Identification of Potent Inhibitors against Potential Drug Target for Schizophrenia Trough Virtual Screening Approach

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Abstract- **Background:** Schizophrenia is related with physiological condition. It affects brain sections including the prefrontal cortex, the basal ganglia as well as limbic system. There are certain neurotransmitters present in the brain such as dopamine. When level of dopamine is disturbed it causes schizophrenia. Environmental factors like stress, depression and anxiety also contribute to schizophrenia. Several drugs including first and second generation are available to provide treatment against schizophrenia. Second generation drugs have better results and fewer side effects than first generation. Clozapine is second generation drug which was recommended as best drug for schizophrenia. It rebalances dopamine and helps to treat the patients of schizophrenia as well as diminishing of suicidal thoughts. Therapies and counseling also help to overcome this disorder. Dopamine Beta Hydroxylase catalyzes the synthesis of norepinephrine. Dopamine beta hydroxylase activity was suggested as a biological marker for schizophrenia alongside other psychiatric disorders.

Objective: To identify best inhibitors for schizophrenia by using ligand and target based virtual screening with Dopamine Beta Hydroxylase as target and to predict 3D structure of the target protein.

Material and Methodology: For this study, different databases were used. Structure and potential lead molecules were identified by the help of structure as well as ligand based virtual screening. Auto Dock is used for virtual screening based on target. For ligand based virtual screening ZINC 15 database was used. Compounds were selected and further screened by using PyRx software. The ligands with less binding affinity were selected for analysis. Structure and interactions are viewed in DS visualizer tool and protein ligand explorer. Virtual screening is used for computational screening of huge library of chemicals for the compounds which supplement targets of known structure by testing them experimentally. Ligands were screened by using PyRx server and ZINC 15 server. AutoDock is used to dock ligands against drugs and display results. On the basis of binding energy and affinities, ligands were Chosen.

Results: Binding Affinity and Root Mean Square Deviation of Ligands obtained by using PyRx Virtual Screening software Binding energy of ligands with target ranges from -10.1 to -6.7. The binding affinity of ligand ZINC 000095550333 is - 9.7. ZINC 000036089409 binding energy is -9.5. Ligand ZINC 000036089465 has binding energy of -9.3. Binding affinity of ligand ZINC 000095550333 is -10.3. Binding affinity of ZINC000004292831 is -10.1. Binding affinity of ligand ZINC 000036089410 is -9.8. Ligand ZINC 000036089465 has binding affinity of - 9.3. On the basis of interactions and best ligand binding energy with target Ligand 4zl_ZINC000004292831 is consider as best. Virtual screening of ligands against target by PyRx ligands are placed in the lowest to the highest order on the bases of their Interactions and Binding Energy binding energy ranges from -10.3 to -6.7 Ligand ZINC000095550333 is shows best interaction and ligand energy with target having Binding Energy of -10.3.

Conclusion: As for computational analysis, it emphasizes diverse ligands by using different servers which can act as best target against schizophrenia. Further nature of ligand will be explored by in-vitro and vivo analysis. Interaction of ligands with lead molecules suggests that auxiliary analysis of these drugs will give a way towards the treatment of schizophrenia.

Index Terms- VS (Virtual Screening), CADD (Computer Aided Drug Design), LBVS (Ligand Based Virtual Screening), SBVS (Structures Based Virtual Screening),

I. INTRODUCTION

Schizophrenia is associated with mental illness and considered as messy thoughts with anomalous behaviors as well as anti-social behaviors (1). It affects around 1.1% of the world's population. In 19th century about 3.5 million people have been affected with schizophrenia (2). Accurate causes of Schizophrenia are unknown but researchers claim that it can be parent's genetic transfer to children. Environmental stressor, neglected home environment, disturbed level of chemicals like dopamine and changes in brain chemistry like size of brain also add up to schizophrenia (3). "Psychosis" is a common state in schizophrenia in which mental damage is marked by hallucinations. In these state conflicts of sensory perception as well as delusions occurs which affects person's ability to differentiate real from unreal experiences (4)."

II. DISCOVERY

Kraepelin was the first person to classify schizophrenia and further forms of psychosis in 1887. The Swiss psychiatrist, Eugen Bleuler, coined the term, "schizophrenia" in 1911 transforming the name. The word "schizophrenia" originates by the Greek era where schizo (split) and phrene (mind) define thinking of people by the disorder (5). Both Bleuler as well as Kraepelin define the diverse subtypes of the disorder. Over the years, those working in this field have made continuous efforts to classify schizophrenia types. As a result five types were demarcated in the DSM-III: disorganized, catatonic, paranoid, residual, and undifferentiated (6). The first three kinds were formerly proposed by Kraepelin. Such categorizations being still engaged in DSM-IV, have not yet revealed to be supportive in expecting conclusions of the disorder (7). In 20th century four major classes of symptoms were recorded by many experts who agreed to ensure it in schizophrenia: positive, negative, cognitive and affective symptoms (8).

Genetic Variation:

Schizophrenia is also a multi factorial disorder followed by several genetic susceptibility elements. Family linkage as well as chromosomal anomalies is supplementary to schizophrenia. Genetic studies confirm that dysfunction of dopaminergic or N-methyl-D-aspartate (NMDA) receptor-mediated signaling were main donating aspects in schizophrenia pathogenesis (9). Minor deletion in the area of chromosome 22 called 22q11 might be involved in causing schizophrenia. Susceptibility for schizophrenia on loci 13q, 22q11-12 and 8p21-22T being minor deletions might count up to 2% of schizophrenia (10). More genes such as Val66, Met GAD1, DISC1 Neuroregulin 1 (NRG1), COMPT (catechol-O-methyl-transferase) (10) DTNBP1 (dysbindin), ALC6A3, DRD3 and SLC184 also contributed in schizophrenia (11). C4 appears to be involved in excluding connections among neurons. Variations in number of copies of C4 gene helps in predicting how the gene were arranged in the brain and could cause schizophrenia (12).

Pathophysiology:

At macroscopic level it has been founded that the brain of schizophrenia patients is different from normal person's brain so the total cerebral volume is reduced, total ventricular volume is more, the total cortical grey matter is less and the hemispheric asymmetry is reduced. The brain of schizophrenia patients has irregular distribution of neurons. Behavioral and neural system effects of computerized social cognitive training exercises in schizophrenia. Schizophrenia Research, 153, S4. In synapses, synaptic terminals show slight morphological modifications (13). Dopamine being broadly considered as a neurotransmitter which is important in the pathology of schizophrenia (14). At molecular level neurotransmitter systems seem to be affected by dopaminergic neurons (9). Dopamine being broadly considered as a neurotransmitter which is important in the pathology of schizophrenia (15).

Dopamine β- Hydroxylase:

Dopamine-β-hydroxylase catalyzes the formation of norepinephrine by dopamine (16). Mutations in such gene cause Dopamine β-Hydroxylase deficiency in patients. Polymorphisms in this gene play a part in a range of psychiatric disorders (17). Dopamine hydroxylase deficiency leads to genetic disorder that affects patients so norepinephrine, epinephrine, and octopamine cannot be synthesized in both central nervous system and peripheral autonomic neurons (18).

Treatments for Physiological Disorder:

Psychosocial treatments and antipsychotic medication will help patients suffering from schizophrenia. According to the current Diagnostic and Statistical Manual of Mental Disorders (DSM 5), there were about 400 different psychological disorders (19). Common examples of that include bipolar disorder and schizophrenia. Several forms of psychotherapy like cognitive therapy or behavioral therapy are founded to effectively treat several disorders (20). Effects of medication and psychotherapy often seem to provide long-term treatment. Psychotherapy and medication along with family members support by humility courage is advised to be the best choice. Since families will make sure their loves ones keeps their treatment as well as medications (21).

Drug Designing:

Drug designing is the innovative development of finding new medicines base on the knowledge of biological target. Such methods are used to identify novel inhibitors against Schizophrenia. Clozapine is considered as inhibitor for schizophrenia."

The Dry Lab Technique:

Dry laboratory technique de novo a drug making procedure in which the drug is designed by using computer."

Computer Aided Drug Design (CADD):

Drug discovery and emerging with a novel medicine is difficult, costly and a risky procedure. However, CADD provides numerous tools which helps in several stages of drug design thus reducing the cost of research as well as progress time (22). This is one of the reasons why computer-aided drug design (CADD) methods are being broadly used.

Importance of CADD:

1- To provides efficient and safe targets for drugs.

It determines new tools and techniques to support drug discovery process which in turn helps in reducing both cost as well as time taken for developing drug rather than the conventional methods. It also assists in improving different phases of drug (23).

Types of Drug Designing:

There are two major types of drug designing
1. Ligand based drug designing
2. Structure based designing

1- Ligand Based Drug Designing:

In lack of 3D structures of possible drug targets, ligand-based drug design is one of the best methods for drug discovery and lead optimization. 3D structure-activity relationships (3D QSAR) and pharmacophore modeling are significant and generally used tools in ligand-based drug designing (23). They provide decisive insights into the nature of interactions among

drug target and ligand molecule. Moreover, they also help in providing predictive models appropriate for lead compound optimization.

2- Structure Based Drug Designing:

It combines power of several scientific disciplines, such as X-ray crystallography, NMR Molecular modeling, Enzymology and Biochemistry, in a functional model of drug development (24). SBDD lies in parlaying enzyme inhibitors into drugs.

Aims of Drug Designing:

1. It provides better understanding of drug discovery and development process.

2. It covers the basic principles on how new drugs are discovered.

III.   RESEACH QUESTION

How Dopamine β- Hydroxylase Causes Schizophrenia?

Dopamine hydroxylase catalyzes the synthesis of norepinephrine from dopamine in schizophrenia, tyrosine hydroxylase is overactive that results in an even higher concentration of dopamine so this causes imbalance of chemical in brain and leads to schizophrenia."

Which genes are Susceptibility Genes for Schizophrenia?

Table 1.1 Susceptibility Gene for   Schizophrenia

S.NO	GENE	PROTEIN
1	DISC 1	Disrupted in schizophrenia 1 protein
2	DRD2	D(2) dopamine receptor
3	DRD3	D(3) dopamine receptor
4	DRD4	D(4) dopamine receptor
5	BDNF	Brain-derived neurotrophic factor
6	COMT	Catechol O-methyltransferase

IV.   MATERIAL AND METHODS

Virtual Screening:

Virtual screening is a technique currently used in drug discovery. Millions of molecules are verified using silico (25). The main goal is to only choose the best targets for drug and testing them in later stages.

It is evaluated that an individual drug discovery cycle through lead identification passes via clinical trials and takes 14 years costing approximately 800 million US dollars. In 1990s, fast progression was made in the fields of combinatorial chemistry and HTS technologies which presented great potential to accelerate the drug designing process (26). It permitted massive libraries of compounds that were being synthesized and screened in a short period of the time. It introduced a dominant technique for identifying hit molecules as a starting point for medicinal chemistry (27). VS method is used in the area of drug designing.

Types of Virtual Screening:

VS were divided into following categories:
1. Ligand-Based Virtual Screening
2. Structure-Based Virtual Screening

1- Ligand Based Virtual Screening (LBVS):

Ligand-based virtual screening tools take existing active molecules as preliminary point for verdict novel drug candidates (28). LBVS approach employs structure-activity data by a set of known actives to identify candidate compounds for experimental evaluation (29). LBVS approach includes different methods like similarity, substructure searching, (QSAR), pharmacophore and 3D shape matching (30). Clozapine is inhibitor of schizophrenia it blocks the expression of excess dopamine and stable level of dopamine. Ligand based virtual screening was preformed via ZINC database. "

2- Structures Based Virtual Screening (SBVS):

SBVS consumes the 3D structure of biological target (determined either experimentally through X-ray crystallography, NMR or computationally through homology modeling) to dock the candidate molecules and ranked them centered on their expected binding affinity or by complementarity to the binding site .VS show the series of likely targets to deliver an account of broad approach and to expose potentials for accurate goals. DBH model is used was used for structure based virtual screening. The structure based virtual screening helps in identifying such molecules that have capability to attach with active site of Dopamine Beta Hydroxylase. Auto dock is used for structure based virtual screening."

Tools for Virtual Screening:
PyRx Based Virtual Screening:

PyRx is Virtual Screening software for Computational Drug Designing which is used to screen libraries of compounds for drug targets. It permits Medicinal Chemists to run VS from every platform. It comprises of docking wizard that marks a valued tool for CADD (31). PyRx also includes visualization engine which are vital for structure-based drug design. Library of lead molecules were screened by PyRx beside protein DHB. The molecules were docked and placed according to scores energy and RMSD. The top molecules were chosen for further study. All ligands were evaluated by docking through PyRx. The docked ligand molecule complexes were arranged on the basis of binding affinity with lowest energy to the top. In virtual screening on the basis of ligand Molecule were screened by using Dopamine beta hydroxylase as precedence model for this purpose.

Zinc 15:

ZINC 15 is a server which has publically accessible compounds for virtual screening. It encompasses about 35 million available ZINC 15 screened 140 ligands compounds in ready-to-dock; 3D formats (32). The drug binds to the protein and the binding affinity of drugs obtained by virtual screening ranges from 6.7 to 10.3.

DS Viewer (Discovery Studio):

DS permits a mechanistic sympathetic of a molecule's structure which can be viewed so clearly in sight. It also shares among computational modeling authorities and helps cooperating

team associates. It comprises Quality Graphics as well as handled huge macromolecule systems (i-e Ribosomes). It supports variety of stereo graphics selections (E.g., split screen, hardware stereo)**.** The interactions of ligand and protein dopamine Beta Hydroxylase were seen along the types of boding and bond distances in DS Viewer."

Swiss Similarity:

It is a web tool designed for quick LBVS to unprecedented ultra-huge libraries of small molecules. It includes screen able compounds, drugs and bio actives. Predictions are carried out by using different screened approaches, such as 2D molecular fingerprints super positional and 3D similarity methodologies (33). It allows to do LBVS of various libraries of molecules by using several techniques. Virtual library were screened in Swiss Similarity. Its screening reference molecules form virtual screen library against target. Drug clozapine is given in Swiss Similarity. It generates possible outcomes against the target by using its virtual library.

Secondary Structure Prediction:

Dopamine Beta Hydroxylase contains amino acid having polypeptide chain. The 3, dimension model confines containing 4 helices; 41% beta sheet 44 strands 4 turns and 4 alpha helix. This observation can be done by secondary structure prediction tool. Total atoms are 8927. It can be predicted that this model have 2 disulfide bonds.

Ramachandan Plot:

For the assessment of the predicted model quality, several validation tools have been used. It is a way to visualize energetically allowed regions for backbone dihedral angles ψ against φ of amino acid residues in protein structure. According to the Ramachandran plot statistics of Dopamine Beta Hydroxylase, total number of residues are 1094 including end residues, non-glycine and proline residues. Disallowed regions having 2 (0.2%), generously allowed regions having 11(1.2%), additional allowed regions having 66 (7.1%) and favored regions having 856 (91.6%) number of residues." "

Dopamine β- Hydroxylase Model:

Dopamine Beta Hydroxylase is used for structure based virtual screening. The structure based virtual screening provides assistance to recognize molecules and has competence to attach the active site of Dopamine Beta Hydroxylase protein. Library of lead molecules is made for screening by PyRx so that it is energy minimized can be further analyzed and examined in Discovery Studio Visualizer.

Docking: "

Drug clozapine was docked with the protein molecule by using Auto dock. It was chosen as a best drug due to its large number of interactions and fewer side effects. Interaction of ligands by protein is evaluated by Auto dock with PyRx. It can be done on particular pocket that are predicted by different software and literature studies. AutoDock is set of automated docking tools. It intends to predict how small molecules, such as substrates or drug candidates, bind to a receptor of identified 3D structure. It screened more than 200 drugs. A library of all lead molecules was

created for screening with PryR. Library of lead molecule was energy minimized. Auto dock is use with PyRx software to dock the drug with target and analysis result. Top 10 models were docked as the result of that docking score given clear idea about their interaction with dopamine beta hydroxylase."

Analysis of Interaction:

To analyze interaction between docking ligand and protein, Ligplot software was used. This software helped to view type of bonding between protein and ligands**.** Ligand was docked with dopamine beta hydroxylase to check for hydrogen bond interaction with LEU B: 424 LYS B: 452, SER B: 455 and VAL B: 456.

V.   RESULTS

To analyze interaction between docking ligand and protein, Ligplot software was used. This software helps to view type of bonding between protein and ligands**.** Ligand was docked with dopamine beta hydroxylase to assess for hydrogen bond interaction with LEU B: 424 LYS B: 452, SER B: 455 and VAL B: 456. CADD tool was used to explore new ways to design the cost effective drugs more rapidly via computer technology. Through conventional method, a drug takes 10-15 year to be released in the market. However, virtual screening is vital step in early-stage drug discovery. It encompasses 10 million accessible compounds Library of lead molecules which are made for screening by PyRx. Swiss similarity and ZINC15 servers are used to perform virtual screening of molecules where ZINC 15 can screen 140 ligands. The drug binds the protein where the binding energy ranges from 6-6.7 to 10.3. The binding energy of ligand ZINC 000095550333 is -10.3. The binding energy of ZINC000004292831 is -10.1.The binding energy of ligand ZINC 000036089410 is -9.8. The binding affinity of ligand ZINC 000095550333 is - 9.7. ZINC 000036089409 binding energy is - 9.5. Ligand ZINC 000036089465 has binding energy of -9.3. Binding affinity of ligand ZINC 000095550333 is -10.3. Binding affinity of ZINC000004292831 is -10.1.Binding affinity of ligand ZINC 000036089410 is -9.8. Ligand ZINC 000036089465 has binding affinity of - 9.3. On the basis of hydrogen bonding interaction which were analyzed. Dopamine Beta Hydroxylase combined with ligand showed interaction with residues VAL B: 456 having bond length 4.18 and Leu B: 424 having bond length of 1.48 and SER B: 456 having bond length of 2.27 and VAL B: 454 having bond length of 5.36. Ligand ZINC 000036089409. It also showed hydrogen bond with residue ASP B: 163, TYR B: 67, LEU B: 450, LYS B: 452, PRO B: 592, LEU B: 589 and ASN B: 435 with bond distance of 4.60. Ligand ZINC000036089410 showed interaction with ASN B: 435, LYS B: 452, ASP B: 163, MET B: 449, LYS B: 453, LEU B: 450, PRO B: 592 and GLN B: 436 having bond length of 4.61. Ligand ZINC 000095550333 showed hydrogen bond with HIS B: 493, GLN B: 445. GLU B: 265 TYR B: 491, VAL B: 298 and ILE B: 364 with the distance of 5.03. Ligand ZINC 000004292831 showed interaction with GLU B: 366, ARG B: 296, VAL B: 298, ILE B: 364, TYR B: 491, ARG B: 243 and LEU B: 502 with the bond distance of 5.01. Ligand ZINC 000004292831 showed interaction with ligand PRO B: 442 at bond length of 2.10 HIS B: 412 showed interaction having a distance of 4.95. GLU B: 265 showed interaction with

bond length 4.63 VAL B: 298 showed interaction having bond length of 5.25. HIS B: 414 showed interaction with bond length of 5.14. TYR B: 491 showed interaction having a distance of 4.56. HIS B: 493 showed interaction having bond length of 3.91. ARG B: 296 showed interaction having bond length of 5.45. PHE B: 267 having bond distance 4.63. ILE B: 483 showed interaction of ligand with receptor having bond length of 5.05. The results of system biology might vary in lab situation but most results were approximately good under appropriate circumstances. As for computational analysis, it emphasizes diverse ligands by using different servers which can act as best target against schizophrenia. Further nature of ligand will be explored by in-vitro and vivo analysis. Interaction of ligands with lead molecules suggests that auxiliary analysis of these drugs will give a way towards the treatment of schizophrenia.

VI. DISCUSSION

The name schizophrenia, as discussed earlier, is derived from Greek which implies "split mind" Schizophrenia is a disease characterized by a complex symptomatology, affecting most aspects of cognition, emotion and behavior. Schizophrenia affects approximately 1% of the population worldwide and is a chronic, severe disorder, lacking curative treatment. The suicide rate is as high as 9-13%, with the incidence of suicide attempt reaching 50% of diagnosed patients over a lifetime. The onset of schizophrenia usually occurs around 18- 25 years of age and is often preceded by premorbid behavioral deviations, such as social changes (34).Schizophrenia encompassing the different factors including several different susceptibility genes, multiple neurochemical system implications, and diverse clinical symptoms. Current understanding of the molecular, functional, and pathophysiological nature of schizophrenia, group of symptoms of illness and new pharmacological and treatment approaches which target specific stages of pathogenesis may prevent illness progression at different stages of schizophrenia. Making it is essential to develop community-based and clinical strategies. Dopamine β-Hydroxylase primarily contributes to catecholamine and helps trace amine biosynthesis. It also participates in the metabolism of xenobiotic related to these substances. Dopamine Beta Hydroxylase has been implicated as correlating factor in conditions associated with decision making and addictive drugs. Catecholamine storage vesicles provide a convenient biochemical marker for subcellular fractionation work and studies of the cellular regulation of catecholamine synthesis, storage, and release. It also plays an essential role in catecholamine synthesis. Schizophrenia includes a relative block or dysfunction at the Dopamine Beta Hydroxylase step in norepinephrine (NE) synthesis. Increased level of dopamine can cause schizophrenia. For the assessment of the predicted model quality several validation tools are being used. According to Ramachandran plot contain total 1094 residues. Out of 1094 residues 856 residues lie on the Most favored region of plot with 91.6 %, 66 residues are present in additional allowed regions having percentage of 7.1, 11 residues are present on the generously allowed regions of the plot with 1.2%, 0.2% are disallowable regions of plot having 2 residues, Non-glycine and Proline residues are 935, end residues 12 whereas glycine are 71 and Proline residues are 76 in number. The measure of standard distance between the backbone atoms of obligatory protein is called the root mean square distance. Secondary structure prediction helps in identifying secondary structural elements i.e. α-helices, β-sheets coils. Analysis of binding site can be made through COACHs software. Ligand Explorer is used to study the proteins and ligands interactions. The interactions are hydrophilic, hydrophobic, metal interactions and water molecules interactions. Ligand explorer was used to show interactions. Discovery studio visualizer tool and Milagros molecular viewer were used to study the protein-ligand interaction. Discovery studio tool was used to visualize the structure that identify and analyze protein-ligand interactions. It monitors the hydrogen bonds, ligand binding sites. (23).Method of molecular docking in the process of drug discovery uses computational approach to dock small molecules into huge structured molecules with targets concerning docking algorithms. The aim of molecular docking program is the exact positioning of small molecules within the binding sites of protein and also the biological inferences of this process. There are several other software's used for docking as well. The drugs screened by virtual screening might resemble Dopamine Beta Hydroxylase through its interactions. Top 10 models are docked as the result of that docking score gives clear idea about their interaction with dopamine beta hydroxylase. These interactions were further viewed by ligand explorer. There are 14 different drugs for treatment of schizophrenia including first and second generation. First generation drugs have more side effects than second generation drugs. Clozapine is a second generation drug considered as best drug for schizophrenia because of its less side effects. Clozapine rebalances dopamine and serotonin to improve thinking, mood and behavior. Clozapine is observed as the "gold drug" for treatment against schizophrenia. This is merely appropriate for treating the 20 - 30 % of those patients who are not able to give response to other medications and mainly those who have suicidal or violent thoughts.

VII. CONCLUSION

As for computational analysis, it emphasizes diverse ligands by using different servers which can act as best target against schizophrenia. Further nature of ligand will be explored by in-vitro and vivo analysis. Interaction of ligands with lead molecules suggests that auxiliary analysis of these drugs will give a way towards the treatment of schizophrenia.

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