Computer Aided Drug Discovery (CADD) in Plant Pathology: An overview of 'structure based drug discovery'.

By

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DECLARATION

I, Yogeesha G G (2018-11-147), hereby declare that the seminar report entitled "Computer Aided Drug Discovery (CADD) in Plant Pathology: An overview of 'structure based drug discovery'." has been completed by me independently after going through the reference cited herein and I have not copied from any of the fellow students or previous seminar reports.

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CERTIFICATE

This is to certify that the seminar report entitled 'Computer Aided Drug Discovery (CADD) in Plant Pathology: An overview of structure based drug discovery' has been solely prepared by Yogeesha G G (2018-11-147) under my guidance and has not been copied from seminar reports of seniors, juniors or fellow students.

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1. Introduction

The study of plant diseases is important as they cause economic loss. Various types of losses occur in the field, in storage or any time between sowing and consumption of produce. The diseases are responsible for direct economic loss and material loss. Further, these diseases are harmful for the society as they cause stomach disorders, paralysis and liver diseases. Hence, the diseases are required to be prevented and controlled to avoid loss of valuable food. Control of plant diseases is one of the challenges that human being face in present as well as future (Strange and Scott, 2005). To overcome these challenges there are methods which have been used separately or in combination to prevent or control the plant diseases. The farmers are depending heavily on pesticides and chemicals in order to control the plant diseases, which eventually increasing the cost of production (Chandler *et al.*, 2011). In recent days the improved research on molecular attributes of pathogens that confer ability to cause disease on their host, created hope in molecular plant pathologists that provide targets for molecular breeding and discovery of agrochemicals (Boyd *et al.*, 2013). In the last two decades the computer aided drug designing has become popular method of choice for drug developer in pharmaceutical industries that offers advantages in terms of cost and time effectiveness of the technique (Taylor, 2015). However, this method of drug discovery not been extensively used in agriculture in the development of chemicals or drug molecules that can act as a pesticide in control of diseases. The use of this technique in plant pathology is carried by a few researchers (Kandakatla and Ramakrishnan, 2014; Pathak *et al.*, 2016; Soundararajan *et al.*, 2011; Zhou *et al.*, 2015; Shanmugam and Jeon, 2017).

Drug (ligand) - Small chemical compound or small chemical molecule that can bind to macromolecules such as protein or enzyme and modifies the protein or its synthesis.

Receptor (target) - Biological macromolecules which are nothing but proteins or enzymes involved in catalysis.

Drug discovery - It is an effort to produce new drug molecules from a lead compound by applying variety of approaches of design. Drug design approach is the prerequisite for drug discovery.

Drug development - Drug development is the process of establishing and marketing a biologically active compound obtained by drug design, as a suitable drug by observing pharmacokinetics (ADME), toxicological and clinical parameters.

2. Traditional drug designing/discovery

Historically, drugs were discovered by identifying the active ingredient from traditional remedies or by serendipitous discovery and which are developed based on research conducted on Indigenous traditional knowledge (ITK). The discovery of penicillin made drug discovery as an adventurous research activity because of uncertainty of discovering chemical compound that acts as a drug. The use of computer software is not needed for screening and optimization of chemical compound in traditional discovery process. The screening is the most tedious step in case of this method because, in order to get a drug, we have to screen millions of compounds which is time consuming and it is uncertain that we may end up with the compound or may not. The cost of development in this process may reach up to 800 billion of rupees and it needs more than 12 years (Kraljevic, 2004). In order to overcome these drawbacks pharmacists looking for more opportunistic approaches such as Computer aided drug designing, which is time and cost effective.

3. Modern drug designing/discovery

It is the use of Computational resources and tools in therapeutic drug discovery process. The softwares and biological databases are essential components of a successful drug discovery process. Basically this approach used to study the aspects of structural biology to know how protein control living processes, molecular structure - function relationship between drug and receptor molecules and their complementarity with respect to shape and charge. The modern drug discovery speeds up the screening and optimization of drug molecules with specially designed biological softwares and statistical approaches.

4. Computer Aided Drug Discovery (CADD)

It is the use of Computational resources and tools in therapeutic drug discovery process. The technique is a multidisciplinary approach and it involves several areas such as Bioinformatics, Medicinal Biochemistry, Toxicology, Pharmacology, Biophysical Chemistry and Information Technology. The technique mainly utilizes the biological databases and designed softwares which are meant to handle the biological processes. In plant pathology, especially plant diseases caused by the pathogens such as fungi bacteria and viruses, produces pathogenic proteins, enzymes, toxins, polysaccharides for the disease development. These macromolecules serve as a better target for the development of drug or an agrochemical through this approach.

The modern drug discovery involves mainly 4 important steps they are,

1. Target identification
2. Lead /drug identification
3. Lead /drug optimization
4. Final compound

5. Principle

There are several principles behind the technique of CADD they are,

- ✓ Quantum mechanics - CADD uses principle of quantum mechanics in order to understand the nature and stretching of atoms present in the drug as well as in target.
- ✓ Molecular mechanics –molecular mechanism of drug molecules and its action on the target of our interest and also biological function small chemical molecules inside the cellular system.
- ✓ Property of complementarity- CADD works well only when the target responds to the property of complementarity in between the drug and its active site.
- ✓ Biochemical Mechanism: In order to understand the nature of activity inside the cellular level the following principle is used.
    - Enzyme - substrate interaction: In this interaction the enzyme either activated or inactivated. In a similar manner the drug either activate or inhibit the protein of our interest.

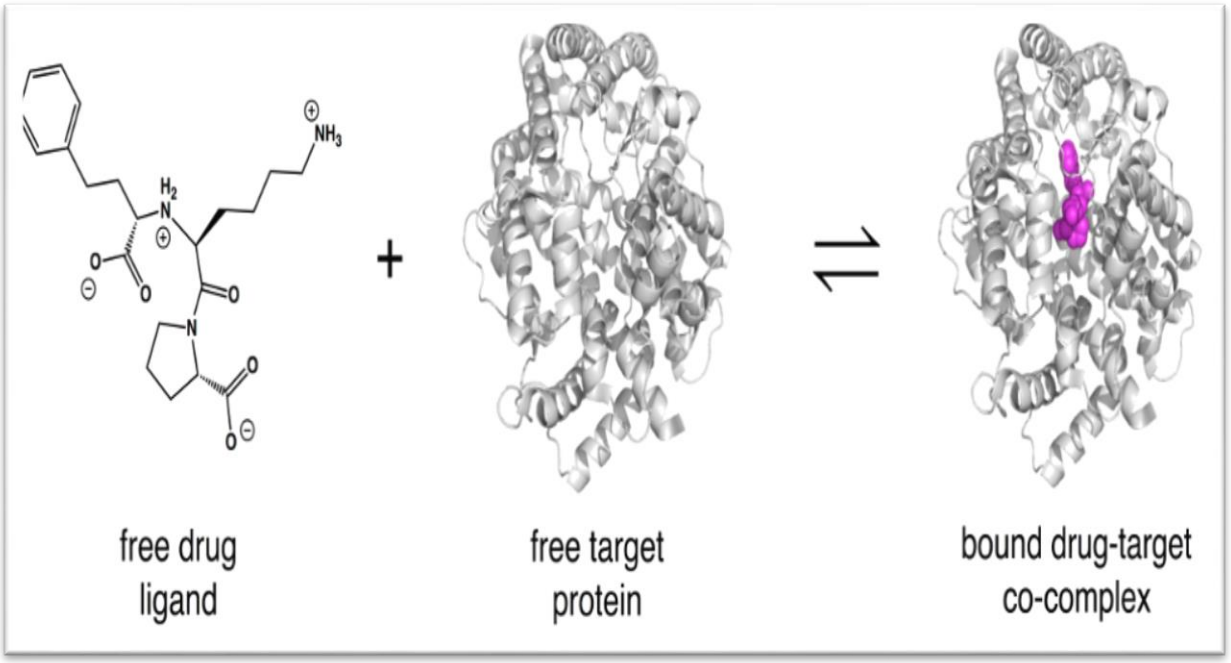

Plate 1: Representing principle of protein drug interaction

6. Types of Computer Aided Drug Discovery (CADD)

The CADD approach is broadly classified into two types based on the molecules discovered and the purpose. They are,

1. Ligand based drug discovery

2.Structure based drug discovery

6.1. Ligand based drug discovery: The method which involves the discovery of drug molecules based on knowledge of drug molecules. In this method, drug molecules and their information is well known but the target is unknown. Drug molecules discovered based on drug's physicochemical properties. This method is less established and have least importance in terms of agrochemical discovery because, the plant pathogens secrete macromolecules in order to develop diseases in plant.

These macromolecules act as a biological target for development of drugs. knowledge of ligand with drug properties may not be targeted always to these pathogenic proteins and it is uncertain that they can be used on pathogenic target. The chances of success in the screening of drug molecules

that specifically acts on plant pathogenic substances is very rare therefore this method is not popular in agrochemical drug discovery process.

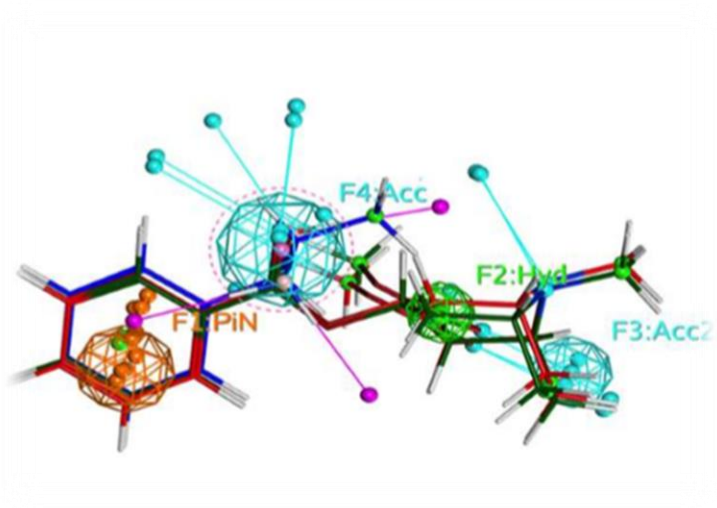

Plate 2: Pictorial 3D view of ligand structure

6.2. Structure based drug discovery: The method which involves the information of biological target and its active site. Here the drug molecules are screened in order to find the suitable compound that can bind to the active site properly and in turn modifies the biological target. Here the software needs the 3 Dimensional structure of the biological target that can be obtained through

- ✓ X-ray crystallography
- ✓ Nuclear Magnetic Resonance(NMR) spectroscopy

The 3D structure information is very essential in drug discovery. The 3D structure is useful to identify binding sites present in the target and the exploration of ligand binding pockets.

3D structure depicts information on hetero atoms, no of H bond donor, H bond acceptor, anionic and cationic properties as well as amino acid residues in the active site. Candidate drugs with high affinity and selectivity to the target is designed based on the physicochemical properties of the active site of the target. The drug discovered can be used as an agrochemical by improving the drug features. This method is most promising in agrochemical drug discovery process as it is target directed drug discovery.

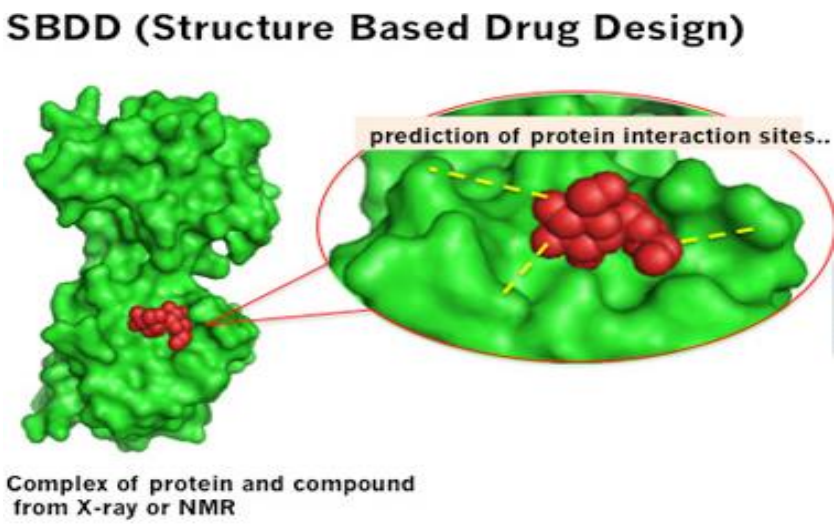

Plate 3: Pictorial representation of structure of protein and its active site

7. Importance of CADD in Plant Pathology: The CADD technique in agriculture especially in plant pathology is mainly in the field of fungicide discovery and development process. There are several studies on fungicide resistance development in fungi. The development of fungicide resistance in fungi is common against new generation fungicides such as triazole and strobilurins (Zulak *et al.*, 2018; Walker *et al.*, 2009). According to recent report from Insecticide resistance action committee (IRAC, 2010) there is a tremendous increase in the no. of unique cases of resistances in fungi against fungicides. .

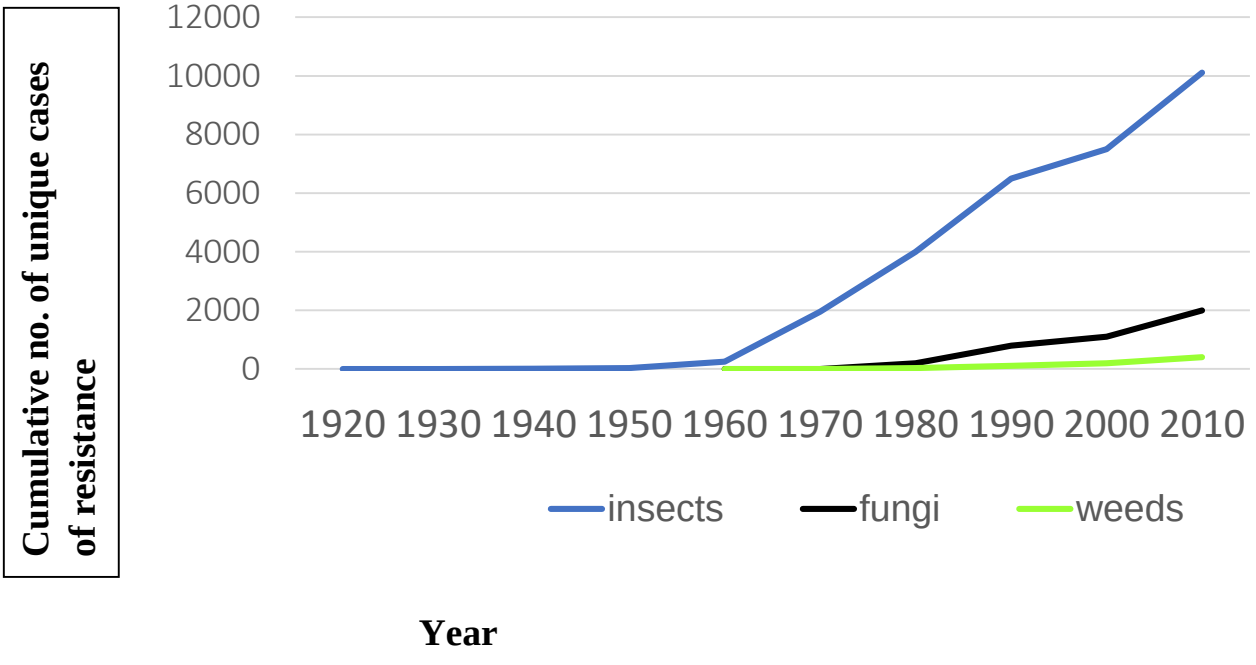

Figure 1: Above graph indicate unique cases of resistance development in different organisms against pesticides (**source: IRAC**)

The main objectives of use of this CADD approach in Plant Pathology includes

- To speed up fungicide discovery process by improving screening of fungicide molecules through computer aided softwares
- To avoid fungicide resistance problem by understanding deep into the molecular mechanism of action of fungicide on fungi
- To understand molecular events of disease management
- Finally to develop effective agrochemicals which is more target specific and effective and efficient in action.

8. Structure based drug discovery (SBDD)

The drug discovery based on the structure of the biological target is made possible through use of specially designed softwares by utilizing the sequences of pathogenic proteins. The primary sequence information is very essential in order to obtain the 3D structure or otherwise one can get the structure of these targets based on previous experimental studies which have submitted the 3D structure of protein under study in the biological databases. The biological databases such as National Centre for Biological Information(NCBI), DNA Data Bank of Japan(DDBJ), European Molecular Biology Laboratory(EMBL) *etc.* The databases are freely accessible and provide information about target as well as ligand molecules.

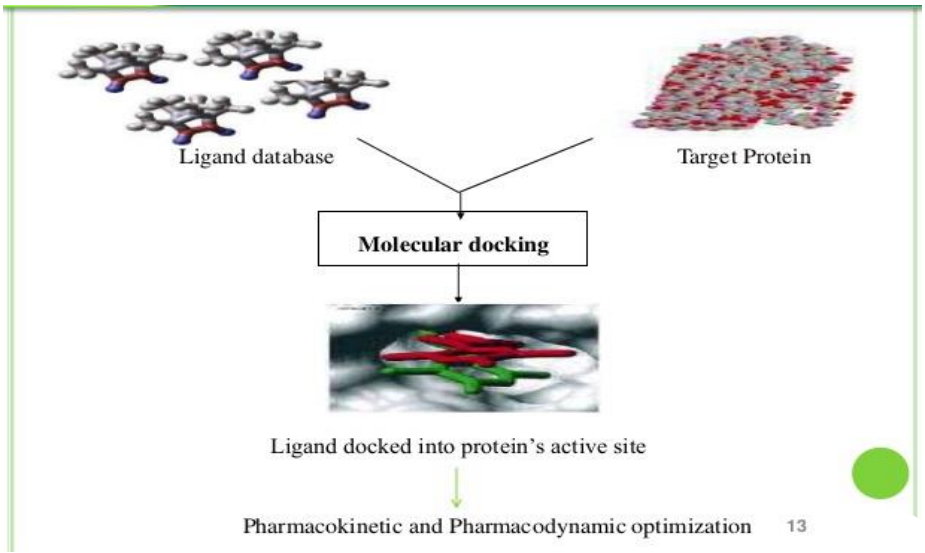

Plate 4: Pictorial representation of structure based drug discovery

9. Typical SBBD process:

The SBDD process involves several steps in drug or agrochemical discovery which are discussed below.

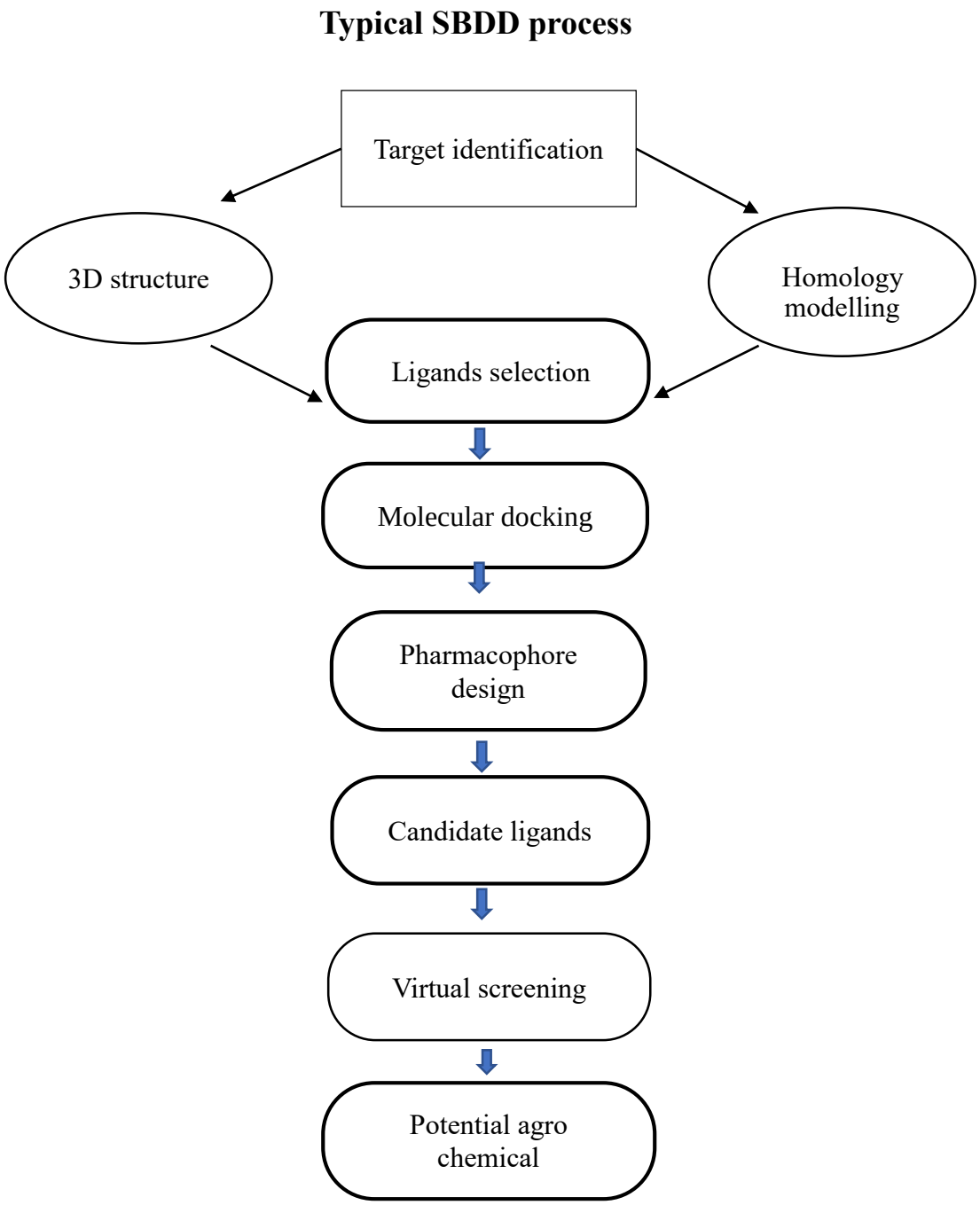

Figure 2: Flow chart of typical agrochemical discovery process

9.1. Target identification

Target is nothing but the protein of our interest against which we want to develop a drug that can to bind with it. Pathogenic macromolecules serves as a best target for the development of agrochemicals. During identification of target some important properties need to be considered. The properties must be possessed by an ideal target given by Chandra in 2011.

- ✓ **Essentiality** – the pathogenic protein or target we have selected should be very essential for the development of particular disease
- ✓ **Specificity** – the target must be highly specific to particular disease/ host
- ✓ **Selectivity** – target should be selective in nature and it should have well defined active site
- ✓ **Druggability** – target should response to small chemical molecule (drug) or it should be modified upon binding with the drug.

There following are some points to be considered while designing the target

- Structure availability : whether the 3D structure for the biological target is available or not
- Chemically active : the target is chemically active or not
- Active sites : the information about the active site and its amino acid residues should be well known to design ligand binding pockets
- No of H-bonds: other additional features of a target such as hydrophobic interaction and no of H bonds in the polypeptide backbone of a target should be well known.

There are several target molecules discovered in plant pathogens which are listed below

Drug target	**Function**	**Pathogen**	**Reference**
Mur Enzymes	Peptidoglycan synthesis	Bacterial pathogens	El Zoeiby *et al.,* 2003
Isocitrate lyase	Virulence	*Magnaporthe grisea Colletotrichum spp. Xanthomonas campestris*	Dean *et al*., 2012
Lanosterol 14α-demethylase	Steroid biosynthesis	Fungal pathogens	Sagatova *et al.,* 2015
Asparagine synthase (Asn1p)	Pathogenecity	*Botrytis cinerea Fusarium graminearum Ustilago maydis*	Ramakrishnan *et al.,* 2016

Table 1: Potential drug targets discovered in plant pathogens

In case the 3D structure of biological target is not available then one can design 3D structure of target by technique called homology modelling.

9.2. Homology modelling

It is also known as comparative modelling of protein, refers to constructing an atomic resolution model of the "target" and an experimental 3D structure of a related homologous protein (the "template"). In general 30 % sequence identity is required to generate useful template model. This technique is based on the observation that protein tertiary structure of protein is better conserved than amino acid sequence.it generate models based on primary sequence similarity of target to homologous protein and builds the 3D structures of proteins based on template sequences in which 3D structure is experimentally known. The accuracy of the built model depends on the choice of template. Generally, the models built with the templates exhibiting over 70% identities are considered to be accurate enough for drug discovery applications.

Prediction of binding sites: Identification of the ligand binding sites in the target active site of the target is very important. It provides us the information regarding selection of ligands that can complimentarily binds to the regions identified and form stable ligand-protein complex. It is essential to know the amino acid sequences and physicochemical properties. Steric and electronic features located in active site helps further modification of drug or ligand molecules for better binding efficiency.

9.3. Ligands selection

Ligands or drug molecules are selected from the available biological databases based on previous reports of drug related activity. Lipinski's rule is a rule of thumb to evaluate drug likeness Molecules should follow the Lipinski's rule of five (RO5). The rule is important to keep in mind during drug discovery when a pharmacologically active lead structure is optimized step wise to increase the activity and selectivity of a compound as well as to ensure drug like physicochemical properties are maintained as described by Lipinski's rule. It state that ideal drug molecule must obey these properties.

1. Molecular mass should be less than 500 Daltons

2. No. of H bond donor molecule should not be more than 5

3. No. of H bond acceptor molecule should not be more than 10

4. An octanol-water partition coefficient (*LogP*) that does not exceed 5.

In addition to this, there are some properties we should consider while selecting set of ligands such as,

- Binding affinity: molecules should have high binding affinity with the target.
- Degree of stability of interaction: stable enough until the target function has been modified.
- Biological half-life: ligand molecules should have least biological half-life and less residual in plant system.
- Analogues of already reported drugs: we can choose the already reported drug molecules and its analogues for the target of our interest only if the mode of action of drug related to our protein of interest.

9.4. Molecular docking

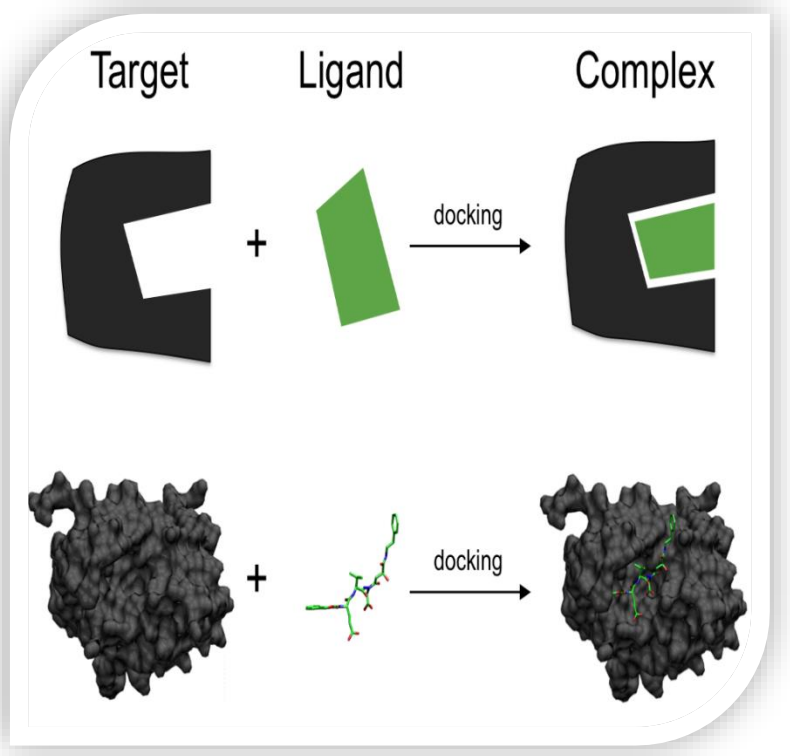

Plate 5: pictorial view of molecular docking

Molecular docking is the preferred orientation of one molecule to another molecule which form a stable complex. In molecular docking the softwares are designed in such a way to find out the possible poses that both target active site and drug molecule fit one another. After designing the possible binding pockets in the active site of template, the protein-ligand interactions can be explored through molecular docking study. The degree of stability of interaction –determines

biological consequences of the interaction. It predicts energetically stable orientation of ligand when it is bound to target protein and the degree of stability of interaction between molecules is the key factor in determining biological consequences of the interaction.

9.5. Pharmacophore modelling

A pharmacophore model is the ensemble of common steric and electronic features that are necessary to ensure the optimal molecular interactions with a specific biological target and to trigger (or block) its biological response. Typical pharmacophore features include hydrophobic features, aromatic rings, hydrogen bond acceptors or donors, cations, and anions (plate 6). These pharmacophoric points may be located on the ligand itself or may be projected points presumed to be located in the receptor. A pharmacophore model can be established either in a ligand-based manner, by superimposing a set of active molecules and deriving common chemical features that are essential for their biological activity, or in a structure-based manner, by probing interaction points between the macromolecular target and ligands and strengthening the interaction between them.

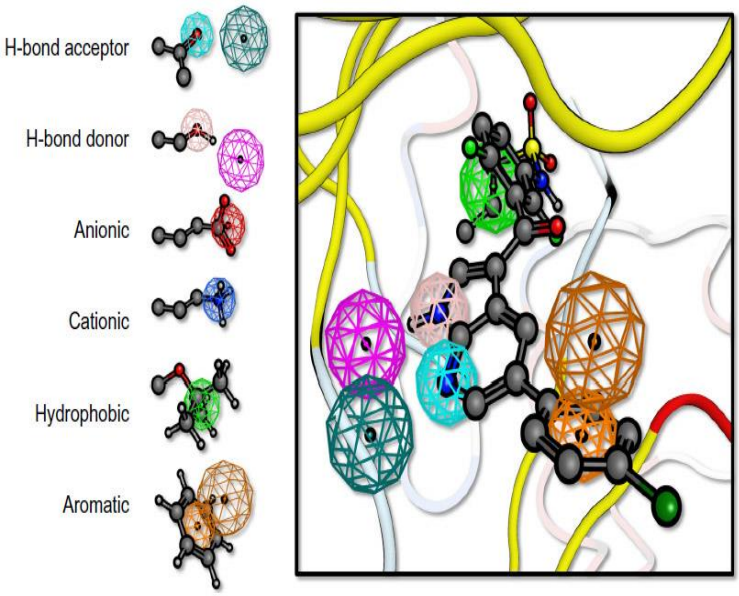

Plate 6: Typical pharmacophore model and its steric and electronic features

9.6. Structure based virtual screening

The search for new chemical compounds as lead molecules is a critical step during the process of drug discovery. Structure based virtual screening screens and evaluates large libraries of compounds and identifies putative hits (leads) through comparison of 3D structures of ligands with the putative active site of the target. The software selects the docked files and within the identified

candidate ligands it further screens the valid ligands. Ranking of compounds with desired binding interactions will be made through this software for identified valid ligands. The top ranked compounds are virtually screened to get final lead molecule. This final molecule can be used as an agrochemical.

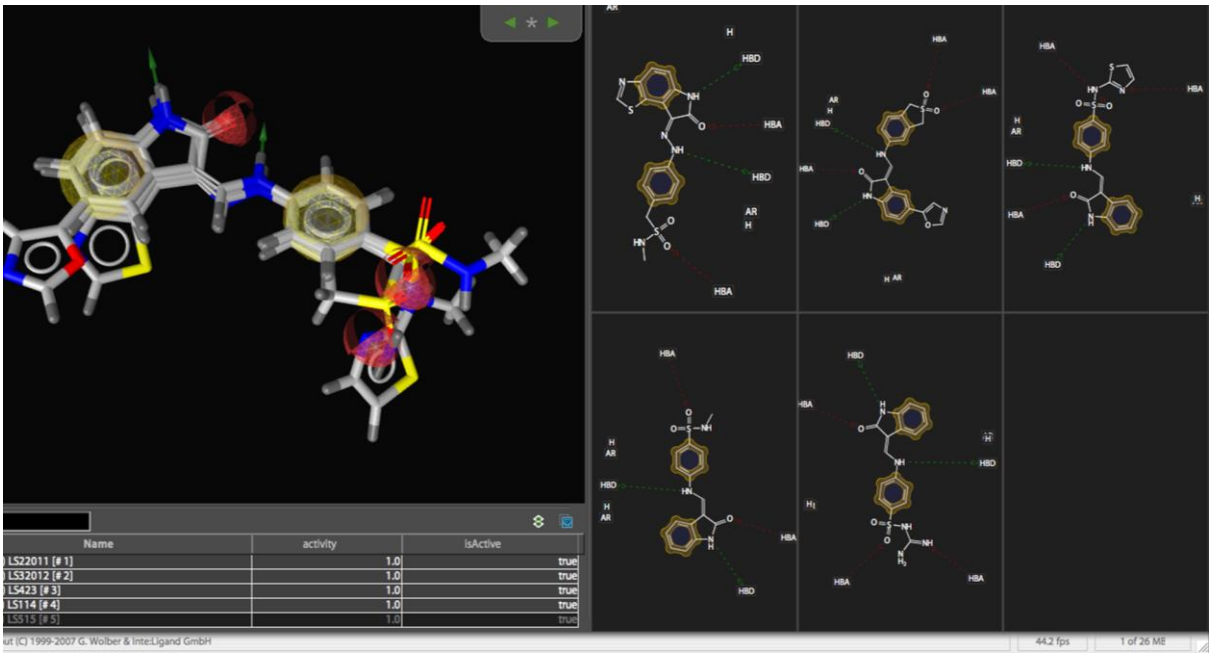

Plate 7: Screenshot of virtual screening software showing screening of top ranked compounds

(Source: Ligand scout developer)

9.7. Agrochemical validation

The compound identified is subjected to the field trials and also ADME (Absorption, Distribution, Metabolism and Excretion) toxicity studies (if discovered agrochemical are used on fresh vegetable, study conducted to check residual nature in humans and animals). Further it should not be phytotoxic or residual in plant cellular system. Dose should be optimized through dose response relationship**.** Formulations are specified and subjected for certification in respected authorities. QSAR (Quantitative structure activity relationship) is also done for the predictors consist of physico-chemical properties or theoretical molecular descriptors of agrochemical. Biological activity can be expressed quantitatively as the concentration of a substance required to give a certain biological response. The biological activity of molecules is usually measured in assays to establish the level of inhibition of particular metabolic pathways.

10. *De novo* ligand design (DnLD)

The development of drug is done *de novo* in case it is not possible through the structure based method. De novo ligand design is one such method which uses 3D structure of target and its active site information for developing drug molecule. Drug development is through the placement of pseudo-molecular probe and addition of functional groups to the active site of target fragment by fragment and this will grow throughout the active site of target molecule to satisfy the spatial constraints of target binding site.

11. Computational tools in the SBDD process

There are several computational tools needed in drug discovery process using SBDD approach. The technique needs softwares to

- To obtain target of interest and set of ligand molecules for the target by using freely accessible biological databases. The biological databases are NCBI, DDBJ, EMBL Protein Data Bank (PDB), Binding MOAD, PDB bind, MAYBRIDGE, DRUGBANK PUBCHEM Database, chEMBL, Jchem *etc.*
- To identify the ligand binding pockets as well as active sites in the biological target molecules. The softwares such as IntFOLD, RaptorX, Biskit, GeneSilico, MODELLER, MOE (Molecular Operating Environment) are preferred for this.
- For visualization and drawing structure of target and ligand molecule in the cases where, if one found the problem while designing, there are some software to handle the structural studies such as Rasmol, Raswin, Pymol for visualization and chemsketch, ACD CHEMSKETCH for drawing 3D structure to identify individual side chains in molecules. For molecular modelling & Homology modelling in case if no 3D structure available for the protein of our interest we need databases to find similar template sequences which have similar sequence identity with target protein. Based on the template 3D structure the drugs are designed. The development of homology models needs softwares such as Bio edit, ProModel, SWISS- MODEL, RaptorX *etc.*
- For conducting molecular docking there are software like AUTODOCK, PyRx, and FleXx *etc.*
- For ligand design screening and pharmacophore modelling, there are softwares such as LigandScout, PharmaGist, and CHAAC *etc.*

(The above mentioned softwares some of the standard softwares based on the users view and still now no software achieved simple user friendly interface in their design).

12. Case studies

12.1. Fungicide discovery using CADD approach

In Plant Pathology there are studies utilising CADD in developing the drug or agrochemical such as fungicide and bactericide which can act on diseases. One such study was conducted by Shanmugam and Jeon in 2017.

Shanmugam and Jeon (2017) developed drug molecule against the plant pathogens using structure based drug discovery approach. They selected bacterial pathogen *Pseudomonas syringae* which is known to cause bacterial blight, bacterial speck canker and gummosis diseases in various crop plants. For fungal pathogen they selected *Colletotrichum gloeosporioides* known to cause anthracnose disease in chilli and mango. In order to get effective control they conducted a study to develop a drug that can inhibit both organisms.

12.1.1. Target identification and ligand selection: During study they selected *MurD* and *MurE* ligases which are involved in peptidoglycan biosynthesis of *Pseudomonas syringae* as a potential target (Bratkovic *et al.,* 2008; Feil *et al.,* 2005). For the *Colletotrichum gloeosporioides* they selected *pelB* gene encoding pectate lyase as a target for rational drug design since it is an important cell-wall-degrading enzyme for pathogenesis (Yakoby *et al.,* 2000). Instead of screening large amount of chemical library, they opted for selective screening of ligand molecules by choosing analogue of curcumin and penicillin. These two drug are already reported to be having antifungal and antibacterial activity.

12.1.2. Materials and methods

There are no 3D structure for the selected target, so they have conducted homology modelling using BlastP against PDB. Based on highest sequence similarity they obtained Pectate lyase B - cedar pollen allergen (*Juniperus ashei*) as a template for *Colletotrichum gloeosporioides,* MurD and MurE from *E. coli* as a template for MurD *and* MurE *of Pseudomonas syringae (*plate8). They developed valid homology models for these template using software called modeller9v9. To select set of ligands against the identified models, screening of curcumin and penicillin analogues was carried out in DRUGBANK and Maybridge software. They selected 52 candidate ligand from Curcumin analogues and 291 candidate ligands form Penicillin analogues. In molecular docking

and pharmacophore modelling they used DISCOVERY STUDIO and for virtual screening PyRx softwares.

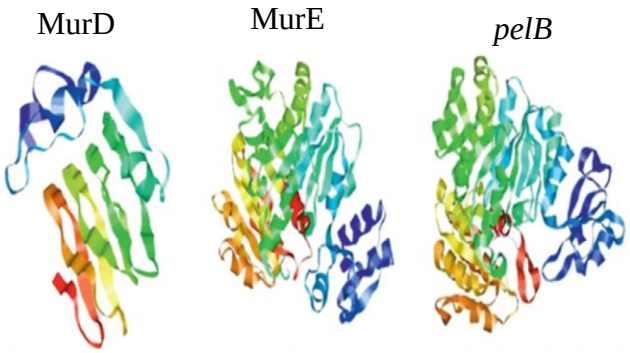

Plate 8: 3D structure of MurD, MurE and *PelB* pathogenic targets

(Source: Shanmugam and Jeon, 2017)

12.1.3. Results

Shanmugam and Jeon (2017) selected 291 and 52 compounds that are similar in their structure to penicillin and curcumin, respectively and screened against identified targets. These new molecules were subjected to energy minimization by using CHARMm force field and collectively retrieved as 3D structures in SDF (structure data file) format for virtual screening against pectate lyase, MurD and MurE. Virtual screening of these 291 and 52 compounds by using FlexX revealed their binding efficiencies through docking in the predicted binding pockets of modelled proteins. The compounds (3,3dimethyl-7-oxo-6-[(2-pyridin-4-ylacetyl)amino]-4-thia-1- azabicycloheptane-2-carboxylic acid (hereafter compound-1) similar to penicillin and (1,7-bis(3,4dihydroxy-5-methoxyphenyl)hepta-1,6-diene-3,5-dione (hereafter compound-2). The docking score (binding energy) were used for the pharmacophore modelling. Here they have employed both SBDD and LBDD approaches. The pharmachophore model was generated against the compound-1 and compound-2 by using Discovery Studio which considers the chemical feature types and resulted in 10 hypothetical pharmachophore models. The best pharmachophore model was selected based on the high correlation coefficient and lower RMSD (root mean square deviation). The final hit compounds from Maybridge database having good fit scores (> 3) for the compound-1 pharmacophore are PD00533, CD01374, CD04888 and CD01278(plate 9 left). While the final hit compounds for compound-2 pharmacophore are CD01278, S10124, HTS05738.These compounds are further used for docking studies against the modelled proteins and found that the compound CD01278(N1-(2,6-dimethylphenyl)-2-[2-(1,4,5,6-tetrahydropyrimidin-2-yl)benzoyl]hydrazine-1-carbothioamide (plate 9) exhibited better binding energies with the three protein models, which

significantly implies that this compound might serve as potential lead molecule to control the disease caused by both the *P. syringae* and *C. gloeosporioides* (Shanmugam and Jeon 2017).

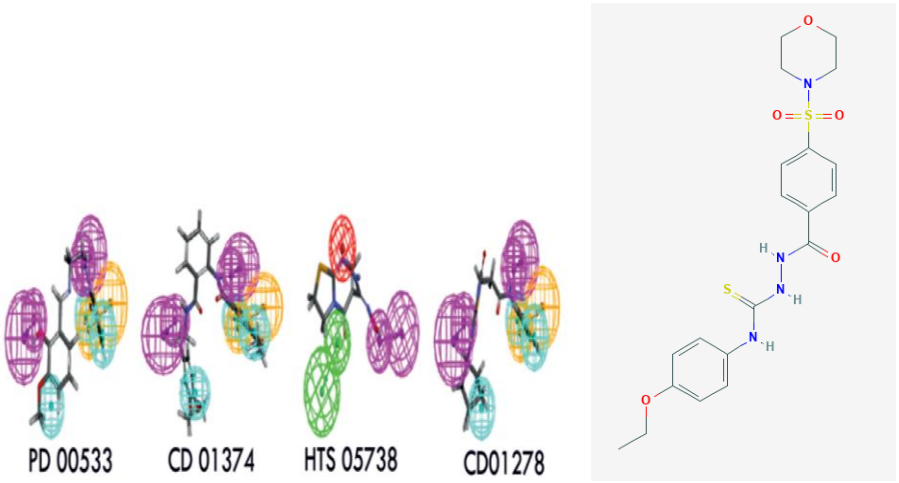

Plate 9: Top 4 validated drugs in virtual screening (left) and the chemical structure of compound CD01278 (right) (Source: Shanmugam and Jeon 2017).

12.2. Fungal Lanosterol de methylase (LDM) as a new generation antifungal discovery

In another case study by monk *et al.* (2019) discovered new binding sites were discovered in the fungal LDM enzyme which can be used as a target by developing drug or fungicide using CADD approach.

In most of the fungi, lanosterol de methylase (LDM) is found as a cytochrome P450 membrane bound enzyme involved in the synthesis of ergosterol biosynthesis. Inhibition of LDM which eventually depletes ergosterol and changes membrane fluidity in the lipid bilayer. This reduces the activity of key membrane bound enzymes and halts fungal cell growth. Most of the new generation fungicides such as azoles and strobilurins are developed to target this enzyme. Repeated crop protection using azole agrochemicals has conferred incremental increases in fungicide resistance in fungi, forcing industry to develop more potent azoles and formulations with other classes of antifungals.

12.2.1. Exploration of LDM mutagenic site of *Aspergillus* sp.

Monk *et al.* (2019) conducted study on one of the storage fungi *Aspergillus* sp. indicated presence of 17 different types of substitution mutations in the active site leads to the evolution and resistance development in fungi against the azole fungicides. Study on mutated LDM structure of *Aspergillus* sp. using a software Schrodinger Prime indicated presence of ligand binding pockets, within which there is a rigid ligand binding pocket in the active site with a heme iron group which

is deeply buried inside the active site (plate 10).The LDM heme iron group binds with N atoms in azole fungicide and forms hydrophobic interaction. But in the mutated enzyme, the head groups of most azole drugs (azole and halogenated benzene rings) partially fill the active site. This in turn leading to the overexpression of *CYP51A* gene. Due to overexpressed CYP51A gene LDM enzyme level gradually increased which makes the drug fails to acts as a substrate for the enzyme in turn leads to drug efflux. Meanwhile they identified other binding sites such as polypeptide backbone and water mediated H bonds which can be used as potential targets (plate 10). In a view to utilize the identified new targets, they modified commonly used fungicides such as tebuconazole, epoxiconazole, prothioconazole, difenoconazole *etc*. These azole drugs are designed and modified by deriving their analogues and by deriving new molecules with slight changes in their structure.

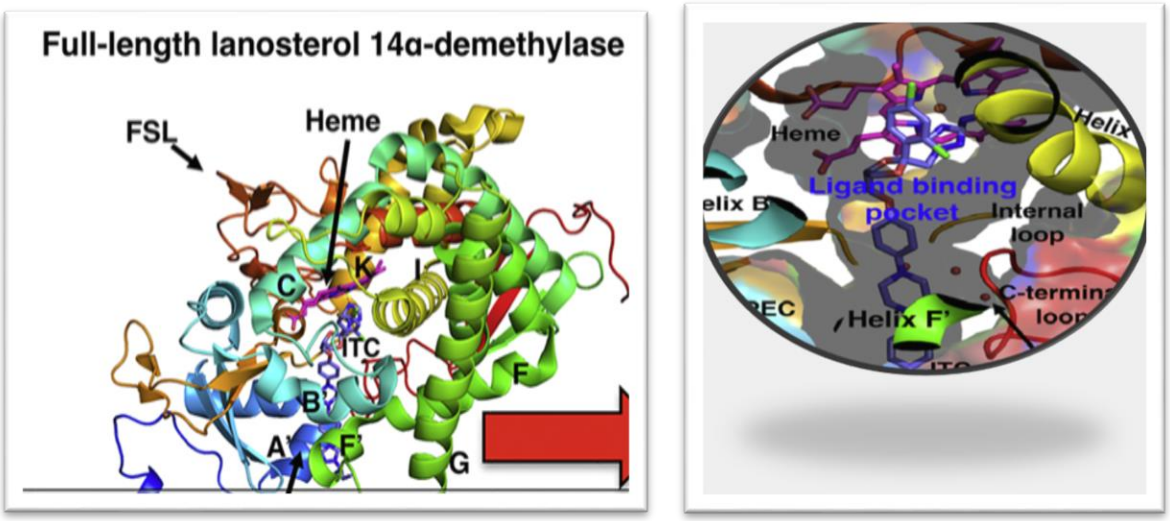

Plate10: 3D structure of active site of LDM enzyme and its newly identified ligand binding pocket

(Source: Monk *et al.*, 2019)

12.2.2. Results

Modification of azole fungicide carried out using Schrodinger Prime software and subjected derived candidate analogues for molecular docking with already identified LDM new targets. Among the azole fungicides difenoconazole where halogen is substituted with phenoxyphenyl group completely inhibited the newly identified sites without giving chance for the mutation to occur (Monk *et al.*, 2019). Due to the new binding site which involve polypeptide backbone where mutation in entire conformation was not possible, made this new ligand binding pocket more promising in designing new fungicides. Even complete binding avoided drug efflux mechanism.

In this way they can able to overcome the resistance in fungi and both the studies discussed above successfully utilized the CADD approach in their work specifically in the area of Plant Pathology

made future research of drug/agrochemical discovery highly target specific using this structure based CADD approach.

13. Future aspects of CADD

Development of lead molecules against viral diseases is need to be addressed. In the development of viral diseases the plant virus also secrets coat protein, movement protein, virulence factors which can be easily targeted by the ligands. There is still less research on identification of 3D structures for plant viral proteins, because of this unavailability of structural information made this area unexplored. There are several reports on the development of drug molecules against human viruses which are targeting the viral proteins (Kumar *et al.,* 2014; Mottin *et al.,* 2018). This method can be used by molecular plant pathologist in developing the drug against plant viruses.

Adoption of technique in pesticide industries will be a major breakthrough in achieving effective chemicals discovery against plant diseases. The technique will empowers researchers in molecular Plant Pathology by deep understanding about the molecular aspects of disease management, fungicide resistance, active site variation *etc.*

14. Pros & cons of CADD

As like other technique the CADD has its own advantages as well as disadvantages they are,

Advantages:

- Cost and time will be less compared to traditional pesticide discovery. In traditional discovery process involves more than 800 millions of rupees and 12-15 years in order to develop an agrochemical. The technique is more accurate because of target oriented approach. It reduces the time needed for screening because of database screening. More importantly it provide molecular information on diseases and how fungicides are acting on target and inhibits it.

Disadvantages:

- There is a lack of information on target and drug molecules, availability of good softwares, handling of softwares and training related to interpretation of molecules. Lack of availability of 3D structure is another limitation whereas, from 2016-19 there are more than 3000 plant pathogenic proteins submitted in the biological databases which makes drug discovery process easier. It includes lengthy process of lead optimization. The bottleneck of this technique is in the Screening of compounds and optimization which needs high technical expertise. Most prominently, accurate simulation of complexity present in the biological

systems is not possible to explore even with state of art techniques and high computational power. Such limitation is the biggest challenge in CADD approach and there are some uncertainties with respect to high flexibility of target with the drug, conformational changes of proteins, and moreover lack of experimental data for absorption, distribution, metabolism, and toxicity of compounds in the cellular systems (Baig *et al.,* 2016; Singh, 2014).

15. Conclusion

This novel method of drug discovery can be used as a rapid supplementary tool for pesticide development. The development and researches in molecular plant pathology made increased availability of genomic and molecular information in biological databases specifically plant proteins and 3D structure availability is rapidly increasing in the databases. Moreover it helps to understand the molecular mechanism of fungicide resistance as discussed in the case studies, the increased understanding of the fungicide resistance mechanism and mutational changes in the active site clearly showed there is a need for effective fungicide molecule that can perfectly bind to active site of a target. There are some limitations in the approach regarding simulation of biological complexity. Despite these, in future it creates possible way to develop chemicals for viral diseases. The adoption of this technique will definitely improve agrochemical discovery in pesticide industries.

16. Discussion

1) Why to choose tertiary structure in protein for CADD?

Tertiary structure are more conservative because 3D structure are more related to function rather than its primary sequence and the molecular and steric features are well understood easily. Active site and its amino acid residues easily altered by designing with use of CADD softwares.

2) Is there any work in Kerala Agriculture University regarding this technique?

No, but there are studies regarding molecular docking in CPBMB to test and screen antioxidant molecules in Moringa leaves using docking software.

3) How it is better than conventional method of fungicide discovery?

In commercial agrochemical companies, the development of agrochemical needs high cost about 800 millions of rupees and it needs about more than 10 years to develop one possible drug compound from millions of ligand library.

4) Any fungicide discovered using this approach?

They are in experimental stages, the top agricultural companies are collaborated with the chemical institute in Canada and North America (maintaining confidentiality) for developing molecule using the approach of SBDD.

5) In the first case study one author mentioned scarcity of information in biological databases for the development agrochemical can u justify it?

Till 2015 there was lack of data regarding major plant pathogenic proteins especially 3D structure of causal agent, but recently since from 2015 tremendous increase in no of pathogenic proteins and its 3D structure repository in biological databases. In PDB itself more than 3000 structures are submitted mainly due to advanced research by X-ray crystallography and NMR spectroscopy.

6) Is there any possibility that newly developed drug using CADD approach that may develop resistance to fungicide again?

There are possibilities based on the type of ligand binding pocket. If the amino acid sequence present in the binding pocket there are chances of resistance, but if we target the whole protein active site especially poly peptide backbone and hydrophobic side chain there won't be mutation (mutation in the entire structure not possible that it changes whole conformity)

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17. Abstract

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Major Advisor : Dr. Sumiya K. V.

Venue : Seminar hall
Date : 01-11-2019
Time : 10:45 am

Computer Aided Drug Discovery (CADD) in Plant Pathology: An overview of 'structure based drug discovery'

Abstract

Drugs are chemical compounds that either activate or inhibit biomolecules which in turn promote health and survivability. Traditional drug discovery process involves steps such as screening, separation and characterization of drug molecules which is time consuming, Whereas, the use of computer software and biological databases makes the drug discovery process time saving and cost effective.

CADD (Computer Aided Drug Discovery) approach is well established in Medicinal Biochemistry and Pharmacology since last two decades. Expansion of this technique to agriculture particularly in process of pesticide discovery was successfully carried out by Doucet-Personeni *et al.* (2001). There are very few studies have been conducted in Plant Pathology utilising this approach (Chandra, 2011; Xue *et al.*, 2014; Shanmugam and Jeon, 2017; Monk *et al.*, 2019). These studies paved way for researchers in molecular plant pathology to explore deep into the molecular mechanism of plant diseases. The evolution of plant pathogens due to inappropriate use of fungicides leads to the development of fungicide resistance in plant pathogens, which in turn leads to the failure of fungicides in management of diseases. Development of agrochemicals that are highly target specific is made possible through this novel computer aided approach.

The CADD approach works based on the principle of molecular and quantum mechanics and complimentary binding process between drug and target molecules. It is mainly classified into two types, structure based and ligand-based approaches. Among the two approaches, structure based drug discovery is more promising with respect to agrochemicals like fungicides because it

involves target identification, homology modelling, molecular docking, pharmacophore modelling, virtual screening of ligands and ultimately final lead compound discovery. Several pathogenic macro molecules such as enzymes and toxins can be used as drug targets. Using structure based drug discovery Shanmugam and Jeon (2017) discovered lead compound CD01278 (N1-(2, 6-dimethylphenyl)-2-[2-(1, 4, 5, 6-tetrahydropyrimidin-2-yl) benzoyl] hydrazine-1-carbothioamide) against MurD and MurE enzymes of *Pseudomonas syringae* and pectate lyase of *Colletotrichum gloeosporioides*.

Recently fungicide resistance is reported in fungi against new generation fungicides such as triazole and strobilurins. Deep understanding of ligand binding pockets present in the active sites of the target will help to overcome the fungicide resistance problems (Zulak *et al.,* 2018). Study on binding mechanism of azole fungicides on active sites of *Aspergillus* sp. showed partial binding of azole group to the heme group present inside the active site of fungi, which is due to 17 different types of substitution mutations. Similar binding pockets were also identified within the active site. In order to utilise these binding pockets, azole fungicides were modified using CADD approach (Monk *et al.*, 2019). Among the triazole fungicides, difenoconazole can overcome the problem of mutations by complete binding within the active site of an enzyme, when its halogen group is replaced with phenoxyphenyl group.

Hence, use of CADD approach will speed up the fungicide discovery process. Despite all these merits, it requires highly expertized researchers from various fields of science. The adoption of this technique in pesticide industries will be a major breakthrough in future for the development of effective agrochemicals.

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