# SULFORAPHANE AND AUTISM SPECTRUM DISORDER: A BIOINFORMATICS APPROACH TO THERAPEUTIC DISCOVERY

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## BIOTECHNOLOGY

**by:**


**Kanchan Kumari**

**2K22/MSCBIO/62**

**Under the supervision of**

**Prof. Yasha Hasija**
**Professor and Head of Department**
**Department of Biotechnology**




**Department of Biotechnology**

**DELHI TECHNOLOGICAL UNIVERSITY**
**(Formerly Delhi College of Engineering)**

**Shahbad Daulatpur, Bawana Road, Delhi-110042. India**


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**Kanchan Kumari (2K22/MSCBIO/62)**

# Delhi Technological University

(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-110042. India


## CANDIDATE'S DECLARATION

I, **Kanchan 2K22/MSCBIO/62** student of M.Sc. Biotechnology hereby declares that the Dissertation Project entitled**"Sulforaphane and Autism Spectrum Disorder: A Bioinformatics Approach to Therapeutic Discovery"** is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Science. This work is original and not copied from any source without paper citation. I have honoured the principles of academic integrity and have upheld the normal student code of academic conduct in the completion of this work.

The matter presented in the thesis has not been submitted by me for the award of my other degree of this or any other institute.

Place: Delhi

**Kanchan Kumari**
**2K22/MSCBIO/62**

Date:

**DELHI TECHNOLOGICALUNIVERSITY**
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-110042. India

## CERTIFICATE BY THE SUPERVISOR

This is to certify that the Dissertation Project titled**"Sulforaphane and Autism Spectrum Disorder: A Bioinformatics Approach to Therapeutic Discovery"** which is being submitted by **Kanchan Kumari 2K22/MSCBIO/62**, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Science is a record of the work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

**Prof. Yasha Hasija**
**Supervisor**
**Head of Department**
**Department of Biotechnology**
**Delhi Technological University**

**Date:**

# SULFORAPHANE AND AUTISM SPECTRUM DISORDER: A BIOINFORMATICS APPROACH TO THERAPEUTIC DISCOVERY

Kanchan

## ABSTRACT

Autism spectrum disorder is one of the serious neurobehavioral disorders. Report of centre for disease control reveals the rise in the rate of ASD diagnosis every year. Present therapeutic options are quite restricted and often prefer symptom management than on resolving the underlying pathophysiological mechanism. The etiology of autism is diverse with several genetic components, environmental factor and epigenetic pathways being identified. Progress in molecular genetics has enabled the identification of distinct medical condition as well as genes and environmental factor associated with pathogenesis of ASD. In order to provide an alternative therapy, intervention strategies using phytochemicals have been proposed. Among phytochemicals isothiocynate sulforaphane is known for its anti-inflammatory and neuroprotective effects in several invitro and invivo studies. Sulforaphane (SFN) is type of organosulfur compound derived from glucoraphanin, a compound found in broccoli, cabbage and Brussels sprouts. SFN has been reported to have some effect in the treatment of ASD. Therefore in this work we employed bioinformatics approach which includes network pharmacology and molecular docking to identify the possible target and mechanism of SFN in treating ASD. Network pharmacology make it possible to conduct a comprehensive analysis of the interactions take place between SFN and multiple biological targets associated with autism. This study brings insights into the signalling pathways, biological functions and complex molecular network. Identification of key nodes and pathways will be identified by analyzing the network of protein-protein interaction. Furthermore molecular docking will be done to predict the binding and interaction of SFN with identified target proteins. Collectively this study suggests that SFN has therapeutic benefits for ASD by targeting many factors and pathways, which offers a preliminary theoretical basis for conducting clinical studies.

## List of Publication

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# ABBREVIATIONS

| | |
|---|---|
| **ASD** | Autism Spectrum Disorder |
| **SFN** | Sulforaphane |
| **Nrf2** | Nuclear factor erythroid 2-related factor 2 |
| **BDNF** | Brain derived neurotropic factor |
| **NP** | Network Pharmacology |
| **MD** | Molecular Docking |
| **SMILES** | Simplified Molecular Input Line Entry System |
| **OMIM** | Online Mendelian Inheritance in Man |
| **PDB** | Protein Data Bank |
| **PPI** | Protein-Protein Interactions |
| **KEGG** | Kyoto Encylopedia of Genes and Genomes |
| **GO** | Gene Ontology |
| **BP** | Biological process |
| **CC** | Cellular Component |
| **MF** | Molecular Functions |
| **SRC** | Proto oncogene tyrosine protein kinase Src |
| **EFGR** | Epidermal growth factor receptor |
| **HSP90AA1** | Heat shock protein HSP 90-alpha |
| **GSK3B** | Glycogen synthase kinase-3 beta |

# CHAPTER 1
## 1.1 INTRODUCTION

ASD-autism spectrum disorder is a genetically heterogenous group of neurobehavioral disorder which is characterized by difficulties in social communication and stereotyped repetitive behaviours or interests[1]. The symptoms of autism usually appear in childhood and persist throughout the life. Studies revealed that in the United States, an estimated 15-20% of children under the age of 18 are diagnosed with these impairment. Moreover ASD has a median prevalence of 1 in 100, with males having a fourfold higher probability of being diagnosed in comparison to females[2][3]. Therapeutic approaches for ASD primarily revolve around behavioural interventions and treatment of symptoms, using Applied Behaviour Analysis to enhance communication and foster adaptive behaviour. Additionally, pharmacological intervention such as antipsychotics, selective serotonin reuptake inhibitors and stimulants employed to manage comorbid conditions[4]. However pharmaceutical interventions frequently exhibit adverse effect and may not yield outcomes for every individual. There is no single treatment that effectively tackles the fundamental symptoms of ASD, highlighting the importance of exploring novel therapeutic approaches. Sulforaphane (SFN) is an isithiocyanate compound obtained from broccoli, the therapeutic potential of SFN depends on its robust capacity to upregulate the function of genes that is taking part in the regulation of cellular protection against inflammation, oxidative stress, DNA-damaging, electrophiles and radiation in aerobic cells[5]. Research on the neuroprotective effects of SFN started in 2004, wherein various studies demonstrated that it can effectively protects neurons and microglia from the detrimental effects of oxidative stress which is carried out through the activation of nuclear factor erythroid 2-related factor 2(Nrf2)[6][2]. SFN also has been proven to have anti-inflammatory, anti-oxidant effects, detoxification effects and neuroprotective properties. The potential effectiveness of SFN showed a significant improvement in social interaction in ASD patients, further suggest the supplementation therapy utilizing the extract from broccoli sprouts abundant in SFN holds promise in improving cognitive impairments among individual diagnosed with ASD. SFN is gaining significant interest for its antioxidant , anti-inflammatory and anti-apoptotic characteristics[7]. In this report we employed network pharmacology method to determine the mechanism by which SFN may treat brain inflammation in neurodegenerative disorder. Researchers are investigating the potential of SFN to treat autism through a

comprehensive analysis of several research and clinical trials but the specific pathways, mechanism and targets are still not widely recognized[8]

## 1.2 OBJECTIVE

The objective of this project is to systematically discover the specific target of SFN that may have relevance to the underlying causes and mechanism of ASD. By comprehending the gene and pathways associated to it, we may acquire insight into the potential therapeutic advantages of SFN in treating ASD from multiple perspectives. The project will utilize network pharmacology to elucidate the interaction between SFN and identified molecular targets. Network pharmacology enables the analysis of the intricate biological networks and pathways offering a comprehensive understanding of multi targets effects. The validation of the expected interactions between SFN and its target will be performed using molecular docking which provide the visualization of the molecular level binding of target and SFN, enables the prediction strength and characteristics of interactions. The goal is to establish a scientific theoretical foundation for optimizing the selection of drugs in the future experimental research.

# CHAPTER 2
# REVIEW OF LITERATURE

## 2.1 ASD

Neurodevelopmental disorder, which affects the brain and nervous system, present an important challenge because they are the main cause of disability and death on a global scale. While chronic and acute neurodegenerative disorders involve multiple complex mechanism, yet shared some common pathogenic features including oxidative stress, mitochondrial dysfunction, neuroinflammation, neural damage , and protein misfolding[9].

The exact cause of ASD are complex and brain development in individuals with autism is very complicated as well, which include a combination of hereditary and non genetic factor such as environmental variables[10]. In addition upto 40% of cases of intellectual disability and 25% cases of speech and language delay with other cognitive impairments are associated to ASD. According to epidemiological administrative and community based research, there is prevalent co-occurrence of other neurodegenerative disorder such as attention deficit hyperactivity disorder, epilepsy, depression, anxiety and obsessive compulsive disorder (OCD)[11][12]. Additionally in the 2010 global burden of disease research showed 52 million people had ASD globally which corresponds to a prevalence of 1 in 132 individuals[13]. Subsequent research has demonstrated that the disorder has a high degree of heritability and susceptibility to autism increases the likelihood of experiencing a wider range of symptoms that range beyond the usual diagnostic criteria[14]. As a result there has been expansion of diagnostic ideas, modification to diagnostic criteria and the introduction of ASD in the most recent edition of Diagnostic and Statistical Manual[11].

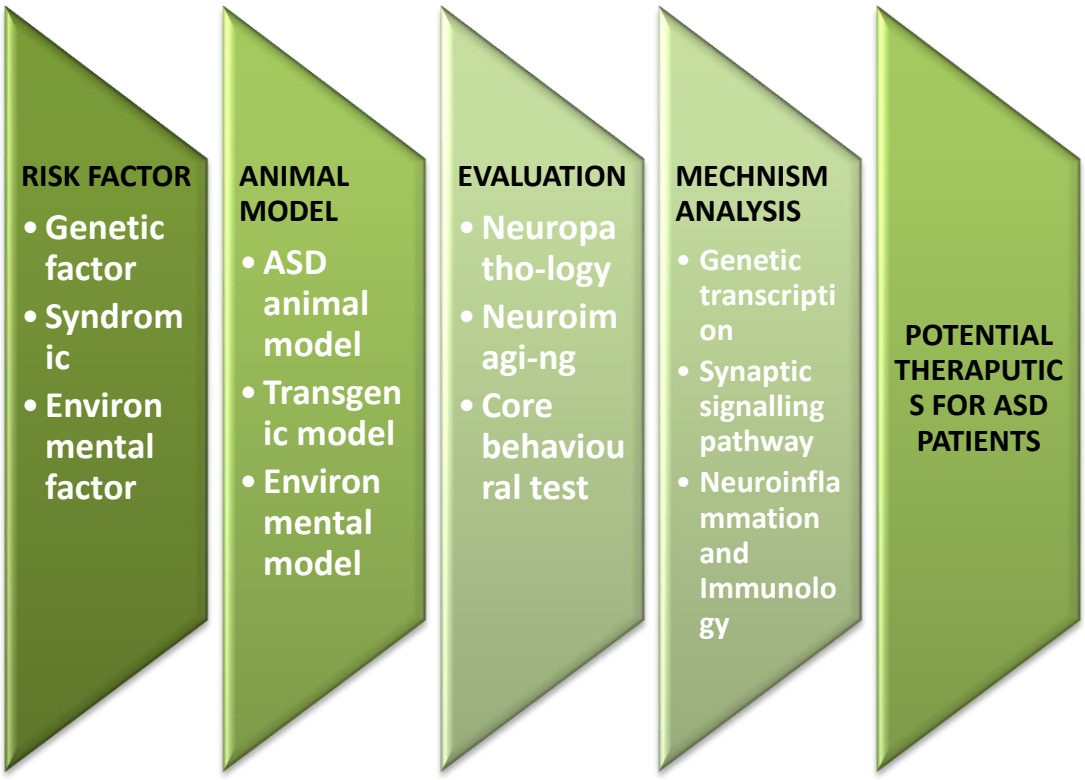


**Fig 1- Epidemiological researches with ASD individual identify the aberrant genes, mutation, copy number variations (CNVs) and environmental factors. By using these data different animal model will created for further studies. After a thorough examination of animal model with human pathology, offers potential and novel therapeutic approach in the treatment of ASD patients[15]**.

various genetic disorders like fragile X syndrome, Rett syndrome and tuberous sclerosis have been associated with an increased susceptibility to ASD[11]. Pathophysiology of autism is influenced by dysregulation of immune system, oxidative stress, mitochondrial dysfunction and inflammatory conditions[16].

MRI studies found the altered neural circuit in multiple brain regions such as visual areas of prefrontal cortex, sub cortex, amygdale, and cerebellum. In addition research indicate the possibility of disturbed neural circuit in autistic children and may offers insights into the underlying mechanism of ASD[13].

### 2.1.1 NEUROBIOLOGICAL VIEW

There are so many gene that is reported to cause ASD are involved in homeostasis, development and plasticity of synapse and mutation in those gene leads to aberrant synaptic pruning, abnormalities in neurotransmission and behavioural symptoms of neurodegenerative disorder[17]. Recent studies demonstrated the dysregulation of important translational signalling pathways, aberrant synaptogenesis, abnormal behaviour of microglia , synaptic protein, scaffolding and signalling molecules that has an impact on the synapses functions and structure , elimination , neurotransmission, synaptic plasticity that exacerbates neuronal dysfunction in ASD[18][19]. Synaptic signalling pathways such as mTOR and FMRP are two primary pathways in translation of mRNA and can straight-up impact the synaptic proteins thus regulate the number and functions of synapse[20]. Mutation in gene results aberrant neural circuits. Increased brain volume has been observed in children with autism. Furthermore post mortem studies suggest the increased level of glia cells, reduced number of GABA neurotransmitter, greater density of dysregulated microglia which leads to less synaptic pruning in multiple brain region[19].

### 2.1.2 IMMUNOLOGY AND NEUROINFLAMMATION

Patients with ASD have been found to have persistent immunological dysfunctions. Research identified the 185 gene with differential expression and most of the gene were enhancing the regulation and associated to immunity response pathways[21]. Inflammatory molecular signalling pathways affect brain connections and functions by affecting complement factor, cytokines, microglia , major histocompatibility complex class I molecules (MHCI)[22][12]. ASD is associated with dysregulated cytokines, which may be sensitive biomarkers for neuroinflammation and immune system disruptions. Furthermore increased level of IL-6,IL-8, TNF-α, TGFβ, CCL2 and IFNγ are found in ASD patients[12]. Maternal autoimmune disorder (MIA) such as fever, asthma, allergy and external exposure to substance including air pollutants can elevate immune response and raise the risk of ASD[18].

### 2.1.3 MICROBIOTA GUT BRAIN AXIS

Recent studies aimed to determine the mechanism of association between microglial neurodevelopmental disorder and gut microbiota. Some studies observed structural changes in brain of germ free mice, the functional link between the microglia and microbiota. Dysbiosis and gastrointestinal tract symptoms such as constipation, vomiting, abdominal pain, bloating, diarrhoea are more prevalent in ASD

children[23]. Dysregulation in immune system has been identified which leads microglia to exhibit aberrant activation with alterations in shape, density and spatial localization. In addition it has been found that people with ASD had lower level of bacteroidetes and increased level of clostridium and firmicutes[24]. Gaining insight into the connection between brain and microbiota could assist in creating potential treatment to reduce phenotype symptoms of ASD.

## 2.2 SULFORAPHANE

Sulforaphane is an aliphatic isothiocyanate derived from glucoraphanin, a compound predominantly present in cruciferous vegetable like cauliflower, broccoli, cabbage and Brussels sprouts.SFN is also known as 1-isothiocyanato-4-(methylsulfinyl)butane[25]. The production of SFN takes place through a hydrolysis, facilitated by the enzyme myrosinase found in plants. This reaction along with the inactive form of epithiospecifier protein (ESP) produces SFN[26][2].several pathogenic process linked to ASD, including oxidative stress, mitochondrial dysfunction and neuroinflammation that involve Nrf2 but the multifunctional SFN can regulate the its expression through cytoprotective enzymes and antioxidant action[27]. Moreover SFN is a sulphur rich phytocompound that exhibit anti-inflammatory, antiapoptotic and antioxidant properties[2]. SFN can upregulate the expression of WNT signalling pathway proteins such as cyclin D1 and also stimulate neurogenesis by raising brain derived neurotrophic factor (BDNF) levels which are involved in proliferation and differentiation of neurons. SFN has a significant level of bioavailability and posses antioxidant properties. It is efficiently digested and eliminated from the body through urine[2]

SFN has been shown to effectively fight various disease and studies shows its potential health benefits in the treatment and prevention of disease. Recent research on SFN has explored its potential neuroprotective effects, indicating its role in regulation of pathways. Evidence from preclinical and clinical studies shows promising novel therapeutic in ASD, schizophrenia, Parkinson’s and Alzheimer’s disease[2]. Additionally SFN has also shown anticarcinogenic , antidiabetic, antiobesity and cardiovascular protective effects resulting the prevention and treatment of such disorders[28].

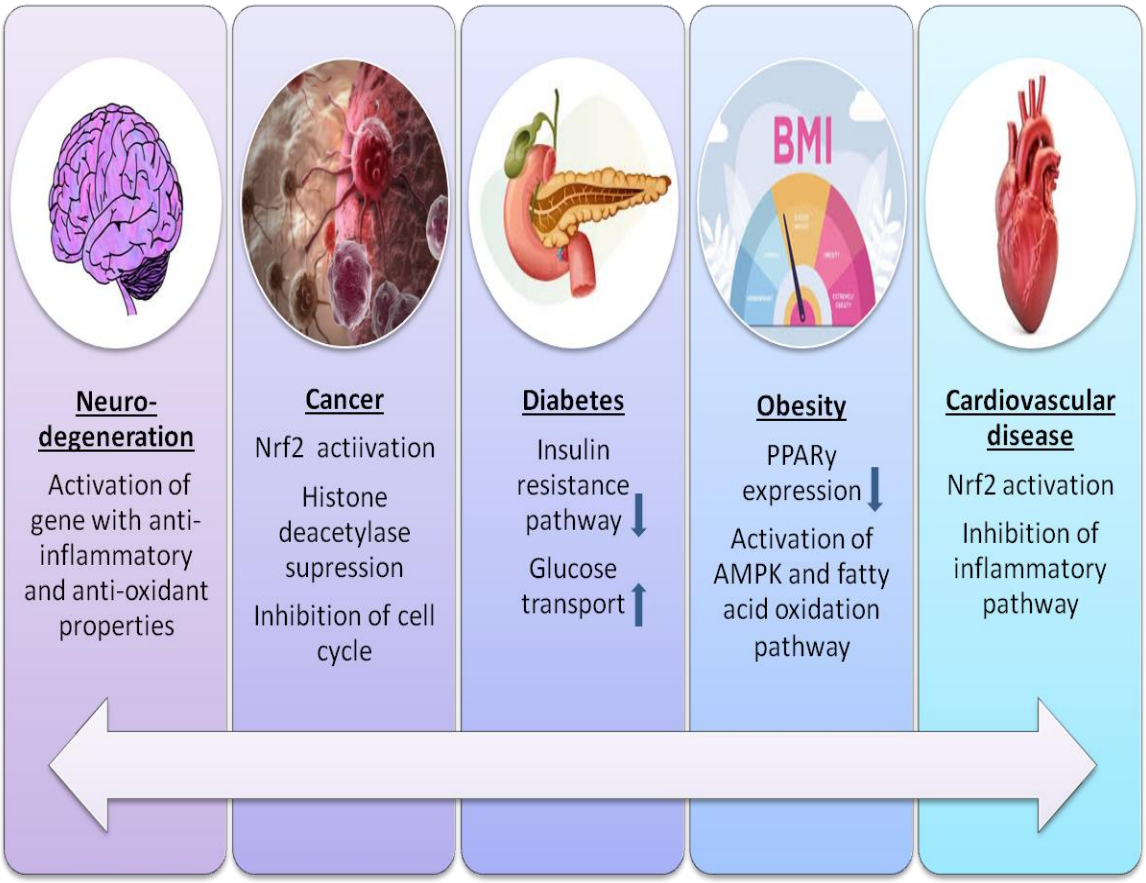


**Fig 2 - Mechanism of SFN in various disease conditions**

### 2.2.1 MECHANISM OF ACTION OF SFN

SFN exhibits an anti-inflammatory impact by deteriorating the build up of pro-inflammatory cytokines, including IL-1β, TNF-α, IL-6. It is accomplished through restricting the nuclear factor kappa B pathway[29]. SFN has anti-inflammatory properties which helps to reduce chronic inflammation, prevalent hallmark of many neurodegenerative disorders[30].

Antioxidant effects- SFN stimulate the production of phase II detoxification enzyme such as glutathione S-transferase (GST) via activation of Nrf2 pathway which leads to cellular defence against oxidative stress[28].

Modulation of neurotransmitter and neurogenesis- SFN influence the dysregulated serotonergic, dopaminergic and glutamatergic systems in neurodegenerative disorders. Moreover studies suggest the activity of SFN to improve synaptic plasticity which promote neurogenesis, memory and learning ability[26].

### 2.2.2 NEUROPROTECTIVE EFFECTS

Several studies indicate the effects of SFN on the reduction of symptoms of neurodevelopmental disorders. Studies on murine models revealed the reduction of depressive behaviour through the serotonin metabolism and transport effects. Furthermore the neuroprotective effects of SFN prevent dopaminergic neurons, leads to improvement of impaired motor function , coordination in ASD patients[28]. Based on preliminary research on animal model, placebo controlled trail found that SFN may reduce the repetitive behaviours and enhance social behaviour ,likely by the reduction of inflammation and oxidative stress[5].

## 2.3 NETWORK PHARMACOLOGY

The system biology technique have gained popularity along with omics sciences, can be considered as new paradigms in pharmacological studies to find new target and treatment of complex multifactorial disorder[31]. Network pharmacology (NP) is an interdisciplinary field which include pharmacology, bioinformatics and system biology to comprehend the interaction of medication with their target in the intricate biological system.

Most of the medications for complex disorder have limited effectiveness and the success rate of discovering new drugs is continuing to decrease[32]. Additionally, the traditional focus on specific organ in medicine and prevailing “one disease-one target-one drug” approach significantly restrict the innovation[33][34]. Moreover discovering and introducing new drugs onto the market was time consuming and expensive. Although the application of traditional technology as employed to produce precise mono targeted molecules that imitate the bioactive properties of natural small molecules rather than focusing on comprehending the basic rationale for their combined effects and devising techniques to extract the component of bioactive compound[35]. Several researchers found that majority of disease arise due to the dysregulation of various proteins. Therefore it is necessary to address many factors that arise from a metabolic cascade associated with a disease in order to get a proper treatment[36]. NP revolutionize the diagnostic and treatment and drug repurposing combined with synergistic multicompound network pharmacology facilitates precise and effective therapeutic intervention[37]. Additionally NP facilitates the personalized medicine treatment by taking into account the distinctive genetic and molecular profiles of patients to enhance the effectiveness of treatment and minimize the side effects.

## 2.4 MOLECULAR DOCKING

MD is a crucial tool in the field of molecular and structural biology and computer aided drug design used to forecast the binding mode of target and protein. Docking has been developed during the last three decades and the process has been significantly accelerated because substantial increase in the availability and power of computers as well as the increasing ease of accessing databases containing small molecules and proteins. The objective of this software is to comprehensively analyze and predict molecular recognition including structural aspect of identifying probable binding modes as well as the energetic aspect of estimated binding affinity[38]. The utilization of in silico methodologies has facilitated the virtual screening of a vast number of compounds within a reasonable timeframe. This has resulted in a reduction of the initial expenses associated with hit identification and an enhancement of the likelihood of discovering the desired drug candidates. Currently, a variety of molecular modelling techniques exist to aid in drug discovery[39]. The utilization of MD technologies in the field of drug discovery encompasses a wide range of applications such as structure activity studies, optimizing leads, identifying potential leads, formulating binding hypothesis to aid in prediction for molecular biology studies and designing combinatorial libraries[38]. MD is a computational method utilized to predict the structure of a complex formed between ligand and receptor[12]. Moreover docking is a two step process that starts with sampling the ligand conformation inside the protein's active site and secondly it involves evaluating these conformations using a scoring function[40]. Fischer's lock-and-key hypothesis explains how the protein fits into the target molecule. The first docking approach viewed the ligand and receptor as rigid entities. In addition, Koshland's induced-fit ides extends the lock-and-key supposition by suggesting, ligand interactions modify the proteins active site. This theory suggest the flexible nature of ligand and receptor[41]. Scoring function can be categorized as empirical, force field based or based on comprehension. In addition search techniques can be classified into two main categories: systematic and stochastic planned research uses predefined intervals to sample the search space and follows a deterministic approach. On the other hand, stochastic search involves making random adjustments to the state variables in an iterative way until a termination condition specified by the user is met[42][38].

Tools and software that we are commonly used for molecular docking including AutoDock Vina, GOLD, MOE-DOCK, SwissDock and PyRx.

## 2.5 DATABASE AND TOOLS USED

### 2.5.1 PubChem

PubChem is a publicly accessible database that stores information about the biological properties of small molecules. It consists of three interconnected database i.e. compound/substance and bioassay. The substance database stores chemical information, the compound database contains distinct chemical structures and the database of bioassay provides data on the biological activity of chemical compounds that have been analyzed in assay tests. PubChem is committed to upholding its role as a vital source of chemical information for biomedical research as it consistently adjusts, evolve and developing new tools and services to utilize continuously improving technology[43].

### 2.5.2 SWISS TARGET PREDICTION

Swiss target prediction provides a user friendly interface for predicting the targets of mini-compounds. Predictions are generated by using the principle of similarity through the reverse screening. Moreover in this tool the screening engine was mainly coded by Python 2.7[44]. The user may input query molecules either as SMILES or drawing them in 2D using ChemAxon. The 2D interface and SMILES input field automatically synchronize. The current version having option to select the species and organism including human, mouse, rat, cow and horse. When the predict target button turns red, the computation is initiated. The expected targets are shown on the result page with their common name along and also provide links to GeneCards, UniProt and chEMBL databases. The target are ordered based on their score regarding target molecule and the table rows were organized automatically based the probability of the corresponding protein[45]. This tool is frequently used in drug discovery, drug repurposing and lead optimization. However, its efficacy is constrained by the quality of the database and the computational demand.

### 2.5.3 SuperPred

It is another powerful computational tool to predict drug target and probable medication interactions. The tools employ both 2D and 3D ligand based similarity metrics and machine learning algorithms. In input we can upload the SMILES or create a molecular structure with Marvin Sketch. The result is a well organized

database that includes predictions which contain similarity scores, compound-Ids, molecular structures, physiochemical parameter and target information of the query compound. Only the targets that meet the criteria of being ‘strong binders’ can be considered known targets and there is also other information and link for visualization of targets are present. In addition target with multiple indications are organized in separate rows in the database[46]. The identification of therapeutic targets now includes not only active binders but also experimentally validated non-binders, obtained through the ChEMBL database. By incorporating the machine learning, this design allows for a significantly precise evaluation of structural groups involved in the protein binding process. Furthermore this approach offers the additional benefit of focusing on substructure features rather than overall structural similarity.

### 2.5.4 GeneCard

GeneCard is comprehensive repository and bioinformatics tool that provide detailed information associated to all annotated and predicted human genes. This tool is an innovative platform invented by the esteemed Weizmann Institute of science. It gather data from various databases, offering comprehensive profiles that cover the sequence of genome, expression pattern, phenotypes, gene functions , phenotypes. The inherent simplicity of its interface allows researchers to conduct gene searches utilizing nomenclature, symbols or keywords, which facilitate the process of gene exploration and clinical investigation.

### 2.5.5 OMIM

It is the primary repository of curated information of genes and genetic disorder that contain detailed overview of all known Mendelian disorder and over 16,000 genes. These database can be searched by MIM number, gene or disorder name and every OMIM entry is assigned a distinct six-digit number where the initial digit signifies its inheritance status such as X-linked,Y-linked, autosomal or mitochondrial[47]. OMIM serves as a dynamic database that provides physicians, researchers and student with up-to-date information on the connections between genes and illness. It aids in the understanding of the complex interconnections between genes and diseases.

### 2.5.6 Venny2.0.2

Venn diagrams are often used for graphic depictions of intersections, union and differences between numerous datasets. Venny 2.0.2 software is widely used to generate Venn diagrams for application in various researches. Sequencing technology, such as genomics, proteomics and transcriptomics has greatly advanced and now extensively utilized in large scale biological investigations to discover fundamental sets of genes[48]. Moreover venn diagrams are commonly employed to visually represent the genes that are either shared or unique across different gene lists.

### 2.5.7 STRING

The database STRING provides precomputed global resource that facilitates the search and study of protein-protein interactions. Protein-protein interactions extend beyond just direct physical binding, proteins can also interact indirectly through many mechanism, such as sharing in a metabolic pathway or influencing each other transcriptionally[43]. As STRING is a pre-computed system, it enables easiest access to all relevant data, encompassing both broad network perspective and the detailed record of individual interactions[49]. It predict interaction score between the proteins in a network based on gene fusion, co-expression, experimental data and co-occurrence across genome. This tool facilitates cross species comparisons and provides methods for functional enrichment analysis, facilitating researchers in identifying crucial biological processes and pathways. The vast integration of data, functional insights, and support for community engagement make it an indispensable tool for comprehending molecular pathways and propelling biomedical research.

### 2.5.8 Cytoscape

Complex networks, characterized by a high number by a high numbers of nodes and edges and can be challenging to understand and the organization of paths within them typically requires a significant amount of time. Thus, a crucial aspect of visualization tools is the availability of suitable layout algorithms that facilitate the organization and alignment of network nodes. Cytoscape has been designed for the display of networks and pathways related to biomolecular interactions. The software offers the capability to represent extremely large networks and to visually display the interactions among the component of various molecular networks[50]. Furthermore cytoscape allows for the modification of an existing network by adding or deleting nodes or edges from a dataset, as well as altering node names and their properties. It

also supports the merging of smaller networks into a single and bigger network. Cytoscape extension mostly focused on the investigation of networks. Several plug-ins enhance the functionality for importing networks and their associated annotations. Cytohubba is powerful plug-in software, specifically developed to identify and rank the nodes in biological network by utilizing different topological parameters such as degree, betweenness, and closeness to examine the network and identify the possible targets for therapeutic interventions.

### 2.5.9 KEGG

It is a comprehensive database if genetic information and genomes. It was first developed to provide a comprehensive resource for interpreting the biological information of completely sequenced genomes using KEGG pathway mapping. It is integrated resources consisting of three type of database for genomic, chemical and network information. This database with the substitutes of metabolic and regulatory pathways searchable maps are included in the pathway information section[51]. There is an organized list of known metabolic pathways accessible via the metabolic pathways links. After clicking on the pathway of interest, an enzyme, product and substrate flowchart appears for the conventional pathways. The large datasets and pathway maps of this tool enhance comprehension of biological systems and increasing advancement in functional genomics and drug development.

# CHAPTER 3
# METHODS AND MATERIALS

## 3.1 Obtain structure and molecular properties of SFN

The molecular properties and structure of SFN was searched in chEMBL and PubChem. The literature was searched for comprehensive study of pharmacologically active component, drug likeness and blood brain barrier permeability information, canonical simplifies molecular-input line entry system (SMILES).

### 3.2 Identification of SFN target

Swiss target prediction and SuperPred tools are used for target prediction, with the species set at HomoSapeins in the search bar. The target we got from both tools was combined and remove duplicate and eliminate the component with no relevant information.

### 3.3 Acquisition of ASD target

GeneCard and OMIM database are used to identify the ASD related target. All obtained target from two databases were integrated in Excel. Deduplicate genes were subsequently eliminated and rectified using UniProt database.

### 3.4 Screening of intersected gene target

The component targets were mapped to each other with ASD target, and then Venn plots were generated to identify the overlapping genes. We used Venny 2.1.0, a tool to get the genes that intersected. Subsequently, a Venn plot was created to visually represent the possible targets of SFN to treat ASD.

### 3.5 Construction of protein-protein interactions (PPI)

To perform a more comprehensive study of the protein interactions between SFN for ASD, the interacting genes were imported to the STRING. The construction of protein-protein interaction is crucial for understanding the degree of interaction between proteins. Organism was set to “Homo Sapiens”, and interaction score was set at 0.4 and results are stored in TSV file. Constructed PPI network were used to derive information for the next step.

### 3.6 Core target screening

In order to determine the essential target genes, we employed network analyzer tool Cytoscape 3.9.0 to examine the topology parameters of network. The TSV file was imported into Cytoscape to build the network including both direct and indirect interactions. By using Cytohubba tools, key target were identified based on their degree value.

### 3.7 GO and KEGG pathway analysis

Bioinformatics tool ShineyGO was utilized to conduct the Gene ontology (GO) enrichment analysis. Upload the key target obtained from Cytohubba and the species was set to “Homo Sapeins”. Analysis was conducted on three aspect including

biological process (BP), cellular component (CC) and molecular function (MF). To further understand the specific target of SFN in the treatment of autism, we conducted KEGG pathway enrichment analysis. KEGG is a database that provides information about the overall functions and uses of biological systems, including cells and organisms. It focuses on molecular level data, generated by genome sequencing and other technologies.

### 3.8 Molecular Docking

By means of KEGG pathway enrichment analysis, we have successfully identified probable genes of SFN that are targeted by the active component of autism. These targets have been confirmed by the utilization of molecular docking technology. Validated component were obtained and crystal structure were taken from RCSB Protein Data Bank in PDB format. Optimization of the structure was done by Chem3D software to minimized the energy of compounds. The structure of target protein was retrieved from Uniprot and PubChem database. After downloading 3D structures upload it on Biovia discovery studio for further preparation. Water molecules were removed from for better binding and add polar hydrogen for better docking calculations.

The chemical, which was treated act as a small molecule ligand and a crucial target protein that acted as receptors. The molecular docking process was carried out through a PyRx version 0.8 software. The grid box was positioned at the middle and had dimensions of $50\times 50 \times 50$ for its length, breadth and height. This experimental setup facilitated prediction of the affinity of binding between the protein and the compound. To visualize the interactions we used PyMOL tools, a software that offers comprehensive information on the interaction between ligand and receptor. Furthermore it facilitates the identification of hydrogen bonds, hydrophobic and pi-pi stacking interactions.

# CHAPTER 4
# RESULTS

## 4.1 Molecular structure and pharmacology of SFN

By using chEMBL and PubChem database we found the 2D and 3D structure of SFN, shown in fig 3. Molecular formula is $C_6H_{11}NOS_2$, molecular weight is 177.3 g/mol, hydrogen bond donor count is 0, hydrogen bond acceptor count is 4 and BBB score is 3.46.

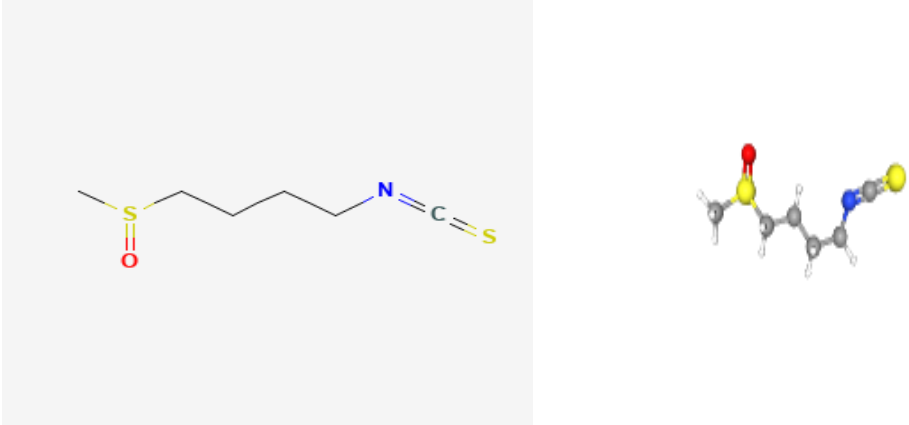


**Fig 3- 2D and 3D Structure of Sulforaphane**

## 4.2 Screening for the SFN and ASD –related gene target

From swiss target prediction we got 707 potential target, one hundred fourteen targets were obtained from SuperPred. The above database were merged and remove the duplicate, we obtained 197 SFN related target. From GeneCard we found 10,2728 genes causes autism and 1133 from OMIM database ,after conducting the screening process, a total of 1701 targets related to ASD were retrieved by merging and removing duplicate entries.

The SFN and ASD associated targets were analyzed using Venny tools, yielding 104 targets which is shown in table- 1 and the venn plot was shown in fig-4

**Table 1 - Potential SFN gene target for the treatment of ASD**

| | | | |
|---|---|---|---|
| EGFR | NAMPT | JAK2 | ANPEP |
| MIF | CTSS | PRKCA | SCN2A |
| NOS2 | CTSL | TYK2 | TOP1 |
| TGM2 | HDAC1 | HSD17B10 | PSMB9 |
| TLR9 | TDP2 | NFE2L2 | AURKB |
| GRM5 | AKR1A1 | NFKB1 | PDE11A |
| SLC6A2 | MPO | CTSD | CNR1 |
| SLC6A3 | MMP2 | APEX1 | RORB |
| MTNR1B | SIRT2 | TRIM24 | NR3C2 |
| PARP1 | ESR2 | AR | CDC25C |
| MAP2K1 | CYP2C19 | GUSB | ADAM10 |
| SLC6A4 | TYMP | PIK3R1 | CSNK2B |
| CYP19A1 | CHRM1 | GRIA2 | ABCC1 |
| MAPK1 | TTR | GRIN1 | PIK3CD |
| GRM4 | STS | CDK1 | CDK5 |
| MAOB | TOP2A | TERT | MC4R |
| CYP11B1 | ADORA2A | CHRNB4 | MDM4 |
| SRC | ADORA3 | BLM | KCNK9 |
| PTK2B | HSD11B1 | SLC2A1 | CTSB |
| HDAC6 | ALDH3A1 | SCN3A | CSNK2A2 |
| MAOA | HSP90AA1 | PTPN11 | GHSR |
| AKR1B1 | PDE3A | F2 | GCK |
| OPRM1 | FLT3 | PSMB1 | TLR4 |
| GSK3B | KDR | KIF11 | FLT1 |
| ACHE | JAK1 | NTRK3 | AHCY |
| ALDH1A1 | ALOX15 | METAP2 | KAT6A |

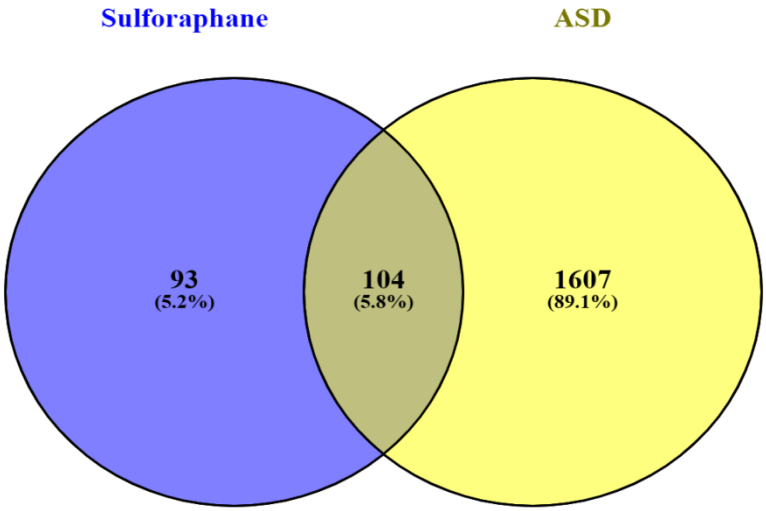


**Fig 4 - Venn map of SFN-ASD intersection target**

### 4.3 Construction of PPI network

The STRING databases provide the protein-protein interaction were further loaded into the Cytoscape tool. It involve 104 nodes and 607 edges, shown in fig-5.Node represent the protein and lines represent the interactions of proteins. The size and color of node shows the degree value. Larger the node larger the degree value and thicker the edge higher the interconnectivity. Additionally key component target are shown in fig- 6 in which the node size is proportional to the degree value.

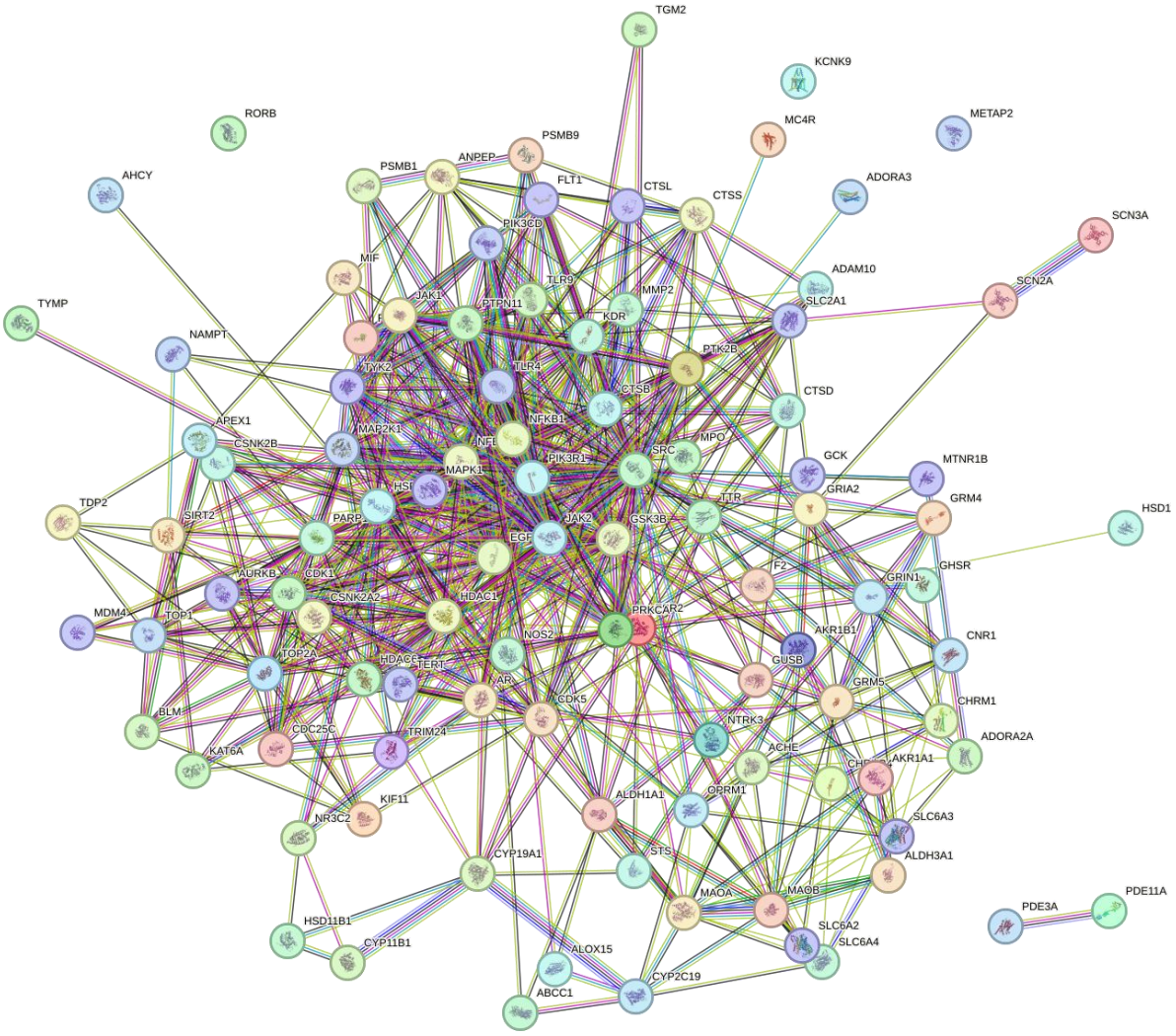


**Fig 5- PPI network**

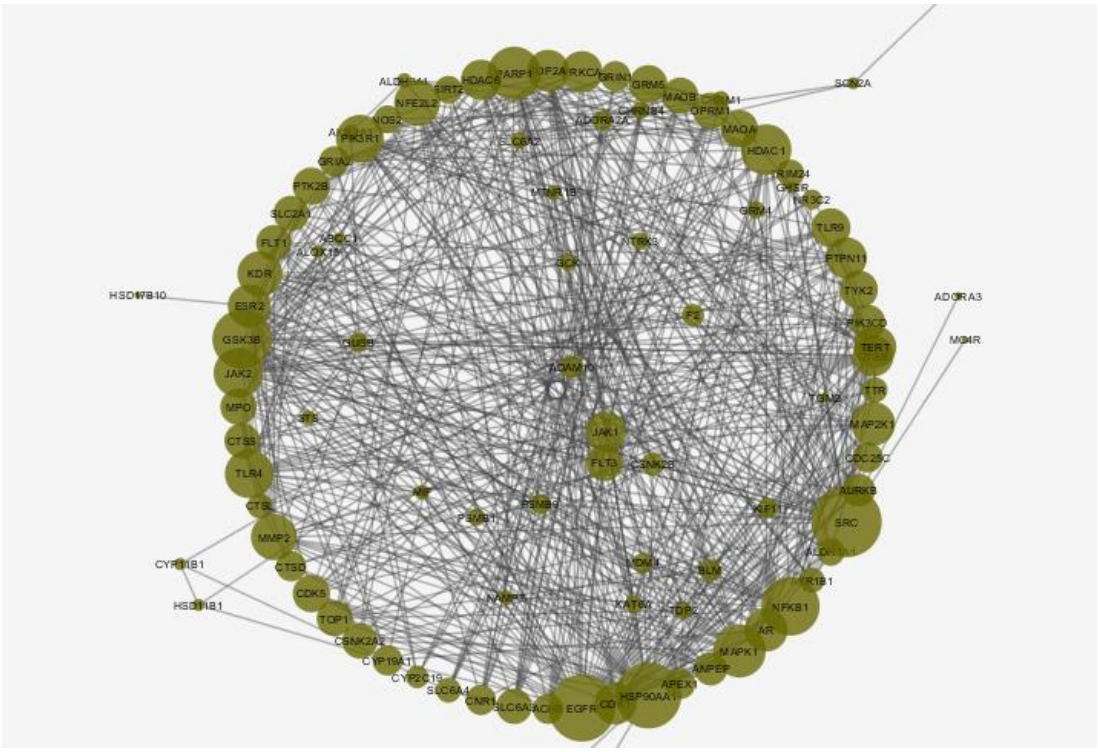


**Fig 6- Key component target network**

### 4.4 Screening results of core target network

In cytoscape software , we use Cytohubba tools to calculate the degree value. The details are shown in Table 4.2.and the network diagram are shown in Fig 7. Target such as SRC, EGFR, HSP90AA1, GSK3B, NFKB1, MAPK1 shows higher degree value. From this, we proposed the hypothesis that these targets are implicated in the pharmacological actions of SFN for the treatment of autism.

**Table 2- Core target network.**

| Rank | Name | Score | Protein |
|---|---|---|---|
| **1** | SRC | 49 | Proto oncogene tyrosine protein kinase Src |
| **2** | EFGR | 45 | Epidermal growth factor receptor |
| **3** | HSP90AA1 | 44 | Heat shock protein HSP 90-alpha |
| **4** | GSK3B | 37 | Glycogen synthase kinase-3 beta |
| **5** | NFKB1 | 36 | Nuclear factor NF kappa B p105 subunit |
| **6** | PARP1 | 31 | Poly [ADP ribose] polymerase 1 |
| **7** | MAPK1 | 30 | Mitogen activated protein kinase 1 |
| **8** | HDAC1 | 28 | Histone deacetylase 1 |
| **9** | JAK2 | 27 | Tyrosine-protein kinase JAK2 |
| **10** | TLR4 | 26 | Toll like receptor 4 |

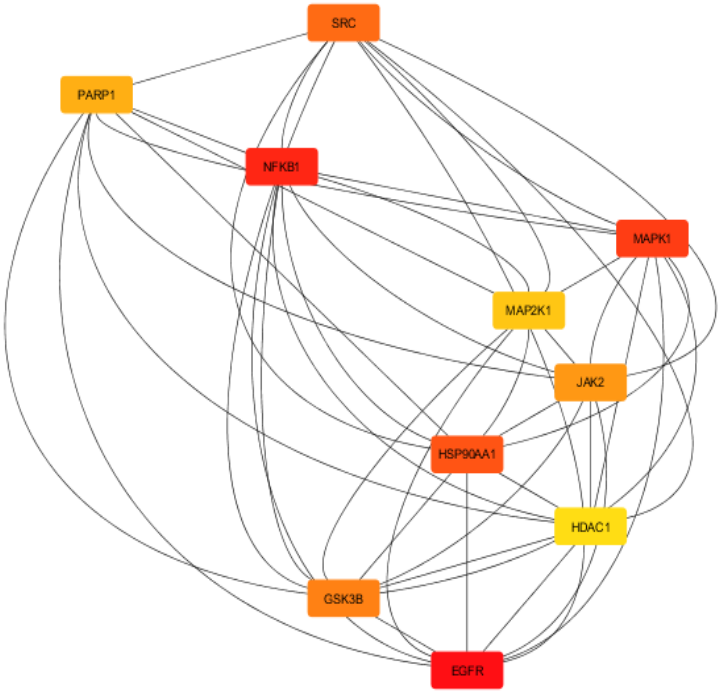


**Fig 7- Core target network**

### 4.5 GO and KEGG pathway analysis

The screened core target was later uploaded into the ShineyGo tool for the purpose of GO gene annotation and KEGG pathway analysis. Keeping $p < 0.05$ as the standard, we took the top ten most significant GO terms, are shown in fig 8-10.Biological process consisted of cell death, cellular response to lipid, positive regulation of protein localization, response to nitrogen compound, cyclic compound, endogenous stimulus etc. Significantly an enriched cellular component GO term involves membrane raft, vesicle lumen, caveola, focal adhesion, mitochondrion etc. Molecular functions GO terms included identical protein binding, binding of protein kinase, binding of nucleotide, protein serine/tyrosine kinase action and enzyme binding.

The KEGG analysis displayed a total of 100 metabolic pathways. The top twenty pathways were chosen for histogram based representation on the p value as shown in fig11.It involves prolactin signalling pathways, prostate cancer, yersinia infection, lipid and atherosclerosis, P13K-Akt signalling pathway etc.

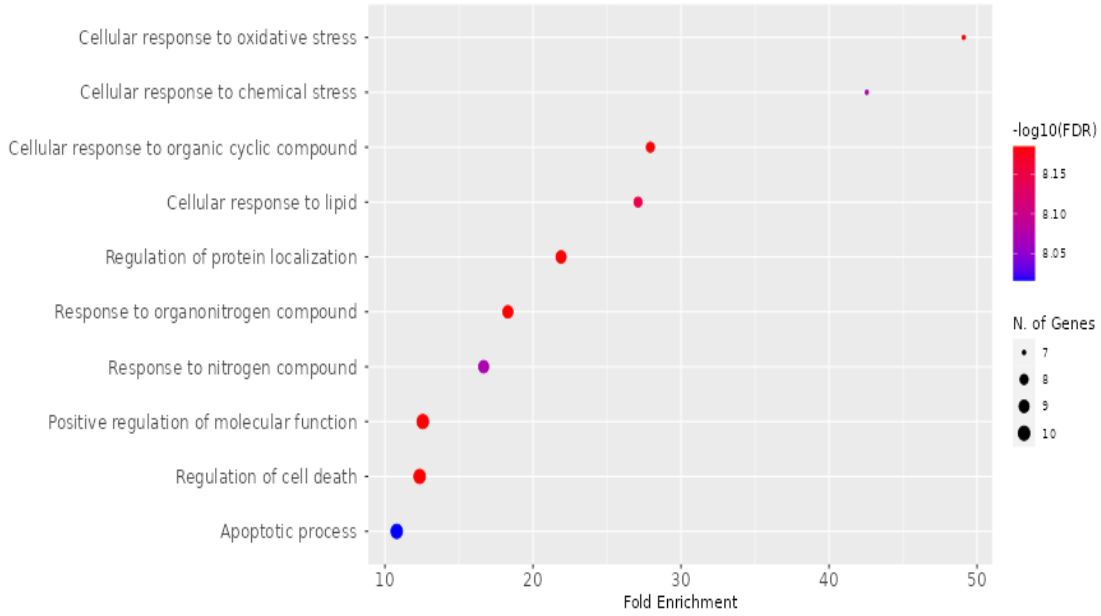


**Fig 8- GO for BP**

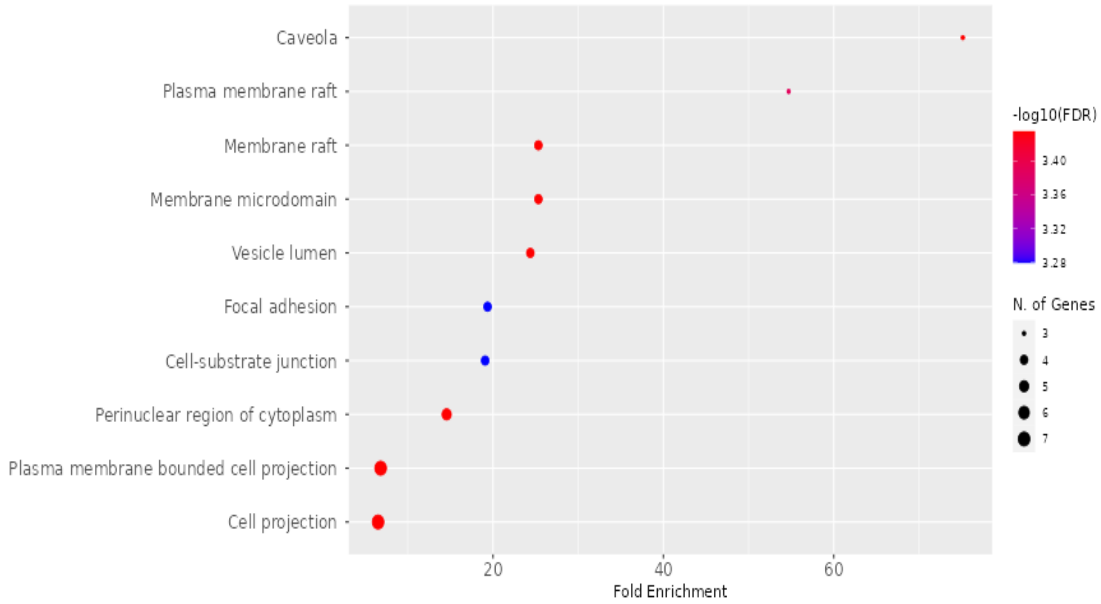


**Fig 9- GO for CC**

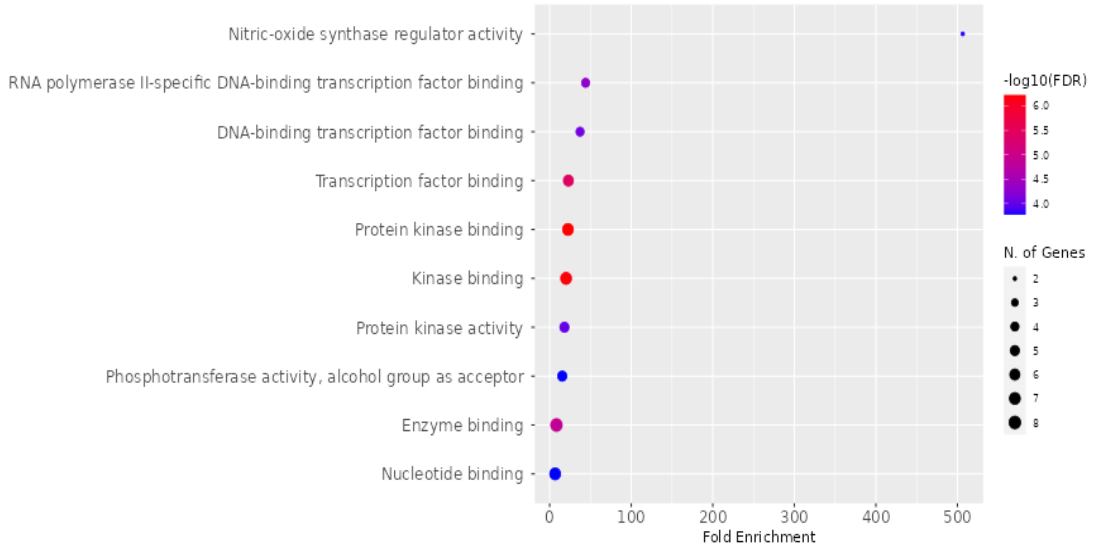


**Fig 10- GO for MF**

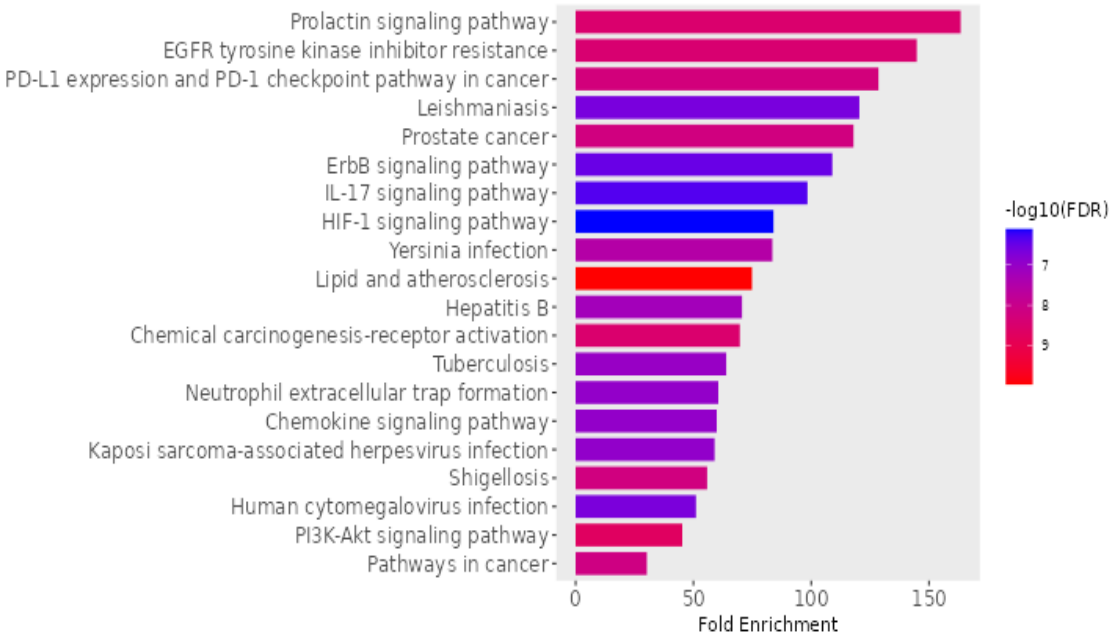


**Fig 11- Histogram of KEGG enrichment pathway analysis**

### 4.6 SFN target ASD pathway PPI network

The PPI network of SFN target ASD pathway were construct from Cytoscape software, shown in fig 12. The purple node represents the compound of SFN and yellow node represents the top twenty signalling pathways where the target is located. After topological analysis we found that MAPK1, EGFR, GSK3B and NFKB1 involved in p13K-Akt signalling pathway, shigellosis, thyroid hormone signalling pathway, chemokine signalling pathway etc. as shown in Table 3

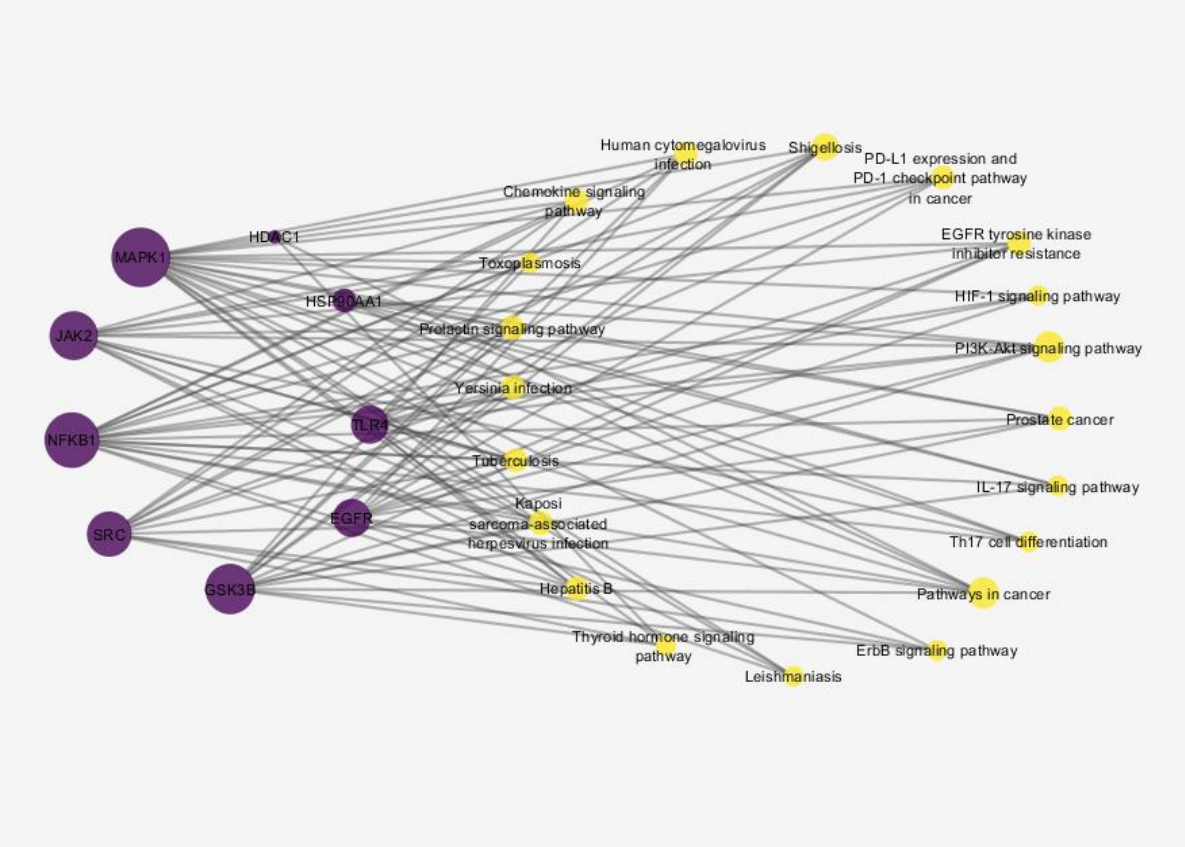


**Fig 12- Target-Pathway PPI network**

**Table 3- KEGG metabolic pathway enrichment analysis**

| ID | PATHWAY | GENE | COUNT |
|---|---|---|---|
| **hsa04151** | P13K-Akt signalling pathway | MAPK1,NFKB1,EGFR,GSK3B,HSP9 , AA1,TLR4,JAK2 | 7 |
| **hsa04917** | Prolactin signalling pathway | MAPK1,NFKB1,GSK3B,SRC,JAK2 | 5 |

| | | | |
|---|---|---|---|
| **hsa01521** | EGFR tyrosine kinase inhibitor resistance | MAPK1,EGFR,GSK3B,SRC,JAK2 | 5 |
| **hsa05131** | Shigellosis | MAPK1,NFKB1,EGFR,GSK3B,SRC, TLR4 | 6 |
| **hsa05131** | PD-L1 expression and PD-1 checkpoint pathway in cancer | MAPK1,NFKB1,EGFR,TLR4,JAK2 | 5 |
| **hsa05215** | Prostate cancer | MAPK1,NFKB1,EGFR,GSK3B,HSP90AA1 | 5 |
| **hsa05200** | Pathways in cancer | MAPK1,NFKB1,EGFR,GSK3B,HSP90AA1,HDAC1,JAK2 | 7 |
| **hsa05135** | Yersinia infection | MAPK1,NFKB1,GSK3B,SRC,TLR4 | 5 |
| **hsa05161** | Hepatitis B | MAPK1,NFKB1,SRC,TLR4,JAK2 | 5 |
| **hsa05152** | Tuberculosis | MAPK1,NFKB1,SRC,TLR4,JAK2 | 5 |
| **hsa04062** | Chemokine signalling pathway | MAPK1,NFKB1,GSK3B,SRC,JAK2 | 5 |
| **hsa05167** | Kaposi sarcoma associated herpes virus infection | MAPK1,NFKB1,GSK3B,SRC,JAK2 | 5 |
| **hsa05140** | Leishmaniasis | MAPK1,NFKB1,TLR4,JAK2 | 4 |

| | | | |
|---|---|---|---|
| **hsa05163** | Human cytomegalovirus infection | MAPK1,NFKB1,EGFR,GSK3B,SRC | 5 |
| **hsa04012** | ErbB signalling pathway | MAPK1,EFGR,GSK3B,SRC | 4 |
| **hsa04657** | IL-17 signalling pathway | MAPK1,NFKB1,GSK3B,HSP90AA1 | 4 |
| **hsa04659** | Th17 cell differentiation | MAPK1,NFKB1,HSP90AA1,JAK2 | 4 |
| **hsa04066** | HIF-1 signalling pathway | MAPK1,NFKB1,EFGR,TLR4 | 4 |
| **hsa05145** | Toxoplasmosis | MAPK1,NFKB1,TLR4,JAK2 | 4 |
| **hsa04919** | Thyroid hormone signalling pathway | MAPK1,GSK3B,HDAC1,SRC | 4 |

### 4.7 Results of molecular docking

The SFN molecule and key target were molecularly docked in order to further indentify the relationship between ligand and receptor. It is well known that binding energy predict how to entities will bind to one other. String affinity for the target protein is exhibited by the compound with reduced binding energy, resulting in a more stable conformation. Using PyRx software the four target protein with low energy value in molecular docking was connected with the SFN compound, Fig13**.**shows the docking combination. Furthermore the results showed that the ligands had lower binding affinities than expected. The analysis of poses suggests that there is deficiency in important hydrophobic interaction and hydrogen bonding. Moreover we can improve the binding affinities by modifying the functional group, stereochemistry of compound and balancing the hydrophobicity and hydrophilicity.

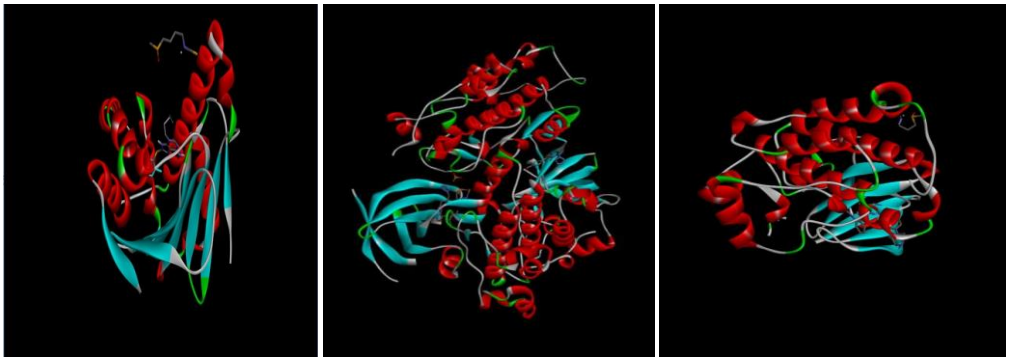

**Fig13- Molecular docking**

## DISCUSSION

Investigating the basic molecular and cellular etiology of ASD has gained more attention. When the etiology remains ambiguous, the approach of network pharmacology enables to examine the efficacious constituents, pathways and targets at molecular level. There is evidence indicating the genetic, environmental, immunological factors in development of ASD related phenotype. Genetic variability include disturbance in mRNA transcription, abnormal gene methylation, copy number variation and single nucleotide polymorphism poses a substantial risk for autism. Moreover other risk factor is drug infection, maternal obesity, preterm birth, and altered gut microbiota.

SFN, 4- methyl sulfinylbutyl isothiocyanate is a phytochemical occurs in broccoli plant and other cruciferous vegetables such as Brussels sprout. SFN is biologically inactive precursor glucoraphanin having pharmacological properties, such as anti-apoptotic, anti-inflammatory, neuroprotective properties[52].

The combination of network pharmacology and molecular docking results offers a thorough comprehension of SFN's impact on treating ASD. It has been discovered through network pharmacology that SFN exhibits interactions with various proteins that play a role in pathways associated with ASD, including inflammation, neurotransmission and oxidative stress. The finding of molecular docking studies was supported by the demonstration of good binding affinities exhibited by SFN, confirming its capacity to regulate the activities of ASD targeted proteins. These multi target approaches indicate the potential of SFN may influence multiple biological processes, and suggest that these compounds may be effective for the therapeutic effect of autism. According to reports, the primary etiology of autism in

children is attributed to the inflammation of the nervous system. The absence of distinct biomarkers and pathogenesis poses a significant challenge to the advancement of efficacious therapeutic interventions[53]. Furthermore the psychopharmacological medications prescribed to patients with autism are unable to effectively treat primary symptoms. Research shows that the utilization of beneficial constituents derived from natural pharmaceuticals has the potential to reduce the phenotype symptoms in ASD patients. Some studies implies that SFN could potentially enhance gene transcription in various dysregulated cell signalling pathways and further cellular level studies could provide valuable insights for discovery of new drugs[5]. Some research revealed that pre-treatment with SFN effectively prevented the rise of tumour necrosis factor-α level and the activation of microglia in various brain areas after a single shot of lipopolysaccharide (LPS). In addition SFN can also enhanced the elevated level of IL-10 in blood and can effectively recovered the protein level of brain derived neurotrophic factor, post synaptic density (PSD), AMPA receptor and dendritic spine density[54]. Thus it is possible that broccoli sprouts rich in SFN might be used s preventive approach to reduce onset of depression in neuroinflammation.

In this study, a total 106 common targets of SFN and ASD were obtained through different database such as OMIM and GeneCard. After screening via cytoscape we find 10 key targets. In PPI network according to the node degree, the main targets of ASD are SRC, EFGR, HSP90AA1 and GSK3B. The SRC,Proto-oncogene tyrosine-protein kinase Src plays a crucial role in the growth and maturation of brain and some studies demonstrated that the regular release of SRC can improve the irritability of patients with ASD[55]. Epidermal growth factor receptors (EFGR) regulate cell differentiation and proliferation and situated on the cell membrane. Scientist observed the significant role of EFGR in neurogenesis, and synaptic plasticity, which contribute to ASD and patients have genetic variants and altered EFGR expression affect brain development and connection[56]. HSP90AA1, Heat shock protein HSP 90- alpha help to maintain the protein homeostasis and is gaining attention in research on ASD because of its role its cellular stress response and protein homeostasis[57]. Disturbance in HSP90AA1 may lead to imbalance in protein regulation which could impact the development of neurons and functions of synapse. GSK3B, Glycogen synthase kinase-3 beta is a serine/threonine kinase play a crucial role in regulating signalling pathways such as Wnt/β catenin, which is vital for the differentiation, development and plasticity of neurons. Dysregulation and changes in GSK3B activity may have effect on brain functions and growth, which leads to the phenotypic symptoms of autism[58].

Biological information is used to explain the pathogenesis and bioinformatics approach reveals that cellular response to oxidative stress and chemical stress, regulation of cell death, focal adhesion, plasma membrane raft, protein kinase activity, kinase binding, transcription factor binding all have an impact on the onset

and progression of ASD. KEGG enrichment analysis showed the various pathways such as prolactin signalling pathway, PI3K-Akt signalling pathway, EGFR tyrosine kinase inhibitor resistance may regulate the neural growth in the brain to different extents.

Although the binding affinities observed in molecular docking study were lower but the whole analysis provides valuable insights into the mechanism of binding and structural properties that affect the interactions. By identifying crucial interaction sites, this study provides possibility for specific alterations to improve the strength of binding. This study provides some findings and limitations and focused on the significance of SFN in autism from a network pharmacology perspective. Hence, it is important to validate the results of this study through pharmacodynamics and further experiments is necessary for better understanding the multi target interactions which is associated in the treatment of ASD.

## CONCLUSION

This work used the network pharmacology approach to examine the mechanism by which SFN treats autism spectrum disorder. The result of this study shows that SFN, a natural phytochemical has pharmacological effects through multiple components, targets and channels. These effects include neuronal control, inflammatory response and immunological regulation. By using bioinformatics method we found the core target such as SRC, EFGR, HSP90AA1 and GSK3B. Furthermore performing GO and KEGG enrichment analysis enabled the exploration functions of gene and signalling pathways, which reveals the possible mechanism of action[8].Although the docking studies emphasize the necessity of making structural alterations to the ligands. The study serves as a point of reference for future research on the treatment mechanism of ASD and offers a specific concept for the conservative treatment of autism with natural medications.

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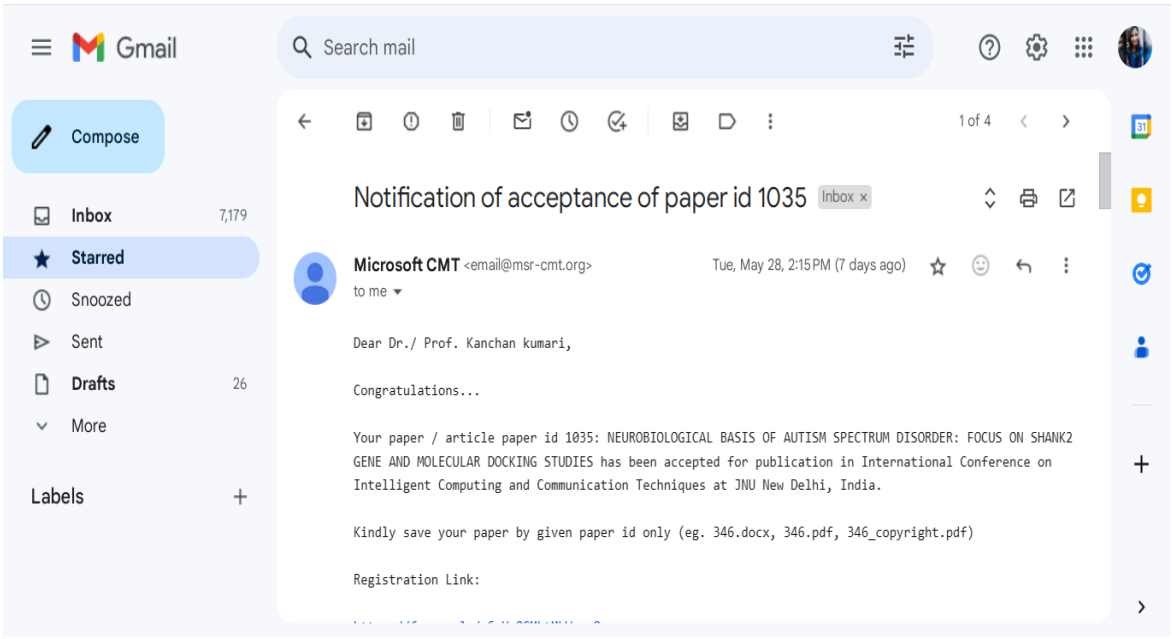

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# CV

**KANCHAN KUMARI**
kanchanchaudhary1110@gmail.com | 9717224308

## OBJECTIVE

To secure a position that offers opportunities for professional growth and advancement, allowing me to continuously develop my skills.

## INTERNSHIP

- **ESIC Hospital, New Delhi** **Dec 2022 - Jan 2023**
  Winter Internship
  Performed training task and gained practical experience with relevant tools and technology in Microbiology and Pathology laboratory.
- **Marpu Foundation**
  Writing Intern
  Work as a content writer in the social awareness wing and contribute to the sustainable development and CSR goals.

## EDUCATION

- **DELHI TECHNOLOGICAL UNIVERSITY** **2022- Present**
  M.sc (Biotechnology)
- **LN MITHILA UNIVERSITY** **2021**
  B.sc (Zoology)
  **65%**
- **VIKASH VIDYALAYA BEGUSARAI** **2017**
  CBSE (XII)
  **74%**
- **SDSVM SAMASTIPUR** **2015**
  CBSE (X)
  **9.4 CGPA**

## SKILLS

- Microbial Technique
  Streaking, Spreding, Gram staining, Biochemical Reaction, Sensitivity Test, Media culture, ELISA, HIV Test
- Pathology
  Worked in Histology and Hematology lab.
- Microsoft office
  Word, PowerPoint, Excel
- Bioinformatics
  Python( Beginner Level)
- Basic Biostatistics tools
  Descriptive and Inferential Statistics

## LANGUAGE

- Hindi
- English

## ACTIVITIES

- Organized events and Campaign in school and college.
- Volunteer work
  Participated in Community Clean up event, Fundraising event.
- Collaborate with school to educate students about human welfare.

## CERTIFICATES

- Microbiology and Pathology laboratory Certificate
- Certification on drug discovery course.
  Online Course - University of California San Diego
- Writing Intern Certificate at Marpu Foundation