to almost all kinds of CADD techniques.

In general, CADDD follows a number of approaches; viz.:

1. Ligand-based drug design

In absence of 3-D structures of biological targets, methods such as Pharmacophore Modeling and Quantitative Structure-Activity Relationship (QSAR) are used for the designing of novel drug targets. These tools give a useful insight of information regarding the nature of target-

ligand interactions, which consequently predict molecular models, which are further used for the lead discovery, and optimization.[6] Ligand-based designing approaches are based on the information about the structure of the active ligand, which can interact with biological targets. The efforts for the designing of the novel drug molecule is based on the concept known as *molecular similarity*; compounds with high molecular structural resemblance with pre-existing ligands, are more likely to have similar pharmacological activity profiles.[7] This approach is considered an indirect methodology as it is usually followed in absence of the 3-D structure of the target or if the target is unknown or cannot be predicted.

There are a number of ways to screen a small molecule library in order to identify key pattern present in a known active molecule or a set of them. The most common and simplest approach is the use of molecular descriptors or physicochemical properties. Molecular features, such as electronic, steric and hydrophobic properties, are obtained from practical experiments or theoretical investigations[8] further, these descriptors are compared with the reference molecule or set of molecules with a large library of compounds at a very low cost.

Another commonly used approach used in the ligand-based design is Pharmacophore Modeling. This approach is more precise than molecular descriptors. A *pharmacophore model* is built by extracting out the key structural information (2-D and 3-D) from the given the set of known active compounds as a reference, which is largely responsible for the interaction with biological targets and the subsequent pharmacological action.

According to the definition carved out by the International Union of Pure and Applied Chemistry (IUPAC), "a *pharmacophore* is the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target, and to trigger (or block) it's biological response.''[9]

The consensus model derived from the structural overlapping of a number of molecules in a particular molecular library represents most suitable and fitting model for the prediction of the new set of compounds. This model defines essential molecular characteristics of the probable novel chemical compounds. The essential features of the compounds contain information about steric interactions, positively and negatively charged groups, hydrogen bond donors and acceptors, or hydrophobic regions, etc. A high degree of complementarity among the new set compounds and the pharmacophore warrants the success of new molecules against the protein target of interest. Pharmacophore modeling has become a key

computational strategy to boost the drug discovery process in absence of biological target structure.[10] This highly efficient modeling approach is now been used extensively for the identification and optimization of lead molecules, high throughput virtual screening of vast molecular libraries across the globe. Various programs such as Catalyst, DISCO tech, Ligand Scout, MOE (its pharmacophore module) and PHASE are widely used for pharmacophore modeling and virtual screening.

Another ligand-based approach, which is more typical and tiresome than the earlier discussed approaches, is QSAR method. This approach consists of developing a simple mathematical equation that can be used to correlate physicochemical properties from the molecular structure of a compound with the biological action. In QSAR modeling, physicochemical properties or theoretical molecular descriptors of chemicals are used as predictors of biological response of a particular molecule. As a result, a simple mathematical relationship is established. Applications of QSAR can be extended to identification of hit compounds, prediction of different kinds biological activities including chemical toxicity, three-dimensional designing of molecules and lead compound optimization. The process of building a QSAR model consists of several steps, which lead to the design of new compounds with the desired activity profile.[11] The commonly used computational tools used for developing QSAR models are comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA).

The well-known examples of drugs discovered by using ligand based drug design include novel COX-2 inhibitors and antimalarial drugs.[12]

2. Structure-based drug design

In contrast to ligand-based methods, structure-based methods rely on the knowledge of the 3-D structure of a macromolecular target or a macromolecule-ligand complex. Both strategies focus on the prediction of the likeliness of the new compound to produce therapeutic action based on the structural target information. Hence, the target is used as a ‘molecular groove’ to computationally simulate interaction with any of the small molecules in the chemical library. Further, the ‘best-fit’ compounds in the ‘molecular groove’ are chosen for advanced stages of the drug development.

These strategies are further extended to improve or refine the alignment of the existing drug molecules with their respective binding sites and subsequently to offer improvement in the

therapeutic action. Such refinements or chemical modification in the ligand molecules largely utilize biochemical information of the ligand-receptor interaction which in turn enhance the steric complementarity with the receptors and resulting in their increased affinity for their receptors. These molecular refinements are often achieved by changing functional groups of the ligand or also by making Vander-Waals interactions more negative of the poorly fitted regions of the ligands within the active sites of the receptors.[10] Software used in structure based drug design are Scigress, SMART, OMEGA etc.

Carbonic anhydrase inhibitor (Dorzolamide) is the classical example of successful application of structure based drug designs methods.[13]

3. Docking

Docking is the process, which essentially comes into play when the multidimensional structure of the macromolecule and the molecular model of the ligand-binding site are precisely available. This approach is quite helpful in the determination of appropriate conformation and orientation of ligand into the binding site of the receptor. With the application of docking, the preferred and stable ligand-receptor interaction can be easily predicted and simulated.[14] Docking begins with the logical posing of small molecules into the active sites of the receptor using docking algorithms. Further, these docking algorithms are supported by scoring functions, which actually are meant to predict the biological activity of the molecule during the energetic process of ligand-receptor interaction. Evaluation of the compound fits by the early scoring functions is mainly based on the computational calculations of the shape and electrostatic features during the interaction. Further, the best compound fits are re-assessed using complex scoring schemes with the employment of some solvation or entropic effects and, with simultaneous comprehensive analysis of electrostatic and Vander Waals interactions.[15]

Thus, docking programs mainly serve three purposes:

- Firstly, to identify potential ligands from molecular libraries.
- Secondly, to predict the binding mode of selected ligands.
- Finally, to provide scoring of compounds for their likeliness of binding with the binding site by calculating putative binding affinities.

Docking programs have shown to be effective in sorting out suitable binding ligands from the vast chemical libraries, which has been further corroborated experimentally. Molecular docking plays a critical role in predicting receptor-ligand interactions leading to the discovery of lead and its optimization.[16] There are a number of docking programs currently available such as Autodock, Autodock Vina, Betadock, Darwin, Dock, etc.

4. Integrated methods

Currently, there has been a surge in integrating both structures- and ligand-based methods. Integration of the both approaches expedites the process of computer-assisted drug design. This method uses information on the structure of the protein or the biological and physicochemical properties of bound ligands, respectively.

The purpose is to enhance the reliability of computational techniques by combining both aspects of the computer-aided design technique that is by using relevant information from both the ligand and the receptor. At the simplest, building a 3-D pharmacophore structure to find potential ligands and performing further docking studies on the biological target constitutes a combined approach. These integrated methods can be classified into two major classes:

a. Interaction-based methods

b. Docking similarity-based methods

Identification of the key interactions between the active sites of the receptor and the ligand using available physicochemical data comes into the category of integration-based methods. These interactions are then used to screen compounds from the molecule libraries, having the potential of producing similar interaction profile.

On the contrary, docking similarity-based methods make the virtual screening very efficient and allow exploring vast libraries of up to 106 small molecules by merging structure-based docking methods with ligand similarity methods.[17- 21]

5. Virtual screening

Screening of a large number of chemical compounds from the molecule libraries against biological targets using *in-silico* methods is called as virtual screening. Researchers had to

carry out this *in-silico* process well-before in the beginning of a rational drug-design process. Virtual screening has been always a daunting and expensive task, many times affordable only to the big pharmaceutical research organization. It is a trial-and-error approach to find potential ligands against biological targets from chemical libraries, which need the high degree of precision and expertise; sometimes-good luck and intuition. This exasperating procedure included testing and screening of around 106 small chemical molecules with the use of powerful computers. The process of *virtual screening* consists of the rapid selection of active compounds from large digital molecule databases that are capable enough of modulating biochemical processes inside biological systems. The cost/benefit ratio justifies the relevance of virtual screening over physical screening in almost every drug-design projects across the globe. However, despite the use of different algorithms and strategies for virtual screening, its success considerably relies on the nature of the project and is a matter of constant debate. Virtual screening offers many advantages over physical screening, for example, it can also be applied for the testing of compounds which do not actually exist. Besides, it is significantly less resource-intensive and faster technique. Thus, millions of compounds can be analyzed by virtual screening. However, it is important to keep in mind that virtual screening is still a relatively coarse filter, which particularly considers structure-based screening because the prediction of binding affinities remains one of the holy grails of computational chemistry.[22] Software packages used for virtual screening includes, DOCK, EUDOC, LUDI, CATALYST, ICM, etc.

Major chemical databases of interest for drug-discovery used now a day are [23]

- Open National Cancer Institute Database
- PubChem
- Relibase
- ChemSpider
- DrugBank
- World Drug Index
- Therapeutic Target Database

- ZINC

CADDD PIPELINE

Computer-assisted drug design can be used at any of the following stages of drug discovery:

a) Hit identification (structure or ligand- based design)

b) Hit to lead optimization (structure- based design, QSAR, etc.)

c) Lead optimization of other pharmaceutical properties while maintaining affinity.

d) Molecular modeling

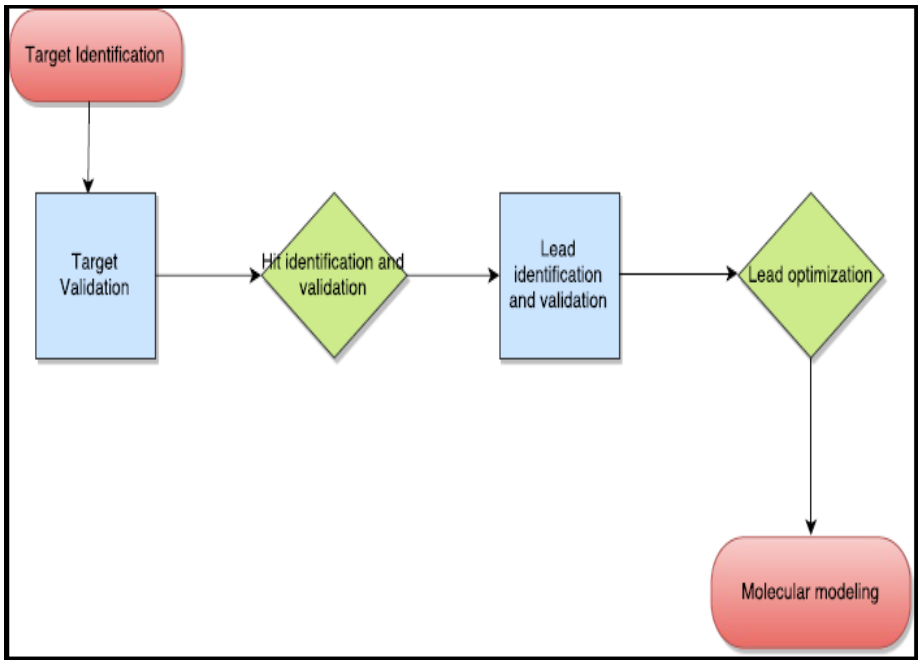
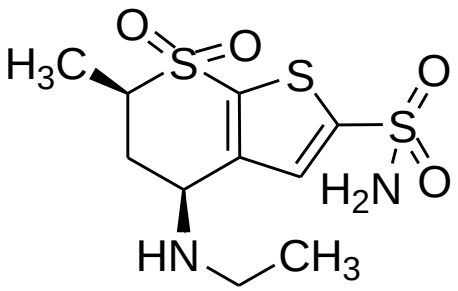


Fig. 1: CADDD Work line

SUCCESSFUL APPLICATIONS OF CADDD: [24-27]

Sr. No.	Biological Target	Drug	Therapeutic Use
1	Acetyl cholinesterase	Zanapezil	Alzheimer's disease
2	Aldose reductase	Lidorestat	Diabetic neuropathy
3	Angiotensin converting enzyme	Captopril	Hypertension
4	Carbonic anhydrase	Dorzolamide	Glaucoma
5	Caspase -1	Pralnacasan	Rheumatoid & osteo arthritis
6	erbB (EGF receptor tyrosine kinase)	Canertinib	Cancer
7	Fibrinogen receptor	Elarofiban	Thrombosis
8	Hemoglobin	Efaproxiral	Radiosensitizer
9	HIV protease	Nelfinavir	HIV
10	HIV protease	Amprenavir	HIV
11	HIV protease	Lopinavir	HIV
12	HIV protease	Tipranavir	HIV
13	Influenza	Zanamivir	Influenza
14	Influenza neuraminidase	Oseltamivir	Influenza
15	Renin	Aliskiren	Hypertension
16	Thymidylate synthase	Nolatrexed	Cancer
17	HIV protease	Mozenavir	HIV
18	IMP dehydrogenase	VX- 148	Psoriasis

1. DORZOLAMIDE



Dorzolamide

Dorzolamide, a carbonic anhydrase inhibitor, is undoubtedly the first evidence of the successful application of structure-based drug-design in the history of mankind.

It was approved by USFDA in the year 1994 for the treatment of glaucoma. It works by reducing (about 20%) intraocular pressure in open angle glaucoma and ocular hypertension.[28]

CADD Work line

Initially, taking into account the proper balance between aqueous solubility and lipophilicity for the enhancement of the activity a structural series of carbonic anhydrase inhibitors, optimization operations were carried out. This solubility balance further permitted topical use in the treatment of ocular diseases. Carbonic anhydrase inhibitors block the local conversion of carbon dioxide to bicarbonate, a critical step in the active secretion of aqueous humor. The isozyme, *carbonic anhydrase* II (CA II), found within the secretory cells of the eye further served for the purpose of designing of drugs targeting their active site.[28] The cone shaped active site of CA II consists of narrow catalytic hydrophobic pocket having an arrangement of zinc in a tetrahedral fashion to three histidine residues, namely, His94, His119, and His96. The two walls of the active site pocket are opposite in nature, one wall is dominated by hydrophobic and the other by hydrophilic residues, which make the active site amphiphilic in nature.[29-33]

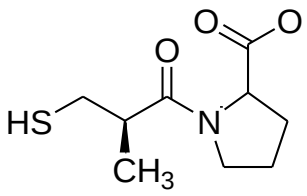
It was found that the prototype compound MK-927, hydrophilic compound, rapidly penetrates ocular tissue, and lowers intraocular pressure (IOP) in animal models. Furthermore, the experimental analysis in competition assay versus dansyl amide, shown the difference (100-folds) in affinity of the two enantiomers of the prototype compound. [28]

The research team of Baldwin *et al.*, successfully decoded the phenomenon, and identified that the sulfonamide group in both enantiomers interacted to zinc within the catalytic site through the presumably deprotonated sulfonamide nitrogen while the heterocyclic thiophene ring was stationed in between the hydrophobic and hydrophilic walls of the active site pocket.[29- 33] However, the alkyl amino group, of both enantiomers, was placed in the less favored pseudoaxial-orientation. *Ab initio* calculations (at the 6-31 *G** level) revealed that the pseudo equatorial conformer would be preferred by about delta H 1 kcal/mol. The overall conformational geometry of the two enantiomers was found to be similar except the N-S-C-S dihedral angle and geometry of the 4-isobutyl amino substituent. First, the N-S-C-S dihedral angle which was differing by about 20° of the thiophene ring in the *R*-isomer (170°) relative to the *S*(150°). *Ab initio* molecular orbital calculations (at the 3-21 *G** level) suggested that the preferred N-S-C-S dihedral angle, which was calculated to be 72° implied that while the angle formed in the *S*-enantiomer is not ideal it is preferred by a delta H of 1 kcal/mol.

A second conformational difference, the geometry of the 4-isobutyl amino substituent, arose due to *trans* position of the side chain (4-isobutyl amino substituent) in *S* enantiomer and *gauche* in the *R.* Further, *Ab initio* measurements (at the3-21 *G** level) recommended that the *trans* orientation should be preferred by 1 kcal/mol.

Therefore, these two conformational features attributed to the 100-fold difference in affinity and potency for the two enantiomers.[28] Given the facts, it was proposed to introduce a methyl group into the 6-position of the thieno-thiopyran ring system, thus leading to the increased affinity. In addition, a 4-isobutyl amino group was replaced by an ethyl amino moiety to counter the enhanced lipophilicity, resulting in the discovery of Dorzolamide. Information obtained after co-crystallized of all enantiomers with human CA II revealed the facts that isomer having Trans *S, S* configuration and a *Ki* value of 0.37 nM exhibited the greatest inhibitor potency and affinity. While data from X-ray crystallography established the fact that thiophene sulfonamide N-S-C-S dihedral angle is the major conformational factor for the optimum affinity within the active site.[28, 34]

2. CAPTOPRIL



Captopril

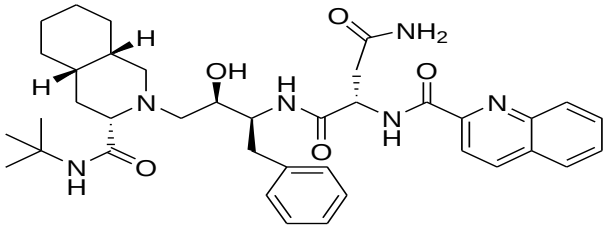
Captopril, an angiotensin-converting enzyme (ACE) inhibitor, is a breakthrough discovery in the medicinal world with the application of concepts of ligand-based drug-design approach. This drug was discovered in 1971 by Nobel Laureate John Vane which was further approved by USFDA in the year 1981. Captopril is now commonly used the drug for the treatment of cardiovascular disorders such as hypertension, congestive heart failure, and myocardial infarction, etc. Captopril reversibly inhibits angiotensin-converting enzyme during the renin-angiotensin cascade.[35, 36]

CADD Work line

Angiotensin-converting enzyme (ACE), a carboxypeptidase with a zinc ion as a cofactor, plays a critical role in the renin-angiotensin mechanism for the regulation of blood pressure in the body. The development of the antihypertensive drug captopril is an example of one of the early endeavors and successes of ligand-based drug design. Preliminary knowledge of the similar enzymatic mechanism of ACE and carboxypeptidase A at the carboxyl end of the protein established the platform for the design and development process of Captopril as a potent ACE inhibitor. Another important piece of information in the process was the discovery of L-benzyl succinic acid as carboxypeptidase an inhibitor, and research analysis of the inhibitory action BPP5a (HOOC-Glu- Lys-Trp-Ala-Pro-NH) against ACE.[37, 38] Further investigations suggested that N-terminal peptide fragments, including tetra, tri, and dipeptide fragments (Ala-Pro) retained inhibitory activity of the parent molecule. In fact, L-benzyl succinic acid was classified as the byproduct-type of carboxypeptidase An inhibitor.[37] In the design process of Captopril, details of the peptidase reaction included were the formation of two peptide fragments in the catalysis. In the case of benzyl succinic acid, the amino functionality is replaced by an isosteric methylene group.Further, considering benzyl succinic acid as a model entity, it was proposed that succinyl amino acids could also behave as

byproduct inhibitors of ACE. Structure-activity relationship (SAR) studies initially performed on the succinyl-proline scaffold culminated to the synthesis of Captopril. In the line of SAR studies, in the initial stages, carboxylic residue of succinic acid was modified with mercapto moiety having stronger zinc-coordinating property and also, a stereospecific methyl group was incorporated to the succinyl moiety. This incorporation of methyl functionality was brought about to emulate the methyl group that is naturally present in the L-Ala residue of Ala-Pro. Finally, these well-coordinated efforts pioneered by Cushman and Ondetti stemmed development of Captopril.[35, 36]

3. SAQUINAVIR



Saquinavir

Saquinavir, an antiretroviral drug, is used for the treatment/ prevention of human immunodeficiency virus (HIV)/ Acquired immunodeficiency syndrome (AIDS). Saquinavir potentially inhibits protease enzyme which is used by HIV for its replication within the cell and release of mature viral particles from an infected cell.[39]

CADD Work line:

Human immunodeficiency virus-1 protease (HIV-1 PR) holds a vital role in the life cycle of HIV. This enzyme is responsible for the replication of HIV inside infected cells and subsequent release of viral particles out of the infected cells to start new life cycle. In the life cycle of HIV protease holds the vital role. This enzyme is responsible for the replication of HIV inside infected cells and subsequent release of viral particles out of the infected cells to start new life cycle.[40- 42] Saquinavir was designed as the protease inhibitor. Designing of saquinavir lies based on Phe-Pro substrates. [39] This substrate is known to be selectively cleaved by HIV-1 PR but not by mammalian *proteases.* Thereafter, crystallographic investigation of the complex between HIV-1 PR (biological target) and peptidic inhibitor Ro 31-8558 (ligand) revealed the expected orientation of ligand within the binding site. Further

analysis of the complex gave the critical clue for the desired modification in the ligand structure.[43]

Furthermore, structure activity relationship (SAR) studies guided the development of saquinavir from the initial compound Ro 31-8558.SAR studies resulted into the introduction of hydroxyethyl amine transition state moiety, replacement of proline at P1 and quinoline at P3 by a bulky (*S*, *S*, *S*)- decahydro-isoquinolin-3-carbonyl (DIQ) group which enables the drug molecule to form a hydrogen bond with the water molecule connecting the inhibitor with the flap regions.[44]

CONCLUSION

The process of conventional drug discovery and development is recognized to be very expensive and time-consuming. Fortunately, due to recent advancement in the *in-silico* methods for developing physicochemical models to simulate bio-molecular processes, discovering and designing of novel drugs is now under the reach of many research laboratories. Earlier, drug development projects were considered highly expensive, demanded greater labor and sophistication, thus affordable only to the big research organization. Now, modern techniques have made this drug development process affordable to comparatively small research institutes and within small researcher groups. Moreover, challenging projects not even conceivable two decades ago can be today tackled with the access to a supercomputer. By using CADDD approaches, researchers are accelerating their steps and projects. A number of potent and widely prescribed drugs for the treatment of several fatal disorders and pathogenic infections have been successfully developed using CADDD techniques; among them, Dorzolamide, Captopril, Zanamivir, etc. are at the forefront. Besides, many others are continuously writing success stories of the science behind these wonderful techniques.

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