Phenyl Caffeate: A Novel Anti-Aging Drug

Major Project - II

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ABSTRACT

The scientific fraternity all over the world has been engaged in solving the internal riddle of senescence and contemplating to discover ways and means which is largely related with the process of aging. Aging is a multilevel and multifaceted process of getting older characterized by physical, physiological, psychological and social changes. It is an inevitable process and is usually marked by the negative deviation of the bodily structures and functions from the optimum. There are currently very limited FDA approved anti-aging drugs in the market. Despite remarkable technological advances in genetics and drug screening, the discovery of new pharmacotherapies has slowed and new approaches to drug development are needed. With this premise, in the present study we explore the chemical-chemical and chemical-protein interactions for finding new and effective treatment strategies for ageing. We have implemented interaction study on three drugs, namely, Resveratrol, Aspirin and Metformin, followed by clustering analysis of interacting partners of these drugs to identify novel candidate drugs for aging. We screened the novel anti-aging drug by ADMET analysis, nondruglikeliness and toxicity prediction. These screening tests indicated 5 drugs having compound ID(CID005910817),(CID4634), (CID5508),(CID26986) and (CID3158) followed all the rules of druglikeliness and Out of five, only one compound i.e Phenyl caffeate or CAP(CID005910817) passed non-drug likeliness filters, and was predicted to be non-toxic also. Drug validation was done using DFT, PPI network analysis and microarray data analysis of wrinkles in young and old aged humans. Docking study proved that CAP has better potential to bind anti aging target SIRT1 than other known anti-aging drugs.Thus we propose that Phenyl cafeate(CID5910817) could be a potential anti aging drug if clinical tested. DFT study also proves that the proposed drug is stable, bioactive and could be used for further clinical analysis.

To the best of our knowledge this is the first time when Docking,Microarray data analysis,PPI network analysis , ADMEt analysis and DFT analysis are done for Phenyl caffeate to validate its anti aging property. The present study would certainly provide new solutions to the pharmaceuticals against aging and age related disorders.

Keywords: Longevity; Clustering; Expectation maximization; ADMET; Candidate drugs; DFT ;

Workflow

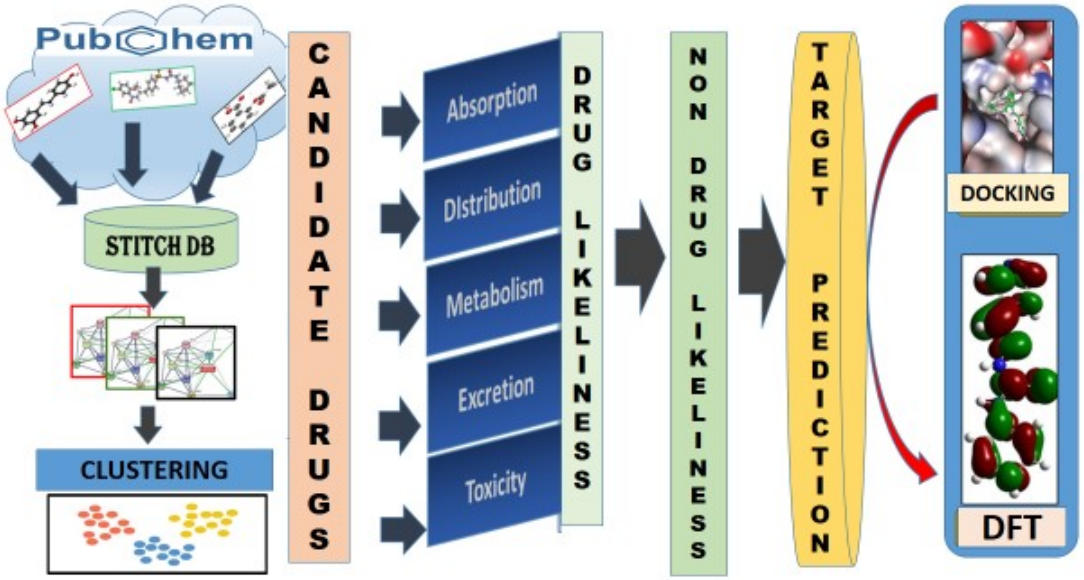

Figure 1: Workflow

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Chapter 1

INTRODUCTION

INTRODUCTION

Aging or senescence commonly known as the process of getting old is a combination of processes of deterioration that follow the period of development of an organism[1]. Aging and longevity are the consequences of the complex interplay of environmental and genetic factors. Old age people struggle with many chronic conditions many at once: heart disease,neurodegenerative diseases, cancer, respiratory disease,arthritis, osteoporosis,diabetes. The biological processes governing these pathologies are myriad. Yet some researchers took another view of this aspect: the of disease in old age is old age itself. So why not discover treatments for ageing. People may never halt the process entirely: ageing is an opaque and complex mixture of molecular pathways. But we should learn how to stop changes that cause the worst chronic diseases. Our main purpose behind this study so far is to stop ageing problem. Now research has taken advancement in this direction by focusing on reversal aging also. Reversal of ageing process in no longer a myth, it is possible and we have available evidences for this statement. In a published paper, research group has showed how transfer of young blood into older people can cause reverse ageing. To check this phenomena scientists transferred the young blood in old mice and observed the result-Mouse heterochronic Parabiosis Model - An experiment was carried out in the laboratory to check how young blood had altered the way genes expressed in old mice[2]. Differential gene activity has been observed for genes governing neural connections which are responsible for cognitive activity[3]. Several approaches have been implemented to identify anti-aging drugs in humans. From previously available studies there are few drugs which are supposed to cause anti-ageing for example Metformin, Adenavir, Aspirin, Idebenone, Nimodipin, Doxycycline, Rapamycin, Monteleukas and Resveratrol[1]. Some are natural products and rest are chemically designed drugs. Drug development is very costly and time consuming process because experimental testing are required for research and development of a drug that can cost millions and even trillions if currency and may take many years to complete and involves several thousand animals[4]. It is very necessary to develop reliable and effecient computational methods, e.g., using structure-activity relationships to predict the anti-ageing activities of chemicals. SAR models of anti-aging compounds could be developed based on molecular descriptors and machine learning methods. In order to do that we need to have ample no. of benchmark SAR data which is unfortunately not available. There are databases available for chemical-chemical and protein-chemical information for various compounds, which may be used to solve this purpose[5]. In this study we have implemented a new approach to discover novel drugs using the chemical-chemical and chemical-protein interactions informationn of three drugs i.e. Metformin, Aspirin and Resveratrol. Metformin is an anti-diabetic drug that helps in the reduction of incidence of type-2 diabetes[6],[?] . Previous research has found its anit-aging role by affecting in various biological processes like genomic instability, cellular senescence etc.[7].Metformin was also found to be involved in slowing down aging and extending life span[8],[9]. Aspirin is an analgesic, anti-inflammatory and anti-pyretic drug and according to scientific report it can inhibit carcinogenesis also i.e. it can inhibit lung, colon and breast cancer[10] and could reduce endothelial cell senescence also[11]. Aspirin can increase lifespan and delays the onset of age related diseases i.e. it can act as an anti-aging agent[12]. Resveratrol is a phytoalexin that could be extracted from number of plants like- grapes, berries and peanuts etc. It is present in red wine and can help in preventing age related muscular degeneration to occur[1]. It can also

act as an anti-inflammatory and anti-aging agent[13],[14],[?] ,[15]. Basic idea behind chemical-chemical and chemical protein interaction study is to discover novel drugs considering fact that interactive compounds share comparatively highly similar functions than non-interactive compounds[16]. In the present study we included not only direct interactions but also indirect interactions in the form of protein-chemical function relationships to discover candidate drugs for anti-ageing.The potential compounds found after clustering were further evaluated and validated with different studies like- ADMET profiling helped to narrow down the candidate drugs based on drug likeliness. Rules for Lipinski filter are: MW $\leq$500, MLOGP $\leq$ 4.15, HBA $\leq$ 10, HBD $\leq$ 5. For Ghose Filter: 160 $\leq$ MW $\leq$ 480, -0.4 $\leq$ WLOGP $\leq$ 130,40 $\leq$ MR $\leq$ 130 ,20 $\leq$ atoms $\leq$ 70. For Veber Filter: Rotatable bonds $\leq$ 10,TPSA $\leq$ 140. For Egan filter- WLOGP $\leq$ 5.88, TPSA $\leq$ 131.6. For Muegg Filter: 200 $\leq$ MW $\leq$ 600 , -2 $\leq$ XLOGP $\leq$ 5, TPSA $\leq$ 150, Number of rings $\leq$ 7, Number of carbon $>$ 4, Number of heteroatom ¿1, Num. of rotatable bonds $\leq$ 15, HBA $\leq$10, HBD $\leq$ 5 Bioavailability score (Abott): Probability of F $>$ 10 in rat[17].
Toxicity is one of the major causes of clinical trial failure or a reason for drugs being removed from the market[18],[19].We have used Chemical Toxicity Predictor-GUSAR which is an online tool for toxicity prediction. This tool provides results which can be interpreted very easily by readers. Based on calculated toxicity, values the tool gives application domain which could be used to predict the use of a particular drug. Geometrical and electronic study of compound are very important to check the chemical stability and bioactivity[20]. Density Functional Theory(DFT) study helps in optimizing the molecular structures . It calculates all the electronic parametrs like- HOMO(Highest occupied molecular orbital), LUMO(Lowest unoccupied molecular orbital) energy and geometric parameters like-Bond length, Bond angle and dihedral angles. Based on the detailed DFT analysis bioactivity of drug compounds were predicted. Wrinkles are considered to be the signs of aging[21]. With age, the skin, underlying connective tissue, and muscle of a person undergo changes that result in wrinkles and sagging skin[22]. In the present study we have chosen Wrinkles(Skin aging) as a case study to show the targeted effect of our newly discovered drug because Skin aging provides an ideal model organ to investigate the aging process.There are two main processes that induce skin aging: intrinsic and extrinsic. Extrinsic aging is caused by environmental factors such as sun exposure, air pollution, smoking, alcohol abuse, and poor nutrition. Intrinsic aging reflects the genetic background and depends on time. Microarray data analysis of wrinkles between old and young age people, has been used as a tool to preidct the main causing genetic factor for aging.[23]. This method clearly depicts the main targets for aging. Thus in this study we investigated the new anti-aging drugs which are interacting with the known anti-aging drugs and could be further explored for their other functions in aging research.

Chapter 2

REVIEW OF LITERATURE

2.1 BACKGROUND

2.1.1 Aging

Ageing is thought to be progressive growth, decline and death. There are many theories regarding ageing[24] :-

1] Wear and tear theory
2] Free radical theory
3] Genetic theory/Telomere theory
4] Cross-linking theory
5] Mitochondrial theory

Wear and theory: suggest that tissues and organs eventually die after years of damage. An individual's genetic makeup (DNA) sustains repeated damage from toxins, radiation, and ultraviolet light throughout the course of a lifetime. Although the body can repair DNA damage, not all of those repairs are accurate or complete. As a result, damage slowly accumulates over time. For instance, one study evaluated the effect of a lifetime of exposure to stress hormones, such as cortisol. The body produces cortisol in response to physical and emotional stress. Researchers found that the amount of cortisol in the body rises with age. Although cortisol levels decline at night in younger adults, the levels do not fall as far in older adults. The researchers concluded that the increased levels of cortisol might be the result of wear and tear of lifelong exposures to stress.

Free radical theory: The most common theory of aging is called the free radical theory. Many researchers believe that molecules, called free radicals, damage the body's tissues. Free radicals are produced when the body fights against infections. Although free radicals are needed for the body to produce energy, maintain immunity, transmit nerve signals, produce hormones, and contract muscles, they may also contribute to the process of aging. Free radical damage begins at birth and continues through adulthood. However, when individuals are young, the effects are minimal because the body has many different ways to repair and replace cells to maintain proper functioning. As individuals age, the damage caused by free radicals increases. Studies have shown that free radicals attack the structures of the body cells and create substances called lipofuscins. When lipofuscins build up in the body, they show up as darks spots on the skin, which are commonly called age spots. Lipofuscins also interfere with the body's ability to repair damage cells and reproduce new ones. As a result, lipofuscins lead to decreased energy levels and they prevent the body from building muscle mass. They also destroy enzymes that are needed for daily functioning. Studies have also shown that free radicals attack substances in the body called elastin and collagen. These substances help keep the skin smooth, moist, and flexible. As a result, free radicals may cause changes in the appearance of the skin, such as folds or wrinkles.

Genetic theory: It has been suggested that an individual's genetic makeup regulates the rate at which he/she ages. This is called the telomerase theory of aging. However, it does not necessarily mean that patients will live to be the same age as their parents. This is because many other factors, including diet and lifestyle, may influence a person's internal biological clock. Telomeres are proteins on the ends chromosomes that carry genetic information. Every time a cell divides in the body the telomeres shorten. The shortening of telomeres is believed to cause cellular damage because the cell is unable to make a correct copy of itself. Over time, this process leads to cellular dysfunction, aging, and death.

Cross-linking theory: The cross-linking theory of aging is based on the observation that as individuals age, the proteins, DNA, and other molecules in the body develop inappropriate attachments, or cross-links, to one another. These unnecessary links decrease the mobility of protein and other important molecules in the body. When proteins become damaged or are no longer needed, an enzyme called protease destroys them. However, cross-linkages prevent protease from doing its job. As a result, dysfunctional and unneeded proteins remain in the body and can cause damage. For instance, cross-linking of the skin protein called collagen has been show to be partly responsible for wrinkled skin. Cross-linking proteins in the lens of the eye has also been shown to cause age-related cataracts. In addition, it has been suggested, but not proven, that cross-linking of proteins in the walls of arteries or the kidneys may be partly responsible for hardening of the arteries (atherosclerosis) and age-related kidney dysfunction.

Mitochondrial theory of agin, a variant of free radical theory of aging, proposes that accumulation of damage to mitochondria and mitochondrial DNA (mtDNA) leads to aging of humans and animals. It has been supported by the observation that mitochondrial function declines and mtDNA mutation increases in tissue cells in an age-dependent manner. Age-related impairment in the respiratory enzymes not only decreases ATP synthesis but also enhances production of reactive oxygen species (ROS) through increased electron leakage in the respiratory chain. Human mtDNA, which is not protected by histones and yet is exposed to high levels of ROS and free radicals in the matrix of mitochondria, is susceptible to oxidative damage and mutation in tissue cells.

There are many factors responsible for ageing which are shown in fig. 2.1.

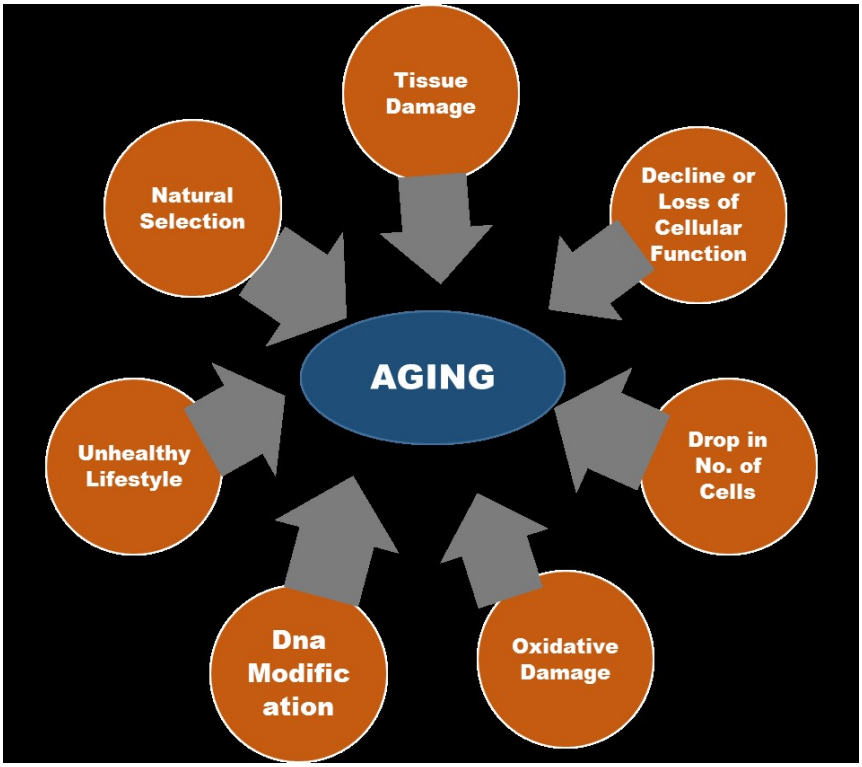

Figure 2.1: Factors causing aging

2.1.2 Death rate with respect to Age and Demography:

Age of dying people varies among the different regions of the world.While in the most developed countries, except eastern Europe, almost 85 percent of its population dies at age of 60 or older as shown in fig. 2.2.. In Africa almost 40 percent of its population unfortunately die before 4 years old and only 22 percent reaches the age of 60Causes od death depend on the age of deceased. Young children die because of infection of genetic disorders but people who die older than 60 years die because of age related disorders or natural death.

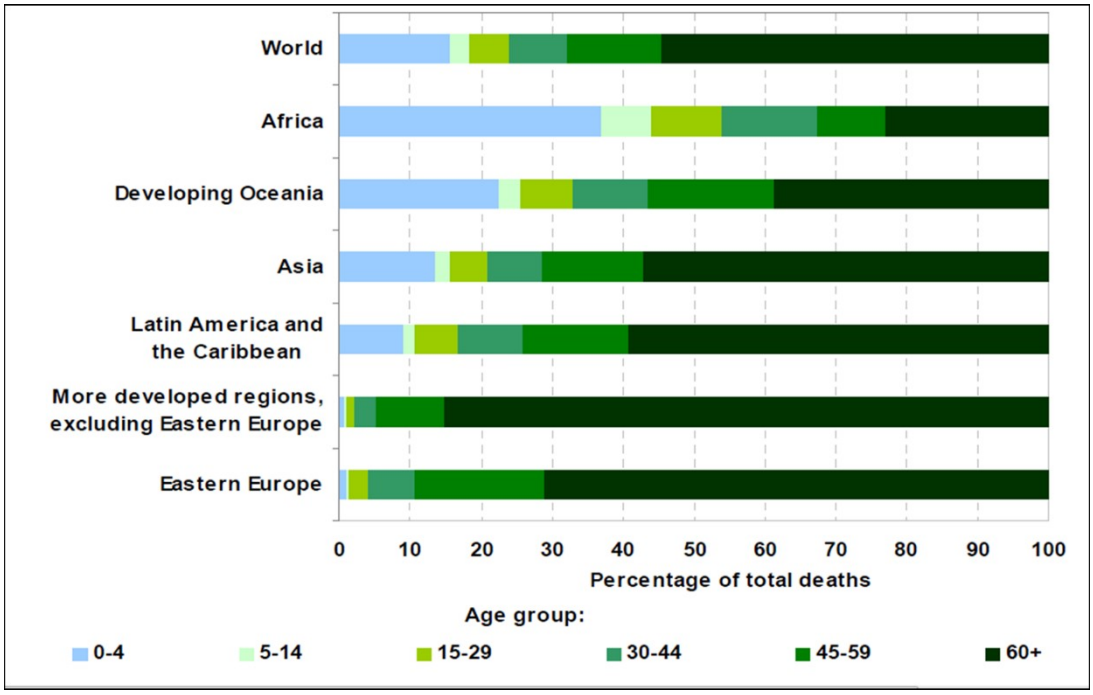

Figure 2.2: Percentage of death in aging population

2.1.3 Terminologies related to drug discovery

This section introduces the commonly used terminology and definitions in this work as described in table 2.1. The chapter ends with a review of recent in-Sillico work on novel drug discovery for age-related diseases.

2.1.4 DOCKING

Molecular docking is an important tool in drug discovery[?] . The molecular docking method could be used to model interaction between a target protein and a small molecule i.e ligand molecules in the binding sites of targets to understand the fundamental biochemical process. This includes two basic steps:- 1] Pose detection in binding site 2] binding affinity calculation These two steps are related to sampling methods and scoring schemes, respectively.

Table 2.1: Terminologies related to drug discovery

Drug discovery:	Processes for the the identification and development of drugs. High-throughput methods that utilize combinatorial chemistry, genomics, and proteomics information are the starting point. Additional research to characterize lead compounds is followed by clinical trials.
Drug:	A molecule used to diagnose, treat, mitigate, or prevent disease.
Ligand	A small molecule that binds to a receptor/protein.
Docking	Computational exploration of the possible binding modes of a ligand to an enzyme, receptor, or DNA.
Pharmacokinetics:	Pharmacokinetics is described as what the body does to adrug, refers to the movement of drug into,through, and out of the body the time course of its absorption, bioavailability, distribution, metabolism, and excretion. Drug pharmacokinetics determines the onset, duration, and intensity of a drugs effect. Pharmacokinetics of a drug depends on patient-related factors as well as on the drugs chemical properties.
Pharmacodynamics:	Pharmacodynamics is described as what a drug does to the body,involves receptor binding, postreceptor effects, and chemical interactions.
ADME	Procedures for evaluating the absorption, distribution, metabolism, and elimination of pharmaceuticals.

2.1.5 Properties of Cinnamic acid and its comparison to CAP

Caffeic acid is one among the hydroycinnamate and phenylpropanoids that are majorly present in plant tissues[?] . These polyphenols are majorly present in food sources like- Apples, Blueberries, Cider, Coffee drinks etc. These can act as carcinogenic inhibitors and has antibacterial properties. These can cure atherosclerosis, cardiovascular diseases, arthritis and other diseases. These compounds can reduce the activity of free radical and oxidizing agents. These structures are resonance stabilized .

The phenolic acids have :- a carboxylic acid group, a benzene ring and one or many hydroxyl groups that provide them the antioxidant property.

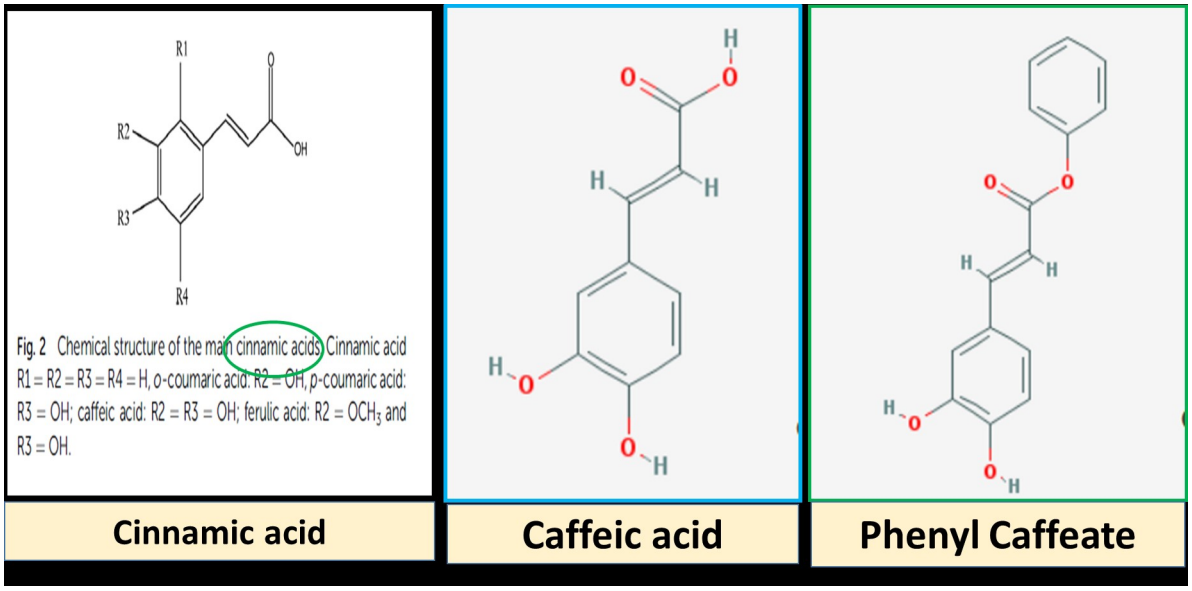

Figure 2.3: Structural details of Cinnamic acid and its derivatives

2.1.6 Activation of SIRT1 activity by resveratrol

SIRT1 removes acetyl group from substrates including H4K16 and H1K26, a no. of transcription factors and cytosoli acetyl co A-synthatase(AceCS1). The reaction requires NAD+ and yields deacetylated substrate, 2-acetyl-ADP ribose(ADPR) and nicotenamide[?] . Deacetylase activity of SIRT1 can be activated by Resveratrol fig. 2.4.. Nicotenamide is an end product inhibitor of SIRT1.

2.1.7 Structural details of SIRT1

SIRT1 has 747 amino acids and five structural domains known so far fig. **??**. Helical domain, NAD and CTR domains are the main binding regions by STACs. These inhibitors bind in these regions to activate SIRT1 and hence helps in increase of longevity.

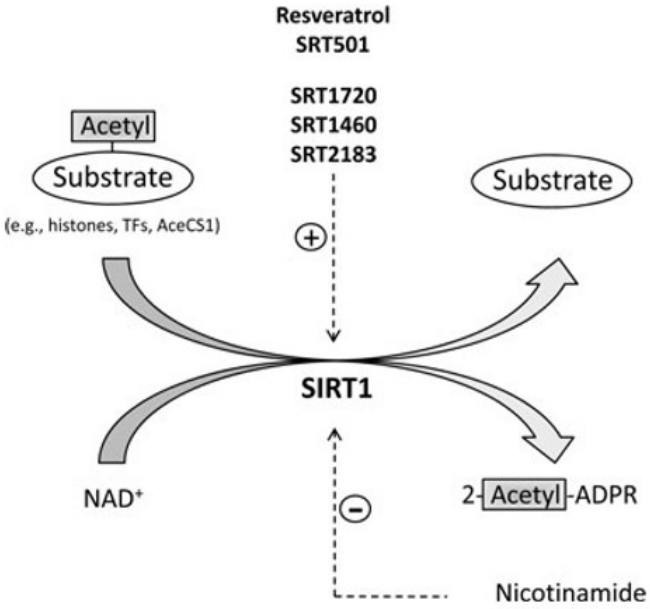

Figure 2.4: SIRT1 activation by Resveratrol

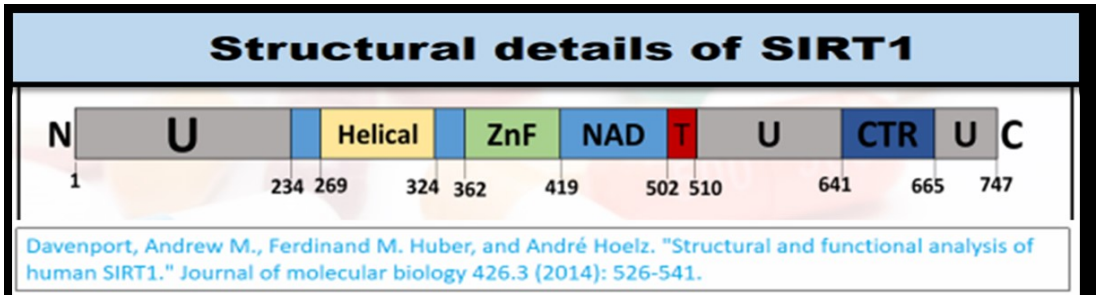

Figure 2.5: Domain structure:- Blue, NAD+-binding domain; yellow, helical module; green, Zn2+-binding module; red, pseudo-substrate peptide (T, tail); purple, C-terminal regulatory segment (CTR);gray, predicted unstructured regions(U).

2.2 RELATED PREVIOUS WORK

Aging is progressive growth decline and death for any individual. It is a cumulative effect of free radical damage to cells and tissues that ultimately leads to death. Any drug that could cure more than 3 or 4 age-related disorder is considered to be an anti-aging drug. Resveratrol and Aspirin are anti-inflammatory and antioxidizing agent. But these are reported to have anti-aging properties. Similarly, Metformin is being used as an antidiabetic drug but it is also reported to increase longevity in humans[1]. Using Chemical-chemical and chemical-protein interaction with clustering study is a new approach to discovering novel anti-HCV(Hepatitis C Virus Drug[?] . In this study we have used three drugs-Resveratrol, Aspirin and Metformin as input to discover novel anti-aging drug.The discovered drug Phenyl caffeate is a polyphenol derivative of cinnamic acids. The phenolic acids have a carboxylic acid group, a benzene ring and one or many hydroxyl groups that provide them the antioxidant property because of resonance stabilization. In our drug CAP(Caffeic acid phenyl) other than methoxy group there is an extra benzophenone group that provides the extra stability to the structure and hence can show more antioxidizing property. DFT and Docking studies have been used as an important tool in drug discovery. In paper[20] DFT and frontier orbital calculation have been used to show the chemical kinetics and energy gap between orbitals for predicting the electronic and geometrical stability of drug molecule. Docking of proposed drug with target molecule at predicted target site and the binding energy calculation provides the insight of interaction strength and its activity against aging and age-related disorders. In[25] a new anti-aging drug DAA has been discovered utilizing Docking study and results were reported in terms of binding energy and No. of HBONDS. The computational approach is one of the newest and fastest developing techniques in pharmacokinetics, ADME (absorption, distribution, metabolism, excretion) evaluation, drug discovery and toxicity. The application of informatics to ADME/PK can provide general guidance on properties such as absorption and BBB penetration and such rules of thumb have been the mainstay of ADME/PK scientists for some time. However, the combination of a wide ADME/PK database and modern computational chemistry techniques enables a more rigorous appraisal of the properties of a molecule as a whole, rather than just relying on knowledge of isolated fragments and functional groups in toxicity prediction[26].

Chapter 3

Materials and Mthodology

3.1 Screening of anti-aging drugs

Drug was screened based on chemical-chemical and chemical-protein interaction data and defining the target antiaging genes table 3.1 which were downloaded from STITCH database and GeneAGE database then they were clustered using EM algorithm[27] using WEKA sofyware[28]. Total 13 drug compounds were proposed to be candidate drugs. Structures of these drug compounds were downloaded from Pubchem database[29]. Thus in this study we are screening the best possible drug for anti-aging and then we have validated the best probable anti-aging drug using Gene expression data analysis, pharmacokinetic and molecular modelling study.

[1] STITCH Database: Stitch database fig 3.1 was used for chemical chemical and chemical protein interaction. Interactions were given with four types of score. Finaly Combined score was used for clustering the drugs for anti-aging.

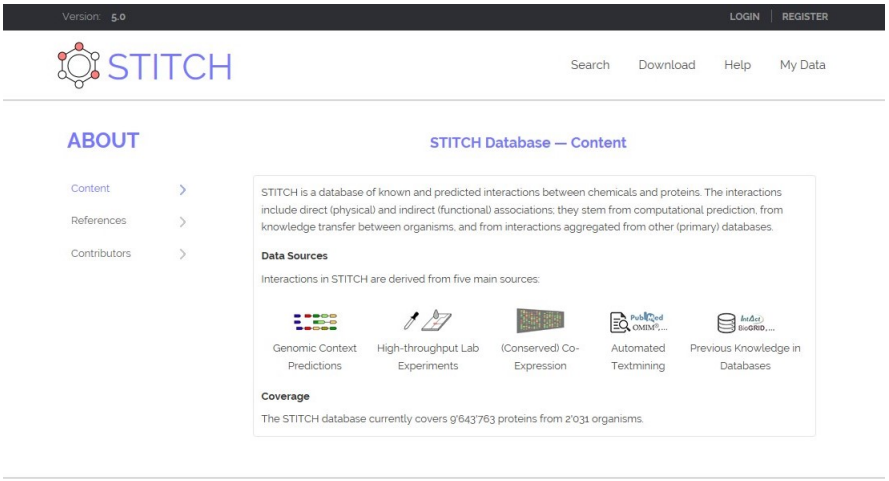

Figure 3.1: Homepage of STITCH database

STITCH is a database where all types of interactions (known or predicted) between chemicals and proteins are stored[30]. The interactions could be direct which is also reffered as physical and indirect which denotes functional associations; they are curated computationally. The five main sources for STITCH interactions are:- 1]Genome Context Predictions 2] High-throughput Experiments 3] (Conserved) Co-Expression 4] Automated Textmining 5] Prvious Knowledge in databases. In search box we can put our chemical or protein interest and we can choose specific organism of interest. Based on the input this database provides all the interaction informations.

[2] WEKA: Weka is a datamining software which is written in JAVA fig 3.1. It consists of all type of datamining algorithms[31]. We have used EM algorithm which is a tool for unsupervised learning. It has the option to provide separate test and training set data or we can split the data as training or testing as per our choice.

[3] Control drug prediction: Random prediction and comparison of result might lead to a biased and wrong conclusion. So to get accurate and better comparison we selected the control drug out of three(Aspirin, Metformin and Resveratrol) based on MCS, i.e., Maximum common substructure prediction and Newick tree generation[?] .

Table 3.1: Target genes for Anti- aging

	IGF1R	IRS1	IL2	PIN1
GHRH	TXN	PTPN1	PDGFB	PTEN
SHC1	KL	IRS2	EGF	CREBBP
POU1F1	E2F1	AKT1	IL2RG	HIF1A
PROP1	PTPN11	PIK3CB	FOS	UBB
TP53	NFKB2	NGFR	PDGFRB	RPA1
TERC	STAT5B	HRAS	EPOR	BLM
TERT	STAT3	MYC	SST	BCL2
ATM	STAT5A	EGFR	PRKCD	S100B
PLAU	NRG1	ERBB2	PPARA	VCP
ERCC2	HDAC3	INSR	RET	POLG
ERCC8	GH1	NCOR1	PLCG2	IGFBP3
WRN	IL7R	NBN	PEX5	HSP90AA1
LMNA	IGF1	JUND	TCF3	NR3C1
ERCC6	IGF2	TOP1	PARP1	EGR1
STK11	INS	RAD51	BRCA1	VEGFA
EP300	NGF	UBE2I	CEBPA	ABL1
APTX	HTT	TNF	CEBPB	BRCA2
PML	PRKCA	PDPK1	MXI1	TOP2A
GSK3B	SSTR3	NFKB1	TGFB1	TOP2B

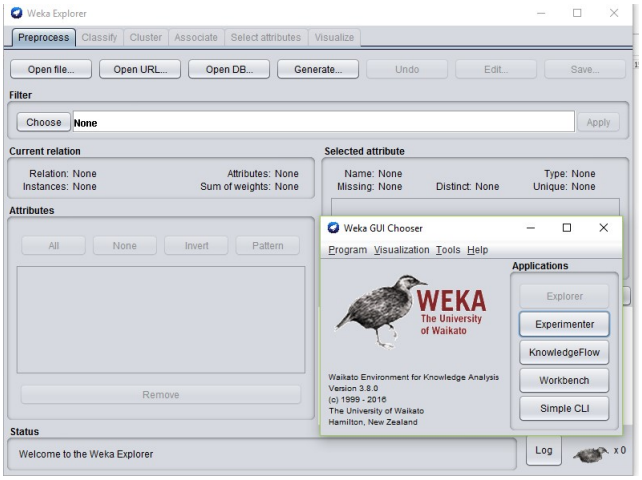

Figure 3.2: Homepage of Weka interface

MCS Prediction: MCS is the similarity measure between two chemical compounds. MCS is calculated using online tool CHEMMINE that takes smile string of two componds as input and calculate the atom pair and MCS similarities in terms of Tonimoto coefficient. The analysis is graph based[32] The compound having maximum of the similarity coefficient value was selected as control for further analysis and comparison of results.
Hierarchical Clustering for structural comparison: Chemmine tool was used to calculate the JoeLib physicochemical descriptors of three input drugs and one experimental drug and these calculated parameters were used for hierarchical clustering. Heatmap was generated and Newick tree was plotted using Treeviewer.

NEWICK TREE: Its a tree generated by Treeviewer to show the similarty measure between three or more than three chemical compounds.

3.2 ADMET Prediction

Most of the candidate drugs fail during drug discovery because of ADME deficiencies during drug development and often fail to reach the market as a result of poor pharmacokinetic profiles[33?] . To avoid this failure from the very beginning, In vitro ADME screening is done to discard compounds in the discovery phase that are likely to fail in later stages. The in-silico approaches of ADMET prediction are relatively cost effective and less time consuming when compared to standard experimental methods[4;34] . SwissADMET tool has been used for ADMET prediction[35]. Lipinskis Rule of Five ,GHOSE, Veber, Egan, Muegg were used to assess drug likeliness of these compounds. Webpae for ADMET prediction tool i.e SWISSADME is shown in fig 3.3

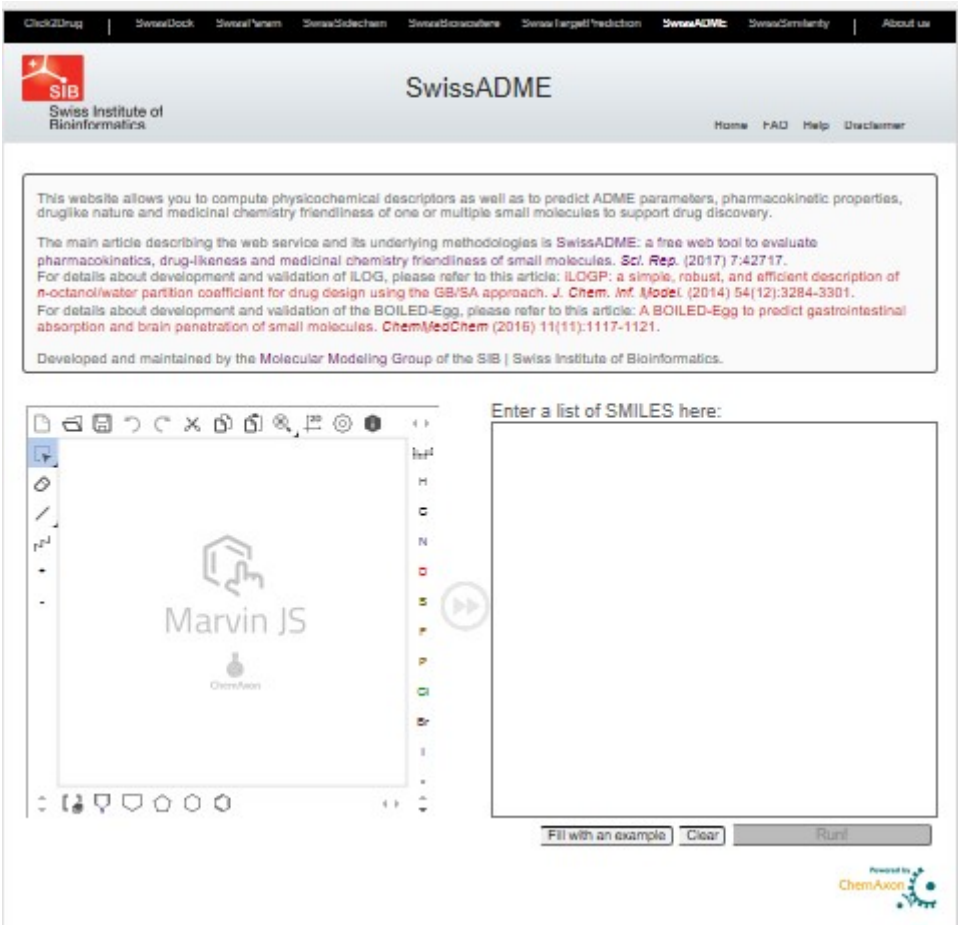

Figure 3.3: Webpage of SWISSADME

3.3 Non drug likeliness prediction

SmartsFilter (Smiles Arbitrary Target Specification) was applied to screen compounds with non-drug like features, so as to avoid potentially undesirable and toxic molecules

(http://pasilla.health.unm.edu/tomcat/biocomp/smartsfilter) homepage of the SmartFilter is shown in fig 3.4. It has five screening filters: PAINS, ALARM NMR, Oprea, Blake and Glaxo. The compounds found to be in the cluster (having the drug) are passed through the filter. If a drug passes all the filters that means the drug has non-drug like properties and if it does not pass 3 filters among 5 that means it has drug like properties and hence could be further evaluated for its application.

Figure 3.4: Homepage of SmartFilter

3.4 Structure similarity bench: COntrol drug selection

Three input drugs Resveratrol, Aspirin and Metformin were used to find out the novel antiaging drugs in human so it was necessary to find out the similarity index of phenyl caffeate with these three drugs . Similarity workbench[36] was used to predict the maximum common substructure(MCS) between novel drug and known drug.With this we were able to conclude that our drug of interest i.e phenyl caffete would behave similar to that particular drug. So to check similarity index Tonimotto coefficient index was used .This was calculated using Chemmine tool.

3.5 Toxicity and application domain

2.8 Toxicity and application domain:- Toxicity is something that is the main reason for failure of a drug in early or later stages of clinical trial. So toxicity prediction is the crucial step of pharmacodynamics study.

Toxicity was predicted using two tools/software:- 1] **ChemicalActivityPredictorGUSAR**(https://ca
was used for predicting toxicity levels and the application domain of predicted drugs. It comprises of two toxicity models
1] T.E.S.T. Toxicity Models and

2] Rat Toxicity Models. T.E.S.T toxicity model predicts 40 hour Tetrahymenapyriformis toxicity, 48 hour Daphnia Magna Toxicity, 96 hour FathheadToxicity, Bioconcentration Factor, Oral Rat Toxicity. Rat Toxicity Model predicts: Acute Rat Toxicity-Intraperitoneal , Intravenous , Oral, Subcutaneous.

2]**TEST software** :- TEST a freely available software is used for toxicity prediction of Phenyl caffeate. It takes SMILE string of a drug as input and gives all types of toxicity, mutagenicity and carcinogenicity.

3.6 Physicochemical properties calculation and hierarchical clustering

ChemMine tool was used for physicochemical properties calculation.(http://chemmine.ucr.edu/)[36] of candidate drug molecule CID5910817 and 3 input drugs(Resveratrol,Metformin and Aspirin) so as to compare the molecular parameters. The input to the ChemMIne server is SDF file and based on that it calculates various physicochemical properties of compounds and hierarchical clutering was done using joeLib descriptors and heatmap was generated to compare these four molecules(Phenyl caffeate and three input drugs-Aspirin, Metformin and Resveratrol).Homepage of ChemMine tool is shown in fig 3.5

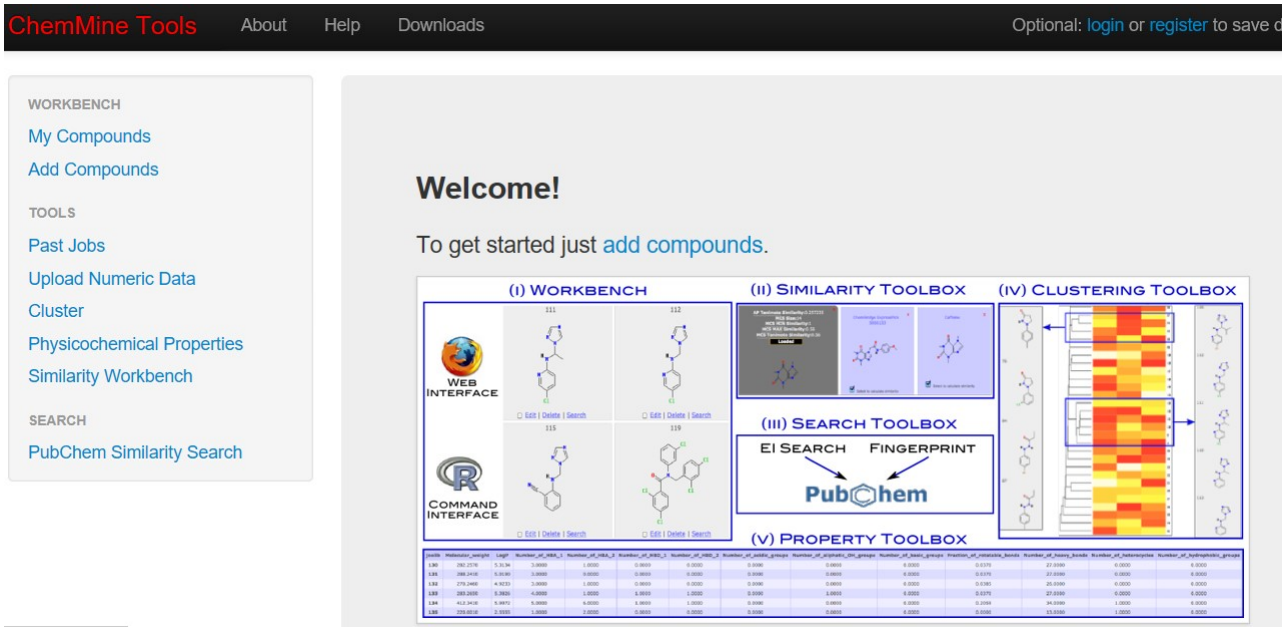

Figure 3.5: Homepage of Chemmine tool

3.7 DFT study

All the studies were carried out at DFT level with Chemcraft molecular visualization program and Gaussian 09 program package. The molecular structure of all the proposed drugs in the ground state were optimized by using Becke's three parameter exchange functional (B3) which is a linear combination of HartreeFock, local and gradient corrected

exchange terms. The B3 hybrid functional was used with the LYP correction (B3LYP) which is the correlation functional of Lee, Yang and Parr. The basis set used for calculations was the People's polarization 6-311G++(d,p) basis[33]. To determine various structural parameters such as bond lengths, bond angles and dihedral angles to verify its geometry the ground state of the compound was optimized[37]. HOMO ,LUMO their band gap and dipole moment were calculated to infer the electron density transfer within the molecule.

3.8 Target Prediction

We used SwissTarget, MIcroarray data analysis and KEGG pathway analysis for target prediction. Target predicted by Swiss target was further crosschecked with GenAge database.If they had relation with aging then they were considered to aging target.

Microarray data analysis for wrinkles data(Young age and Old aged people) was used for target prediction of drug phenyl caffeate(CAP)[38]

3.8.1 Microarray data analysis of wrinkles data

As we are proposing that Phenyl caffeate could be used against aging and age related disorders and to wrinkles which is a skin related problem. we have used Human gene expression data of these problems and tried to find out their targets and checked whether phenyl caffeate could act upon those or not and cure the problems. Methodology is drawn in fig 3.6

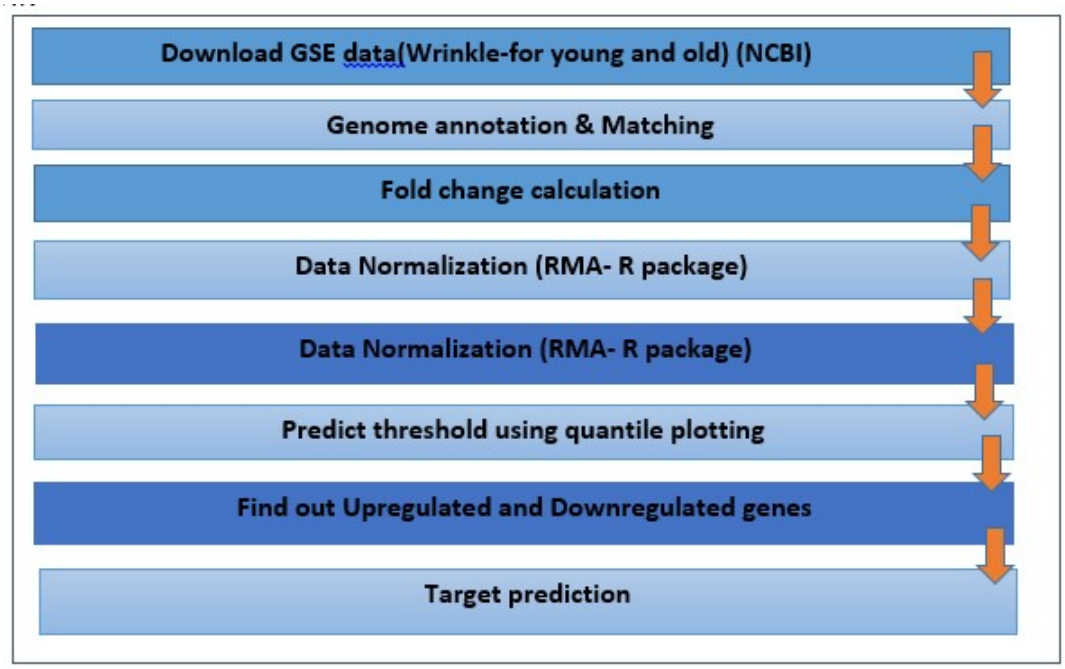

Figure 3.6: Steps followed for microarray data analysis

Microarray data extraction:
GSE85358 microarray data was downloaded from the Gene Expression Omnibus database,12 and a microarray analysis was performed to evaluate the differences in the transcriptome of human wrinkles samples in young individual in comparison with those of samples from old individual. A GeneChip for human genome U133A array (Affymetrix, Santa Clara, CA) was used to process a total of 48 arrays that were available for further analysis, including 25 arrays of young and 23 of old aged person's data. Terminologies related to microarray data is shown in fig **??**.

Normalisation:- The raw expression data was normalised per gene using RMA method in Rstudio version 3.3.1. R script used for data normalization is shown in fig 3.8. **Fold**

```
# load the affy package.
library(affy)
#Set the working directory to the directory
containing #all the .CEL files.
setwd("C:/Users/user/Downloads/Diabetes/extracted"
)
#Read the .CEL file data.
Data<-ReadAffy()
#Compute the RMA measures of
expression. expr=rma(Data)|
#Write the data to a tab-delimited text
file. write.exprs(expr,
file="micro  data.txt")
```

Figure 3.7: R Commands for Obtaining RMA Expression Measures from Affymetrix .CEL Files

Change Calculation:- The average fold change of expression was computed with respect to the median value of the corresponding healthy controls. The fold change for a gene i in condition A with respect to its expression value in condition B was computed as described in equation below:

$$FCi(A/B) = SignalIntensityi(A) \div SIgnalintensity(B) \tag{3.1}$$

Where SignalIntensityi(A) represents the normalised signal intensity for the condition A and SignalIntensityi(B) represents the normalised signal intensity for the condition B.

We fixed the cutoff using Quantile mapping and then found out the upregulated and downregulated genes and the corresponding protein list.

3.9 Protein protein Interaction Network Study

We predicted the protein protein interactions of all the proteins having expression fold change greater than 1.2 using String database and those nodes having maximum no. of

Table 1. Brief textual description of the classes and data structures

Cdf	This structure stores probe identity data read from a *Chip Definition File*. Cdf data is not chip-specific, but is common to all chips of a given type
Cdf environment	This associative data structure is used to map probe identifying information to the corresponding probe intensities
Cel	This structure stores probe intensity data from a single chip. Data is read from the *CEL* data file corresponding to that chip
AffyBatch	This structure groups `Cel` data from a set of chips with common `Cdf` into a single structure. The object also includes variables for experiment documentation
ProbeSet	This structure contains the signal intensity data for a single probe set across several chips

Figure 3.8: Brief description of the classes and data structure Microarray data analysis

hubs were considered to be the new targets for anti-aging.

3.9.1 PPI network analysis for new target prediction

The PPI information data were imported into Cytoscape (an open source software platform 2.8.2)[39], and a PPI network was easily constructed. Network analysis was done using Network analyser present in Cytoscape.

Docking: Docking is the process to check the binding efficiency of ligand with target. This is the method that defines the better ligand for a particular drug. So to check this we have used three tools/softwares for docking and compared the results. SDF file for CAP and Resveratrol were downloaded from PubChem and for SIRT1(Pdb Id- 4kxq) from PDB database[40].

Autodock Vina 4.2.0 which is a freely available software ([41]) with its MGLtool were used for docking of target and phenyl caffeate drug. Two drugs.one is Resveratrol which is a control compound and phenyl caffeate which is a test drug molecule were used for docking purpose and the results were compared to check the better binding capacity of drugs to target. Autodock Vina userinterface is shown in fig 3.9.

2] Docking server is a web based GUI that provides favility of all types of ligand protein docking and gives the result which are easily understandable by any field by researcher of any field of biology. Results are provided in terms of bindng energy, HBplot and 2D map diagrams. **3] Schrodinger 9.4 software** was used to dock target and

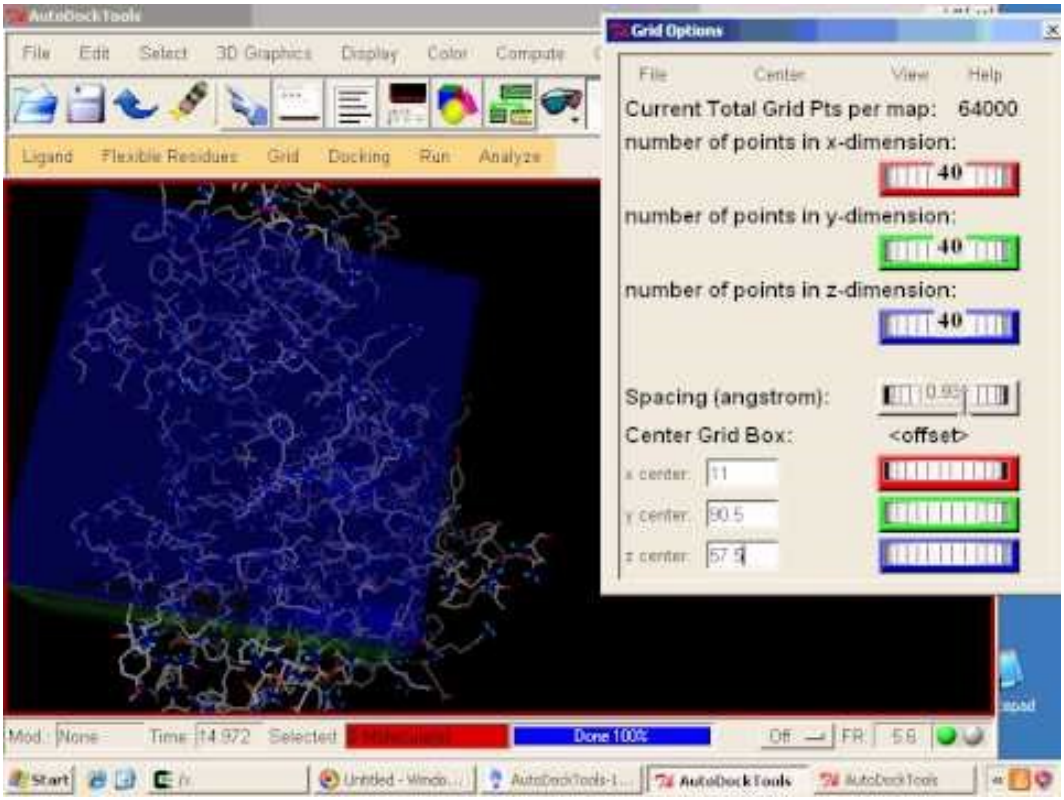

Figure 3.9: Autodock Vina User Interface

Figure 3.10: Docking server Homepage

phenyl caffeate . Schrodinger is the well proved software that gives the result in terms of binding energy and HBplots.

Chapter 4

RESULTS

4.1 Clustering Results

EM algorithm of clustering was used for clustering the input dataset of Chemical-protein interactions of DIc-Target. We find 3 clusters. Cluster(shown in fig 4.1) having one of the Input drug present was chosen for finding drugs for reverse aging. There were more

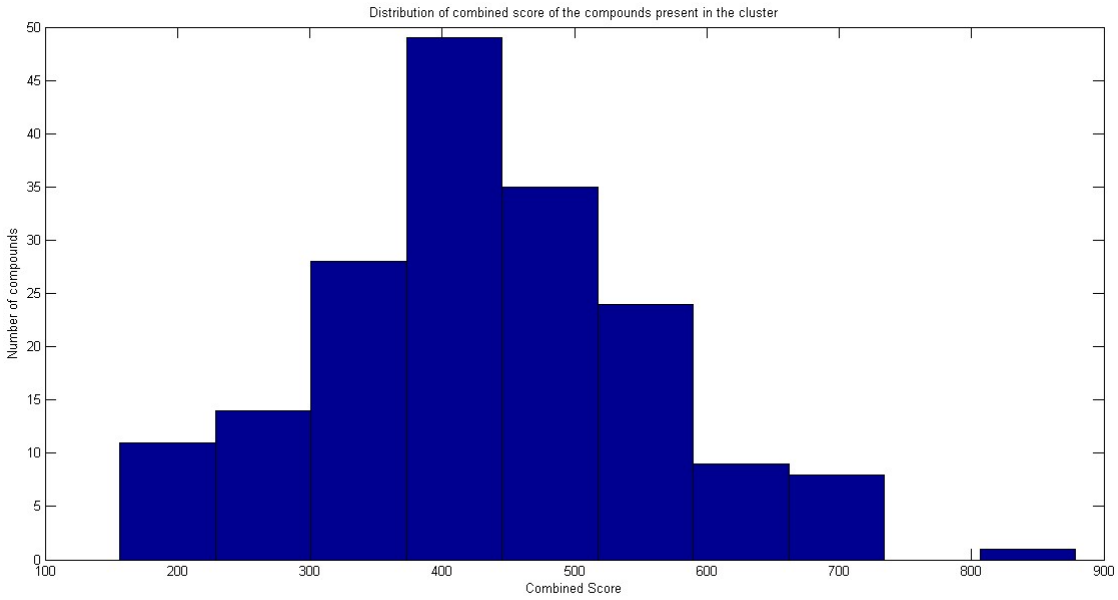

Figure 4.1: Histogram plot for clustered compounds

than 300 compounds in that cluster. Hence we filtered the result based on combined score. We considered probable drugs for reverse aging only to those compounds having combined score ¿=600 (FIG1*). We used a higher cutoff of 600 to select only those compounds having interactive strength with the known drugs for anti aging. Lastly we got 14 compounds which would probably act as anti-aging drugs. All these compounds, their chemical Ids obtained from Pubchem database[37] are mentioned in Tabe shown in Tableclustertable

4.2 Non drug likeliness prediction

SmartsFilter (Smiles Arbitrary Target Specification):- Out of 14 Only, caffeic acid phenyl or Phenyl cafeate (CID5910817), CID00077998 and CID000000985 failed =¿ 3 out of 9 filters which implies that these compounds would be less harmful for human body and could be used for further study to check putative anti-aging properties. Nondruglikeliness prediction by smartFilter in shown in fig 4.2.

Table 4.1: Compound details present in the cluster of interest

S.no.	CID	C_SCORE	Name
1	CID005910817	700	Caffeic acid phenyl
2.	CID000005892	681.64	Dihydronicotinamide , Coenzyme I
3.	CID000000942	677.08	Nicotin, 3-(1-methylpyrrolidin-2-yl) pyridine
4.	CID000004634	676.8	Oxybutynin, Contimin, Cystonorm,Cystrin, Ditropan,Dresplan, Dridase,Driptane,Oxytrol
5.	CID000005508	667.7	tolmetin(1-), [1-methyl-5-(4-methylbenzoyl) -1H-pyrrol-2-yl]acetate
6.	CID000026986	664.93	CLEMASTINE FUMARATE, but-2-enedioic acid
7.	CID000446284	653.1	Eicosapentaenoic Acid, Timnodonic Acid
8.	CID000005202	630.57	serotonin, 5-HYDROXYTRYPTAMINE, Enteramine, Antemovis, Thrombocytin, Hippophain, Antemoqua
9.	CID000077998	610.22	CTK8F0358, MCULE-6489966185
10.	CID000000985	609.85	palmitic acid, Hexadecanoic acid, Cetylic acid, Hydrofol, Hystrene 9016, Hydrofol Acid
11.	CID005280492	606.4	Leukotriene B4, 5,12 diHETE
12.	CID000003158	601.5	Doxepin, Doneurin, Aponal, Deptran, Desidox, Prudoxin, Mareen, Hydrochloride, Espadox, Quitaxon, Sinequan, Sinquan, Xepin, Zonalon
13.	CID000000783	878.59	
14.	CID051508717	722	CommercialnameNA, IUPAC- (2-methyl-1,1,4-trioxothieno [2,3-e]thiazin-3-ylidene)-(pyridin-2-ylamino) methanolate

CID	glaxo_unsuitable_natprod	glaxo_reactive	alarmnmr	lint_blake-v2	oprea_filters	pains_t6	pains_t7	pains_t8
CID005910817	pass	pass	fail	fail	fail	pass	fail	pass
CID000005892	pass	pass	pass	fail	fail	pass	pass	pass
CID000000942	pass	pass	pass	pass	pass	pass	pass	pass
CID000004634	pass	pass	pass	fail	pass	pass	pass	pass
CID000005508	pass	pass	pass	pass	pass	pass	pass	pass
CID000026986	pass	pass	pass	fail	fail	pass	pass	pass
CID000446284	pass	pass	pass	pass	pass	pass	pass	pass
CID000005202	pass	pass	fail	pass	pass	pass	pass	pass
CID000077998	pass	pass	fail	fail	fail	pass	pass	pass
CID000000985	pass	pass	pass	fail	fail	pass	pass	pass
CID005280492	pass	pass	pass	pass	fail	pass	pass	pass
CID000003158	pass	pass	pass	pass	pass	pass	pass	pass
CID000000783	pass	pass	pass	pass	pass	pass	pass	pass
CID051508717	pass	pass	fail	pass	pass	pass	pass	pass

Figure 4.2: Nondruglikeliness prediction of drugs

4.3 ADME Prediction

Pharmacokinetic properties like Absorption, Distribution, Metabolism and excretion in terms of different molecular descriptors were calculated Phenyl caffeate using SwissADMETtool. SwissADMET calculated 44 descriptors. Using these descriptors we predicted the bioavailability score and druglikeliness of Phenyl caffeate and control drug Resveratrol and compared the result for anti-aging properties. Predicted pharmacokinetic parameters are shown in fig 4.3

All pharmacokinetic parameters of CAP are comparable to control drug. After this

Resveratrol	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-5.47 cm/s

Phenyl Caffeate	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-5.71 cm/s

Figure 4.3: Nondruglikeliness prediction of drugs

we predicted the druglikeliness of drugs and result is shown in fig 4.4.

We here followed Lipinskis rule of five, Ghose, Veber and Muegg filters for testing the drug likeliness of compounds for functioning as candidate drug against aging. The

CID	Lipinski's	Ghose	Veber	Egan	Muegg	Bioavailability Score
CID005910817	Yes	Yes	Yes	Yes	Yes	0.55
CID000005892	No	No	No	No	No	0.11
CID000000942	Yes	Yes	Yes	Yes	No	0.55
CID000004634	Yes	Yes	Yes	Yes	Yes	0.55
CID000005508	Yes	Yes	Yes	Yes	Yes	0.56
CID000026986	Yes	Yes	Yes	Yes	Yes	0.56
CID000446284	Yes	No	No	No	No	0.56
CID000005202	Yes	Yes	Yes	Yes	No	0.55
CID000077998	Yes	Yes	No	No	No	0.11
CID000000985	Yes	Yes	No	Yes	Yes	0.56
CID005280492	Yes	Yes	No	Yes	Yes	0.56
CID000003158	Yes	Yes	Yes	Yes	Yes	0.55
CID051508717	Yes	Yes	Yes	No	Yes	0.56

Figure 4.4: Druglikeliness prediction of drugs

results was analysed for those three drugs which had failed =¿3 filters for the nondrug-likeliness proportion and finaly we got only one drug i.e CID5910817(Phenyl Caffeate) is the only drug that has bioavailability score ¿0.55 and follow all rules of five filters used for druglikeliness. So it was hypothesized that CAP(or Phenyl caffeate) has the potential to act as anti-aging drug. To check Gastrointestinal Absorption and Brain Penetration, Boiled egg diagram as shown in fig 4.5 was plotted using SwissADME tool . Just like Resveratrol , CAP falls in Yellow portion which shows that both the compounds crosses the Blood Brain Barrier and access the CNS.

4.4 Chemical Structure details of CAP(Caffeic Acid Phenyl

BAll and stick model of caffeic acid phenyl and its calculated chemical properties are shown in fig **??** CAP is a phenolic compound i.e it consists of phenyl group. Phenolic compounds occur universally in the plant kingdom and are part of a large and complexmgroup of organic substances. CAP is a cinnamic acid derivative. Cinnamic acid derivatives are also known as Phenylpropanoids.

4.5 Control drug selection

MCS is predicted in term of Tonimotto coefficient for all three drugs with Experimental drug i.e CAP shows that Resveratrol can be used as control drug for all the computational studies and comparison of results.

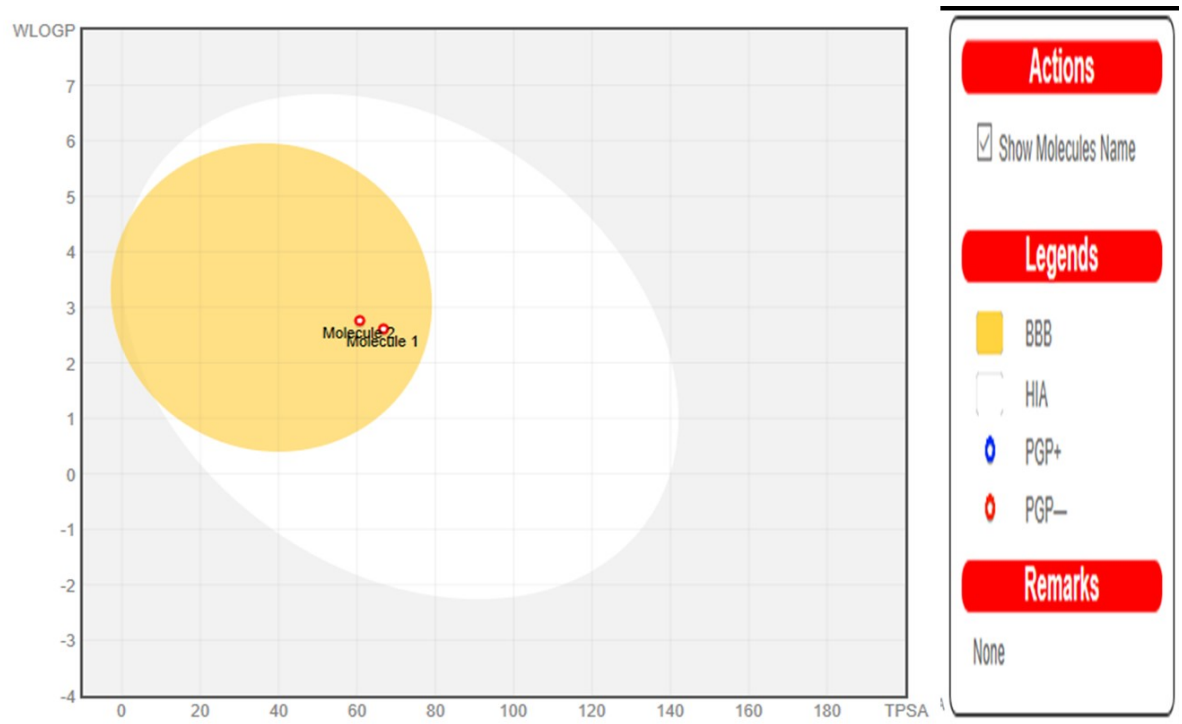

Figure 4.5: Boiled Egg Diagram (WLOGP vs. TPSA):
(Molecule1- Resveratrol, Molecule2-CAP)

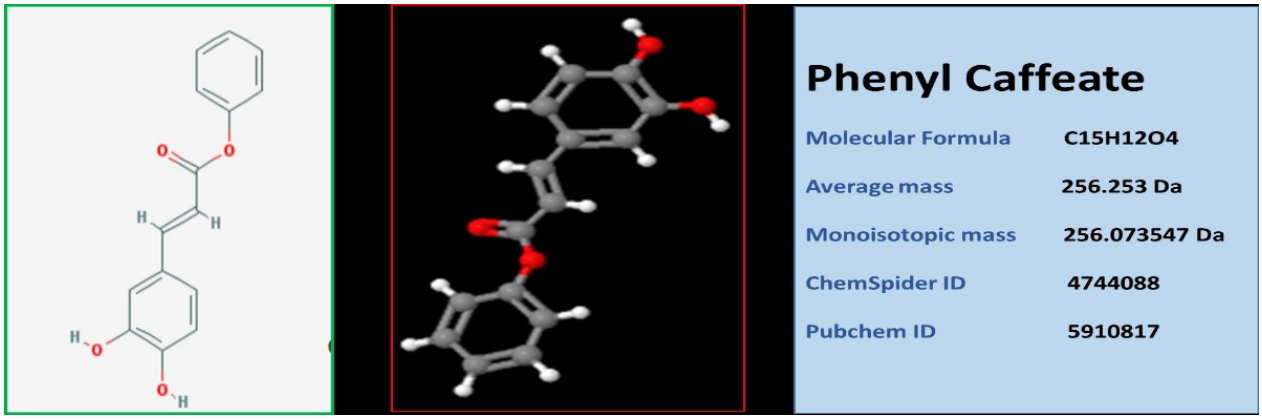

Figure 4.6: Structure details of Caffeic Acid Phenyl

Using Chemmine tool we calculated the JoeLib descriptors for three input drugs and Phenyl caffeate(experimental drug). Then hierarchical clustering was done using these physicochemical descriptors and heatmap and Newick plot were generated to check the

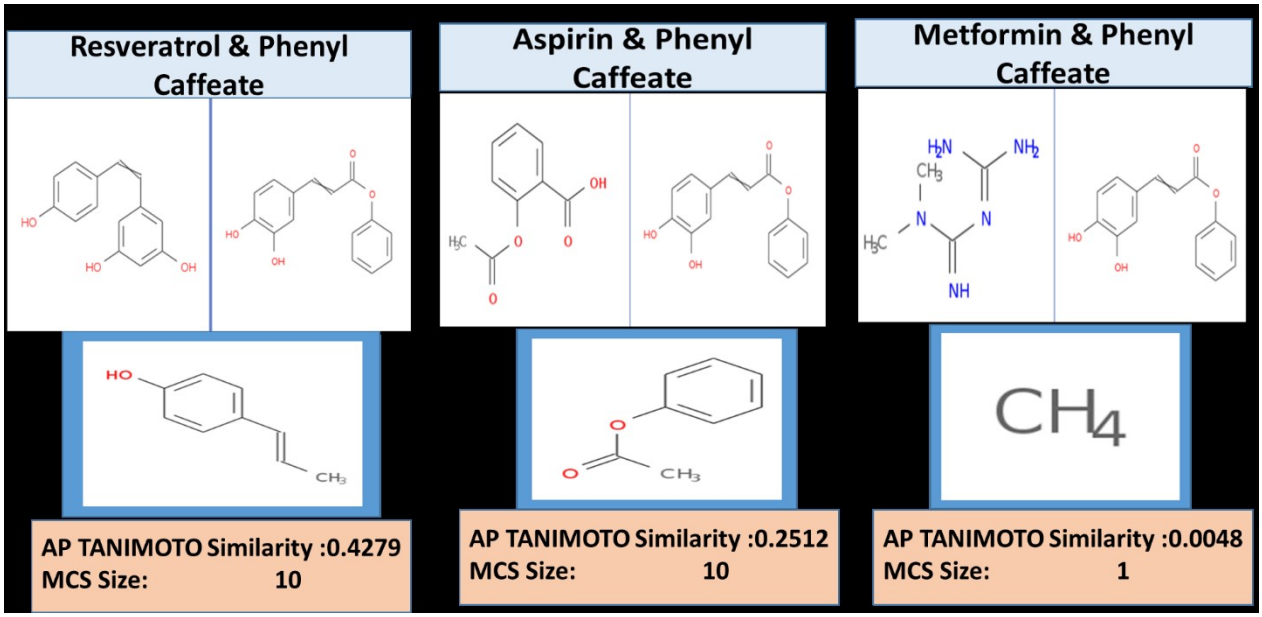

Figure 4.7: Similarity Workbench (MCS)

similar structures. heatmap shows that values of maximum descriptors are same or near

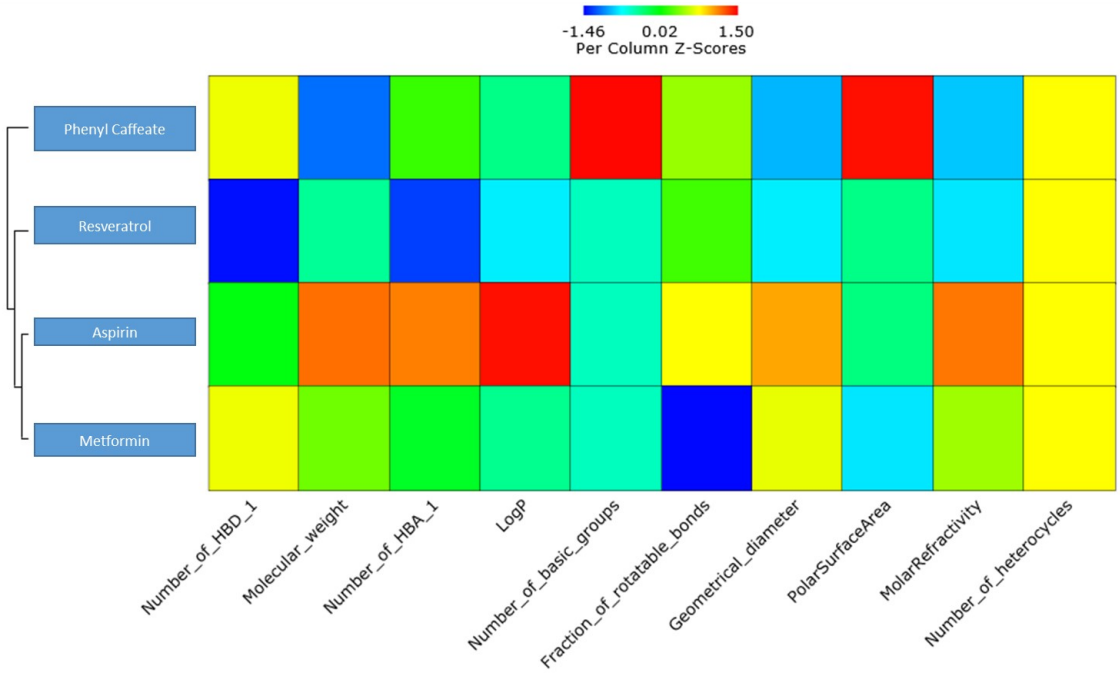

Figure 4.8: Heatmap generated using Joelib descriptors for hierarchical clustering

to each other in case of Resveratrol and CAP. Newick tree shown in fig 4.9 proves this fact.

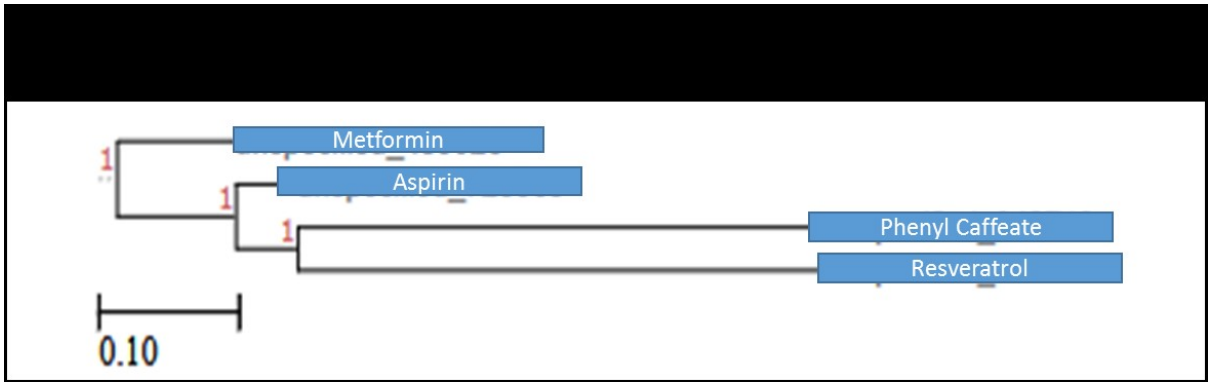

Figure 4.9: newick tree generated for control and experimental drug CAP

4.6 TOXICITY

Chemical toxicity of drug molecue was predicted for both the drugs (Control and experimental) using Chemical toxicity predictor-GUSAR and T.E.S.T software and then results were compared to infer the final prediction of being CAP as anti-aging drug. All entries had application domain- yes which infer that it could be used for in-silico studies and clinical trail study.

[1] Toxicity Prediction using Chemical Toxicity Predictor(GUSAR):- Both types of toxicity were predicted for all drugs. results are shown in fig 4.10 and fig 4.11

T.E.S.T. Toxicity Models	Value	UNIT	Application Domain
40 Hour Tetrahymena Pyriformis Toxicity	4.425	-log10 (IGC50 mol/L)	Yes
48 Hour Daphnia Magna Toxicity	4.628	-log10 (LC50 mol/L)	Yes
96 Hour Fathead Minnow Toxicity	5.256	-log10 (LC50 mol/L)	yes
Bioconcentration Factor	1.572	log10(BCF)	yes
Oral Rat Toxicity	1.696	-log10 (LD50 mol/kg)	yes

T.E.S.T. Toxicity Models	Value	UNIT	Application Domain
40 Hour Tetrahymena Pyriformis Toxicity	4.454	-log10 (IGC50 mol/L)	Yes
48 Hour Daphnia Magna Toxicity	4.546	-log10 (LC50 mol/L)	Yes
96 Hour Fathead Minnow Toxicity	5.171	-log10 (LC50 mol/L)	yes
Bioconcentration Factor	0.836	log10(BCF)	yes
Oral Rat Toxicity	1.979	-log10 (LD50 mol/kg)	yes

Figure 4.10: Test toxicity prediction by GUSAR

[2] Toxicity Prediction using T.E.S.T software:-

We have used an equation generated using QSAR model . In QSAR modelling 1391 data points were used. r 2 which is the estimation measure for training set and q2 which is the estimation measure for test set data. Calculated values of r 2 and q2 are in comparable to each other and are almost same which indiacates the model fit is perfect and the equation can be used for final prediction.

Resveratrol

Rat Toxicity Model	VALUE	UNIT	Application Domain
Acute Rat Toxicity, Intraperitoneal	-0.51	-log10(LD50) mmol/kg	Yes
Acute Rat Toxicity, Intravenous	0.298	log10(LD50) mmol/kg	Yes
Acute Rat Toxicity, Oral	-1.108	-log10(IC50) mmol/l	Yes
Acute Rat Toxicity, Subcutaneous	-0.62	-log10(IC50) mmol/l	Yes

Phenyl Caffeate

Rat Toxicity Model	VALUE	UNIT	Application Domain
Acute Rat Toxicity, Intraperitoneal	-0.323	-log10(LD50) mmol/kg	Yes
Acute Rat Toxicity, Intravenous	0.423	log10(LD50) mmol/kg	Yes
Acute Rat Toxicity, Oral	-1.05	-log10(IC50) mmol/l	Yes
Acute Rat Toxicity, Subcutaneous	-0.424	-log10(IC50) mmol/l	Yes

Figure 4.11: RAT toxicity prediction by GUSAR

Parameter	Value
Endpoint	T. pyriformis IGC_{50} (48 hr)
r^2	0.790
q^2	0.756
#chemicals	1391
Model	Group contribution model

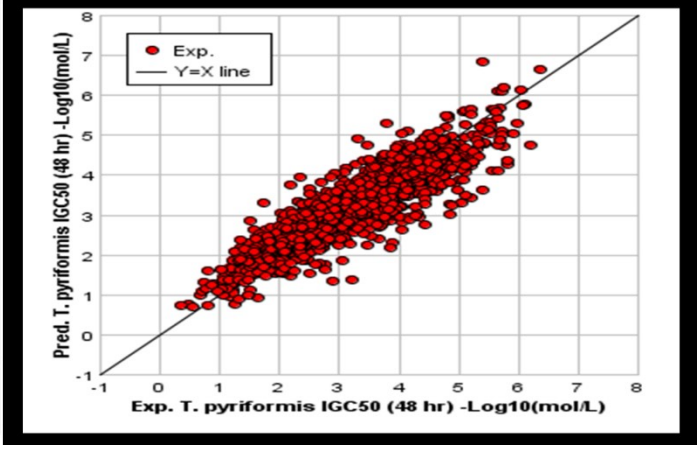

Figure 4.12: QSAR Model For Toxicity Prediction

All the values are comparable to control drug which suggest that CAP is not toxic to the human body or the toxicity value is within the usable range. CAP is Non-carcinogenic and can penetrate the BBB hence could be used as anti-aging drug.

Resveratrol

Endpoint	Predicted value	Prediction interval
T. pyriformis IGC_{50} (48 hr) -Log10(mol/L)	5.43	$4.49 \leq Tox \leq 6.37$
T. pyriformis IGC_{50} (48 hr) mg/L	0.85	$0.10 \leq Tox \leq 7.41$

Phenyl Caffeate

Endpoint	Predicted value	Prediction interval
T. pyriformis IGC_{50} (48 hr) -Log10(mol/L)	5.10	$4.22 \leq Tox \leq 5.97$
T. pyriformis IGC_{50} (48 hr) mg/L	2.06	$0.27 \leq Tox \leq 15.48$

Figure 4.13: T.E.S.T Toxicity Prediction

Compound Name	BBB Penetration(HUMAN)	Carcinogenicity
Resveratrol	Penetrating (P = 0.148, NP = 0.0625)	Non-Carcinogenic (C = 0.16, NC = 0.283)
Phenyl Caffeate	Penetrating (P = 0.17, NP = 0.142)	Non- Carcinogenic (C = 0.172, NC = 0.266)

Figure 4.14: Lazar BBB and Carcinogenicity Prediction (Classification model)

4.7 DFT study

DFT study provides detailed information about the geometry and electronic structure of the molecules. Graph for energy optimization is shown in **Figure 4.15**. To make the difference in structures we plotted the Unoptimized structures and optimized structure of drug which are shown in **figure 4.16, 4.17**. Bond length values for CID5910817 vary from 0.9 to 1.40 A0, CAP is non-planar as maximum of the dihedral angles in its structure are between -1800 and 1800. Table of Atom type, bond angle, bond Length and Dihedral angles for CAP is presented in **Table 4.2, 4.3, 4.4, 4.5** respectively. Biological activity of the molecules were predicted using energy values of HOMO and LUMO and their energy gap **figure 10, 11, 12**. Molecules have a feasible energy gap values which indicates possibility of HOMO to LUMO transition. The presence of small frontier orbital energy gap indicates the polarizability of the molecules. This depicts the high chemical reactivity and low kinetic stability of the drug molecule. From **Figure 13** it is clear that molecules have negative chemical potential hence we can conclude that these drugs are chemically active and could not be decomposed into their constituent elements. **Figure 14,15** ellucidate the high chemical reactivity and low kinetic stability of CAP in comparison to Resveratrol.

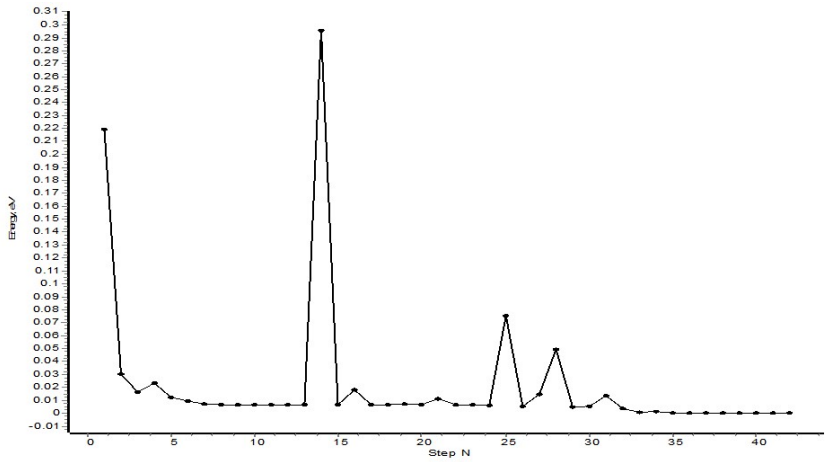

Figure 4.15: Energy values in each steps of structure optimization of CAP)

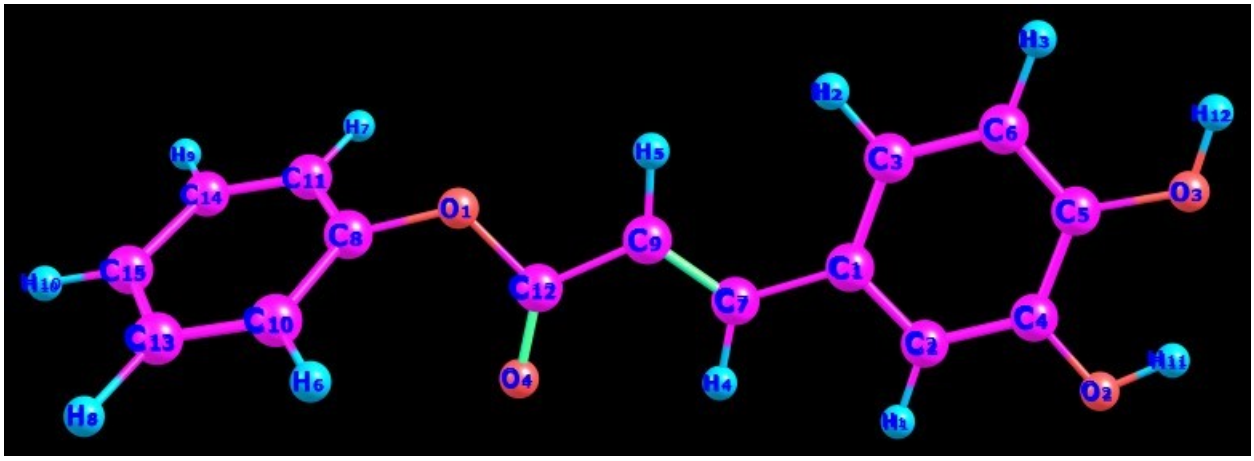

Figure 4.16: CID5910817(CAP) Unoptimized structure)

4.8 Target validation

Targets of candidate drug was predicted using SwissTarget. Targets of proposed drug showed relation to aging. Their entries were present in GenAge database and published research was verified with LibAGe. All for the targets mentioned here are probable target for CAP in combatting aging in humans. All for the targets mentioned here are probable target for CAP in combatting aging in humans.

Other target which is already known is SIRT1. SIRT1 is target of Resveratrol. We did the literature survey for its mechanism of action against anti-aging and pathway analysis using Kegg database and found that Resveratrol act on SIRT1 to increae the longevity in humans. Hence in this study we have considered SIRT1 as main target and focused all our further study like docking with reference to this target only. Other details of SIRT1 is mentioned in section Background and literature review.

Table 4.2: Atom Type

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
O	O	O	O	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	H
21	22	23	24	25	26	27	28	29	30	31									
H	H	H	H	H	H	H	H	H	H	H									

Table 4.3: Table for Bond Lengths

Bond length	Value
R(1,12)	1.3955
R(1,16)	1.378
R(2,8)	1.3616
R(2,30)	0.966
R(3,9)	1.373
R(3,31)	0.9625
R(4,16)	1.2056
R(5,6)	1.4055
R(5,7)	1.4047
R(5,11)	1.4599
R(6,8)	1.3872
R(6,20)	1.0839
R(7,10)	1.3889
R(7,21)	1.0824
R(8,9)	1.4013
R(9,10)	1.3921
R(10,22)	1.0859
R(11,13)	1.3443
R(11,23)	1.0874
R(12,14)	1.3915
R(12,15)	1.3905
R(13,16)	1.471
R(13,24)	1.0826
R(14,17)	1.3931
R(14,25)	1.0816
R(15,18)	1.3935
R(15,26)	1.0833
R(17,19)	1.3946
R(17,27)	1.0841
R(18,19)	1.3937
R(18,28)	1.084
R(19,29)	1.0838

Table 4.4: Table for Bond angles

Bond angle	**value**
A(12,1,16)	120.2479
A(8,2,30)	108.7027
A(9,3,31)	110.8272
A(6,5,7)	118.4822
A(6,5,11)	118.3048
A(7,5,11)	123.213
A(5,6,8)	121.2371
A(5,6,20)	120.5105
A(8,6,20)	118.2524
A(5,7,10)	120.5524
A(5,7,21)	120.4577
A(10,7,21)	118.9899
A(2,8,6)	119.796
A(2,8,9)	120.8552
A(6,8,9)	119.3488
A(3,9,8)	115.3458
A(3,9,10)	124.462
A(8,9,10)	120.1922
A(7,10,9)	120.1872
A(7,10,22)	120.1953
A(9,10,22)	119.6175
A(5,11,13)	127.7173
A(5,11,23)	115.8544
A(13,11,23)	116.4282
A(1,12,14)	121.8224
A(1,12,15)	116.8221
A(14,12,15)	121.2338
A(11,13,16)	120.2849
A(11,13,24)	123.2383
A(16,13,24)	116.4763
A(12,14,17)	118.8988
A(12,14,25)	120.196
A(17,14,25)	120.9026
A(12,15,18)	119.3437
A(12,15,26)	119.2372
A(18,15,26)	121.4189
A(1,16,4)	123.6308
A(1,16,13)	109.3443
A(4,16,13)	127.0241
A(14,17,19)	120.6038
A(14,17,27)	119.3408
A(19,17,27)	120.0555
A(15,18,19)	120.1866
A(15,18,28)	119.5879
A(19,18,28)	120.2255
A(17,19,18)	119.7325
A(17,19,29)	120.1033
A(18,19,29)	120.1641

Table 4.5: Table for Dihedral Angles

Dihedral angle	Value
D(16,1,12,14)	57.2339
D(16,1,12,15)	-126.731
D(12,1,16,4)	0.179
D(12,1,16,13)	179.8735
D(30,2,8,6)	179.9544
D(30,2,8,9)	-0.0613
D(31,3,9,8)	179.8522
D(31,3,9,10)	-0.1774
D(7,5,6,8)	-0.0865
D(7,5,6,20)	179.977
D(11,5,6,8)	179.8956
D(11,5,6,20)	-0.041
D(6,5,7,10)	0.0717
D(6,5,7,21)	-179.89
D(11,5,7,10)	-179.909
D(11,5,7,21)	0.1293
D(6,5,11,13)	-179.346
D(6,5,11,23)	0.4927
D(7,5,11,13)	0.635
D(7,5,11,23)	-179.526
D(5,6,8,2)	-179.984
D(5,6,8,9)	0.0315
D(20,6,8,2)	-0.046
D(20,6,8,9)	179.9695
D(5,7,10,9)	-0.0027
D(5,7,10,22)	-179.996
D(21,7,10,9)	179.9592
D(21,7,10,22)	-0.0346
D(2,8,9,3)	0.0271
D(2,8,9,10)	-179.945
D(6,8,9,3)	-179.989
D(6,8,9,10)	0.0396
D(3,9,10,7)	179.9769
D(3,9,10,22)	-0.0294
D(8,9,10,7)	-0.054
D(8,9,10,22)	179.9397
D(5,11,13,16)	179.7404
D(5,11,13,24)	0.0158
D(23,11,13,16)	-0.0976
D(23,11,13,24)	-179.822
D(1,12,14,17)	176.1729
D(1,12,14,25)	-4.395
D(15,12,14,17)	0.311
D(15,12,14,25)	179.7431
D(1,12,15,18)	-176.207
D(1,12,15,26)	3.626
D(14,12,15,18)	-0.1469
D(14,12,15,26)	179.6864
D(11,13,16,1)	-178.855

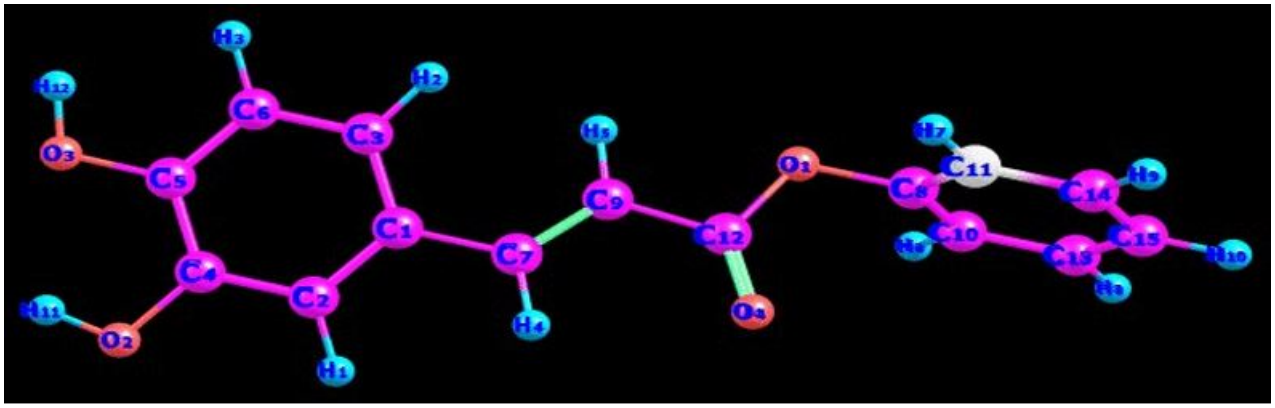

Figure 4.17: CID5910817(CAP) optimized structure(After 42 energy optimization step

ELUMO	EHOMO	ELUMO - EHOMO	η (ev)	μ (ev)	DM (Debye)
8.7792	6.0899	2.6893	1.34465	-7.43455	5.0047

Figure 4.18: The electronic properties of compounds

4.9 Target prediction using pathway analysis

Kegg pathway for longevity in human shows that longevity is increased by activation by Resveratrol and metformin. Pathway diagram is shown in fig 4.22. So taking this pathway as a reference we proposed that as CAP and Resveratrol are structurally similar so their binding partner would also be same or somewhat same. We explored thos study of SIRT1 and did the microarray data analysis to check and confirm the SIRT1 as target for CAP.

4.10 Target prediction using Microarray data analysis for Wrinkles

THe microarray data samples are random and there is much variance in data points. so to minimize it we normalized the microarray data of wrinkles for 42 samples and then calculated the fold change in gene expression values. Data after normalization is shown in fig 4.23. Other plots like QQPlot for normalized data is shown in fig 4.24

To calculate the fold change in gene expression values we found out the threshold using quantile plot and then taking mean of total. The diagram for threshold calculation using quantiles is shown in fig 4.25

After fold change calculation of differentially expressed genes we plotted the heatmap using RStudio 3.3.1. Heatmap is shown in fig 4.26.

Out of all upregulated and downregulated targets SIRT1 is the most downregulated gene which is known to have deacetylase activity . After activation by STACs (SIRT1 activating ompounds) it helps in increase of longevity in humans. But iys role in wrinkles is not reported so far. So to check its anti wrinkle property we targeted it and check

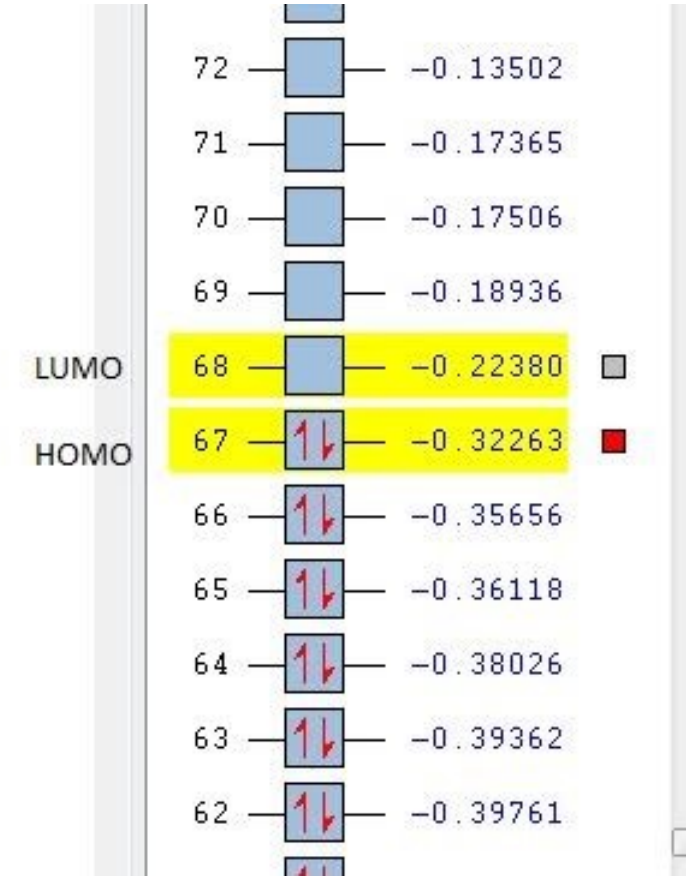

Figure 4.19: Diagram showing molecular orbitals of CAP involved in HOMO LUMO transition

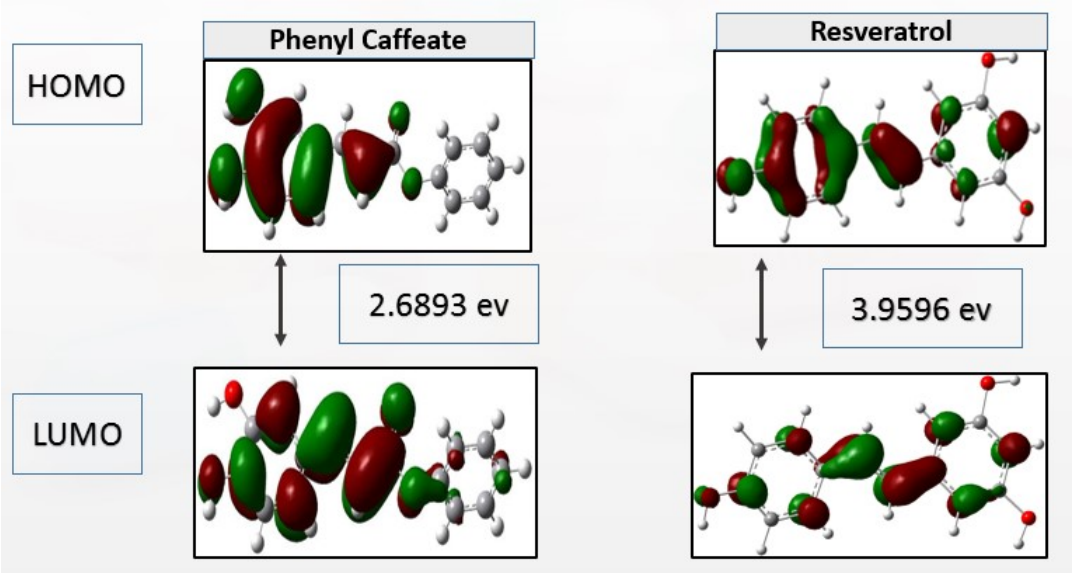

Figure 4.20: Patterns of the HOMO, LUMO of CAP obtained with DFTB3LYP/6-311++G(d,p) method

Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Amine oxidase [flavin-containing] A	P21397	MAOA	CHEMBL1951		53 / 12	Enzyme
Amine oxidase [flavin-containing] B	P27338	MAOB	CHEMBL2039		53 / 13	Enzyme
Tyrosyl-DNA phosphodiesterase 1	Q9NUW8	TDP1	CHEMBL1075138		91 / 17	Enzyme
Carbonic anhydrase 1	P00915	CA1	CHEMBL261		102 / 10	Enzyme
Carbonic anhydrase 2	P00918	CA2	CHEMBL205		102 / 10	Enzyme
Carbonic anhydrase 3 *(by homology)*	P07451	CA3	CHEMBL2885		102 / 10	Enzyme
Carbonic anhydrase 5A, mitochondrial	P35218	CA5A	CHEMBL4789		102 / 10	Enzyme
Carbonic anhydrase 7	P43166	CA7	CHEMBL2326		102 / 10	Enzyme
Carbonic anhydrase 13 *(by homology)*	Q8N1Q1	CA13	CHEMBL3912		102 / 10	Enzyme
Carbonic anhydrase 5B, mitochondrial *(by homology)*	Q9Y2D0	CA5B	CHEMBL3969		102 / 10	Enzyme
Dual specificity tyrosine-phosphorylation-regulated kinase 1A *(by homology)*	Q13627	DYRK1A	CHEMBL2292		57 / 11	Ser_Thr_Tyr Kinase
Epidermal growth factor receptor	P00533	EGFR	CHEMBL203		104 / 6	Tyr Kinase
Receptor tyrosine-protein kinase erbB-2	P04626	ERBB2	CHEMBL1824		104 / 6	Tyr Kinase
Receptor tyrosine-protein kinase erbB-3 *(by homology)*	P21860	ERBB3	CHEMBL5838		104 / 6	Tyr Kinase
ERBB4 intracellular domain *(by homology)*	Q15303	ERBB4	CHEMBL3009		104 / 6	Tyr Kinase

Figure 4.21: Target predcited by SWISSTARGET

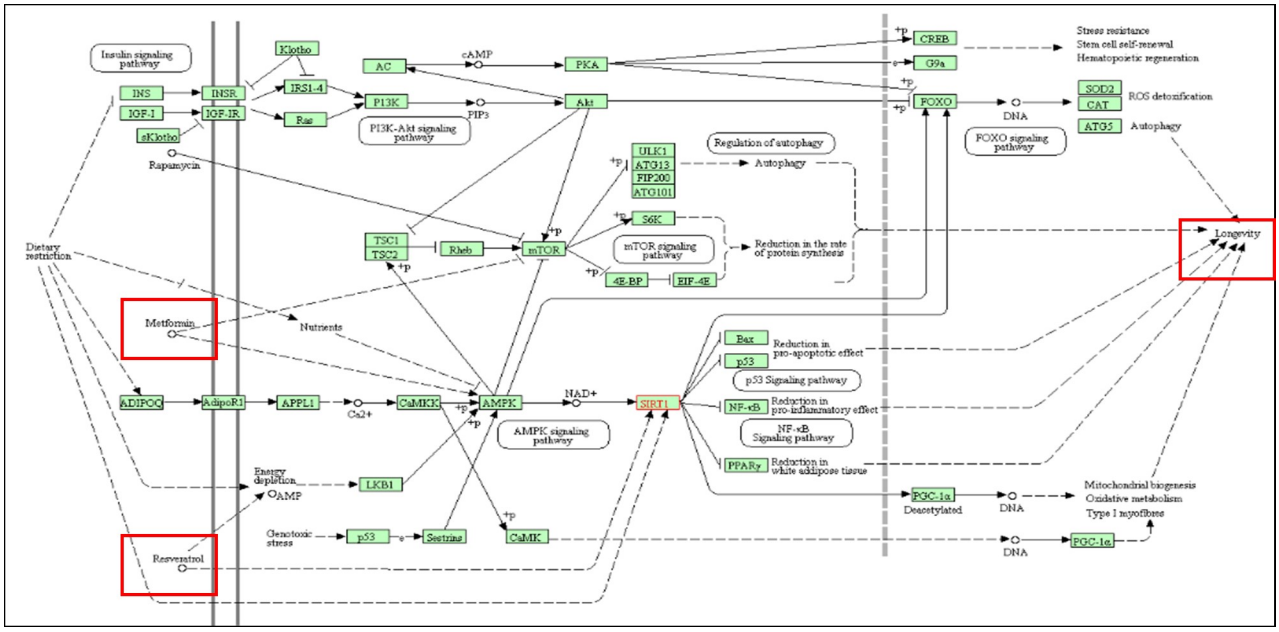

Figure 4.22: Longevity pathway involving SIRT1 activation vy control drug Resveratrol

other interacting partners also which could be related to its action.

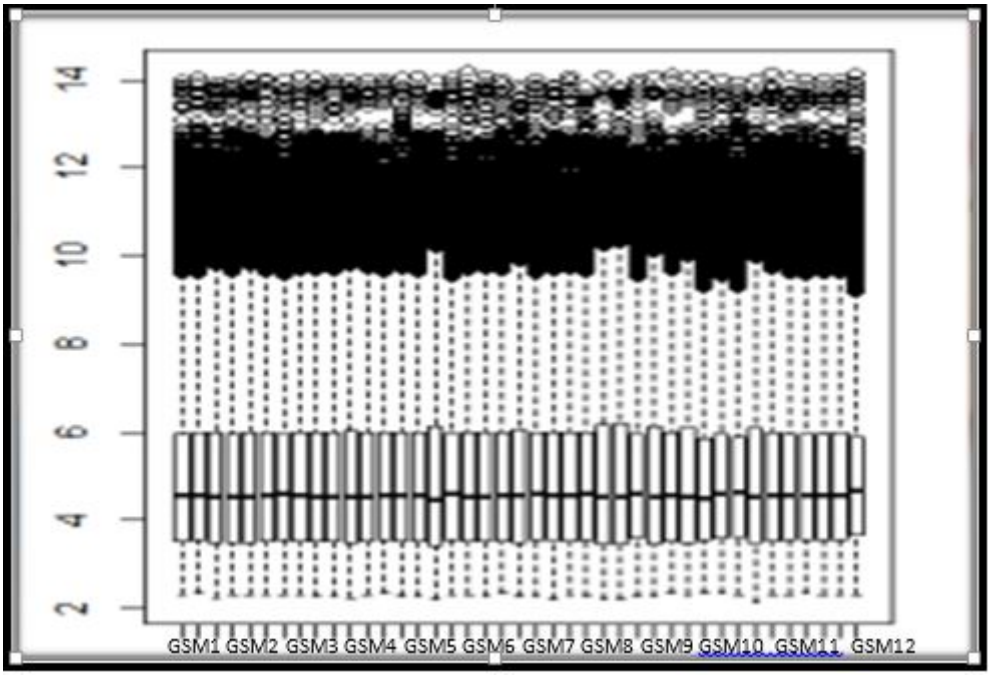

Figure 4.23: Normalized data GSE85358

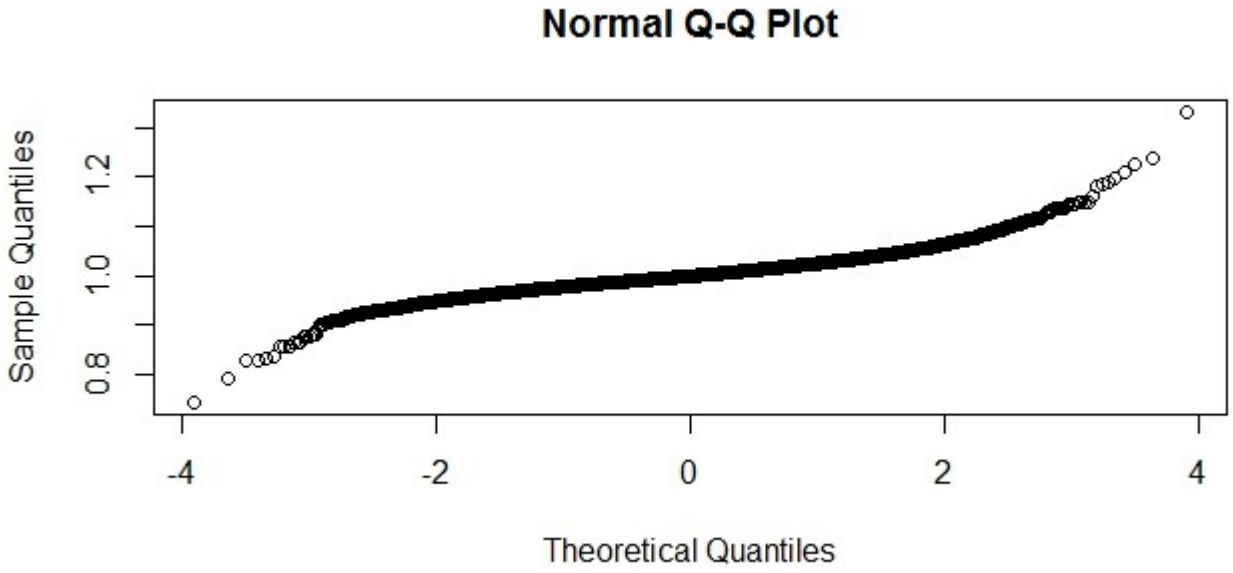

Figure 4.24: QQ PLOT for Normalized data GSE85358

4.11 Protein protein Interaction Network Study

We predicted the protein protein interactions of all the proteins having expression fold change greater than 1.2 using String database and those nodes having maximum no. of hubs were considered to be the new targets for anti-aging.

0%	25%	50%	75%	100%	Threshold
0.74307	0.984411	0.998616	1.015528	1.3309184	1.01450854

Figure 4.25: Quantile plot for threshold selection

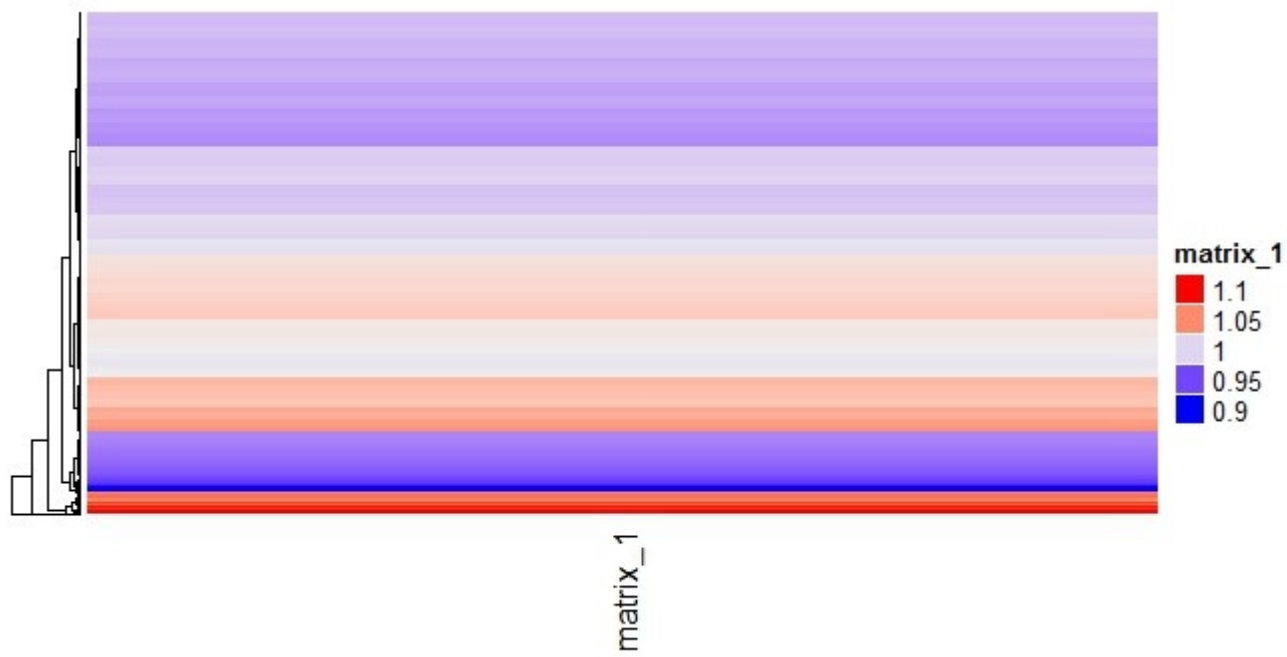

Figure 4.26: Heatmap for differentially expressed genes

4.11.1 PPI network analysis for new target prediction

The PPI information data were imported into Cytoscape (an open source software platform 2.8.2), and a PPI network was easily constructed. Network analysis was done using Network analyser present in Cytoscape.

4.12 DOCKING

There were three tools/softwares used to check interaction of target SIRT1 and ligand CAP. Binding energies and no. of hydrogen bonds between CAP and SIRT1 was much higher than SIRT1 and Resveratrol. This confirmed that predicted anti aging drug could be more potent and effiecient than Resveratrol in curing age related disorders and wrinkles problem.

4.12.1 Autodock Vina 4.0

CAP forms 5 HBOND with SIRT1 but Resveratrol only 3 Hydrogen bonds were formed. Residues are shown in the fig 4.29.We compared the calculated binding energies of SIRT1 with Resveratrol and CAP. Values are shown in fig 4.30. Pymol was used for structure and bonds visualization.

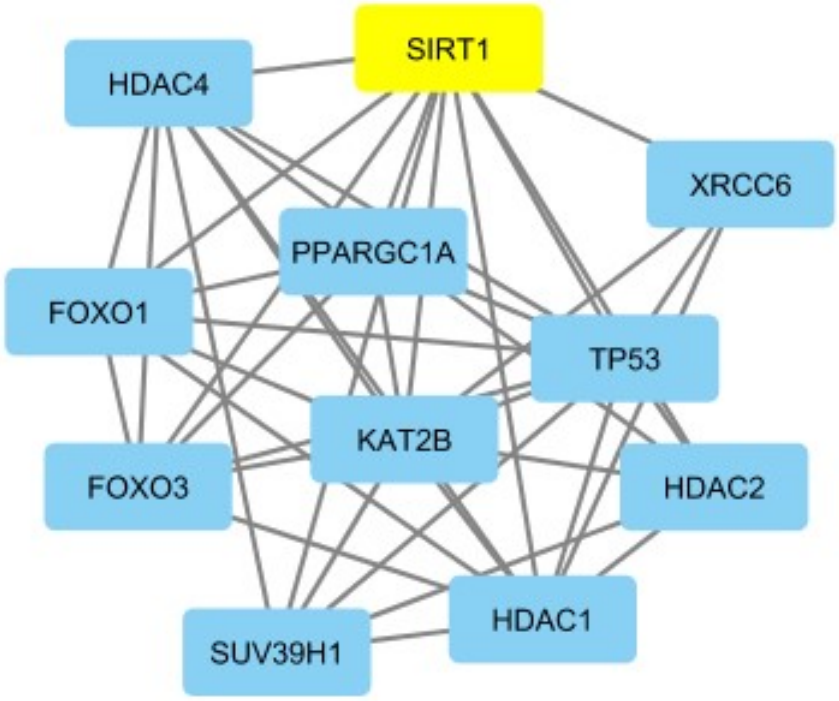

Figure 4.27: Protein Protein Interaction Network for Human SIRT1

Clustering coefficient :	**0.862**	Number of nodes :	**11**
Connected components :	**1**	Network density :	**0.745**
Network diameter :	**2**	Network heterogeneity :	**0.271**
Network radius :	**1**	Isolated nodes :	**0**
Network centralization :	**0.311**	Number of self-loops :	**0**
Shortest paths :	**110 (100%)**	Multi-edge node pairs :	**0**
Characteristic path length :	**1.255**	Analysis time (sec) :	**0.06**
Avg. number of neighbors :	**7.455**		

Figure 4.28: Network Statistics of SIRT1 PPI

4.12.2 Docking Server

Results like binding energy and no. oh hydrogen bonds calculated by docking server are comparable to result obtained by Autodock Vina i.e CAP outperformed Resveratrol. Same results were predicted using dicking server also as using AUtodock. Results are shown as docked structure of Ligand and target compkex **Figure 4.31**, binding energy

Table 4.6: Interacting partners of Human SIRT1

NAME	Average shortest path length	Clustering coefficient	Stress	Degree
FOXO1	1.3	0.9047619	4	7
FOXO3	1.3	0.9047619	4	7
HDAC1	1.1	0.75	18	9
HDAC2	1.4	1	0	6
HDAC4	1.2	0.85714286	8	8
KAT2B	1	0.68888889	28	10
PPARGC1A	1.5	1	0	5
SIRT1	1	0.68888889	28	10
SUV39H1	1.4	1	0	6
TP53	1	0.68888889	28	10
XRCC6	1.6	1	0	4

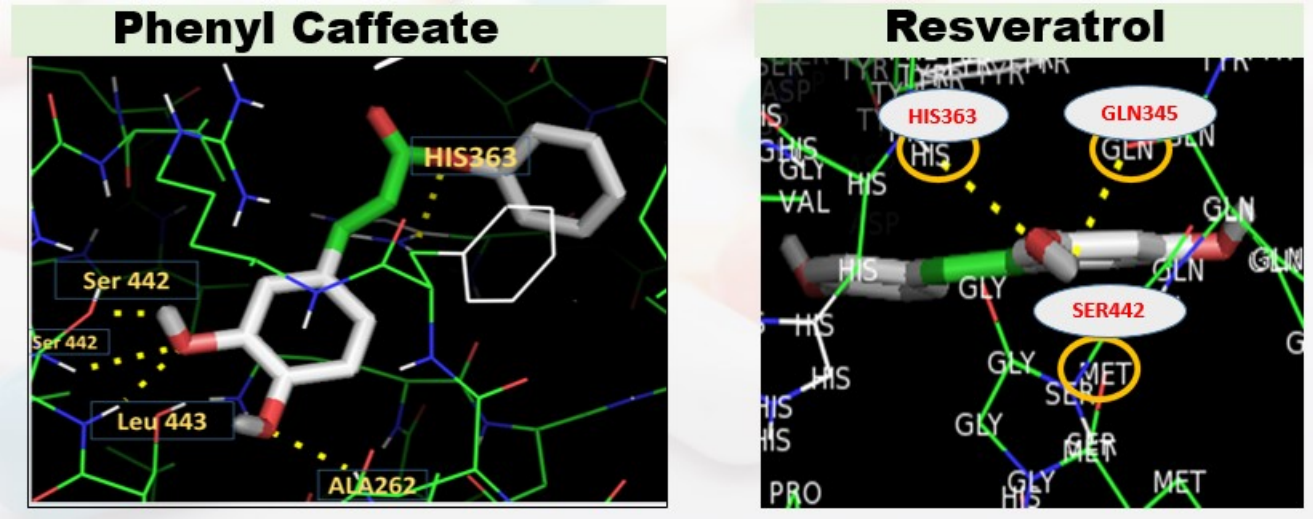

Figure 4.29: Docking of CID5910817 with SIRT1 (AUTODOCK)

in **Figure 4.32**, HBPLOT **Figure 4.33**, and 2D PLot **Figure 4.34**

4.12.3 Schrodinger (Maestro)

Schrodinger(Maestro 9.4,USA) is a chemoinformatics software which is used for molecular docking of SIRT1(target) and CAP(Ligand). Glide module from Schrodinger suite was used to predict the biological activity of drug in terms of binding energy and interaction map which is shown as 2D PLOT in fig 4.36. Docked structure of target and ligand is shown in fig 4.35.

(SIRT1 Vs. Phenyl Caffeate)

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-9.4	0.000	0.000
2	-8.8	2.899	8.223
3	-8.8	7.841	11.384
4	-8.7	2.202	7.564
5	-8.4	4.125	9.252
6	-8.4	2.092	7.452
7	-7.6	10.215	12.389
8	-7.1	13.371	14.973
9	-7.0	6.046	9.020

SIRT1 vs. Resveratrol)

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-6.4	0.000	0.000
2	-6.2	12.332	13.434
3	-6.2	12.328	13.401
4	-6.2	38.527	40.875
5	-6.1	15.640	17.963
6	-5.9	1.218	7.289
7	-5.7	30.045	32.258
8	-5.7	13.048	15.897
9	-5.6	14.588	17.087

Figure 4.30: Energetics of docking(AUTODOCK)

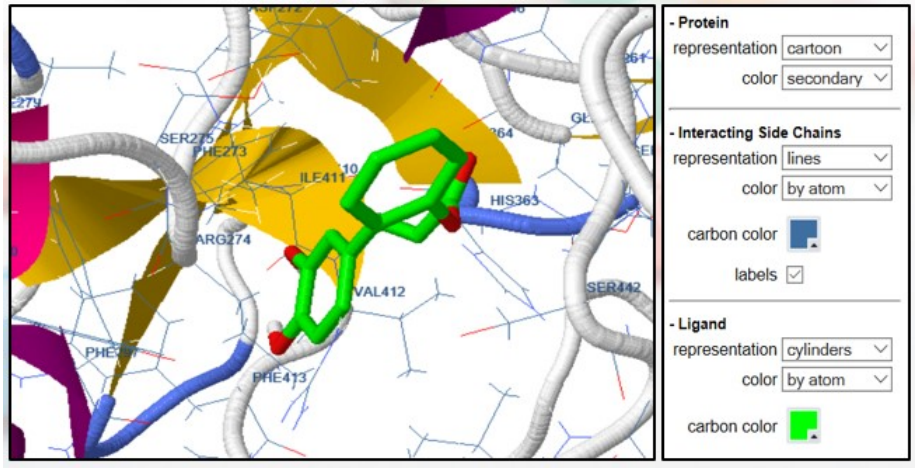

Figure 4.31: Docked structure of Target protein SIRT1 and Ligand CAP(DOcking Server)

Caffeic Acid Phenyl

Rank	Est. Free Energy of Binding
1.	-7.55 kcal/mol
2.	-6.85 kcal/mol
3.	-6.54 kcal/mol
4.	-6.52 kcal/mol

Resveratrol

Rank	Est. Free Energy of Binding
1.	-6.13 kcal/mol
2.	-6.10 kcal/mol
3.	-5.71 kcal/mol
4.	-5.37 kcal/mol

Figure 4.32: Energetics of Docking(Docking Server)

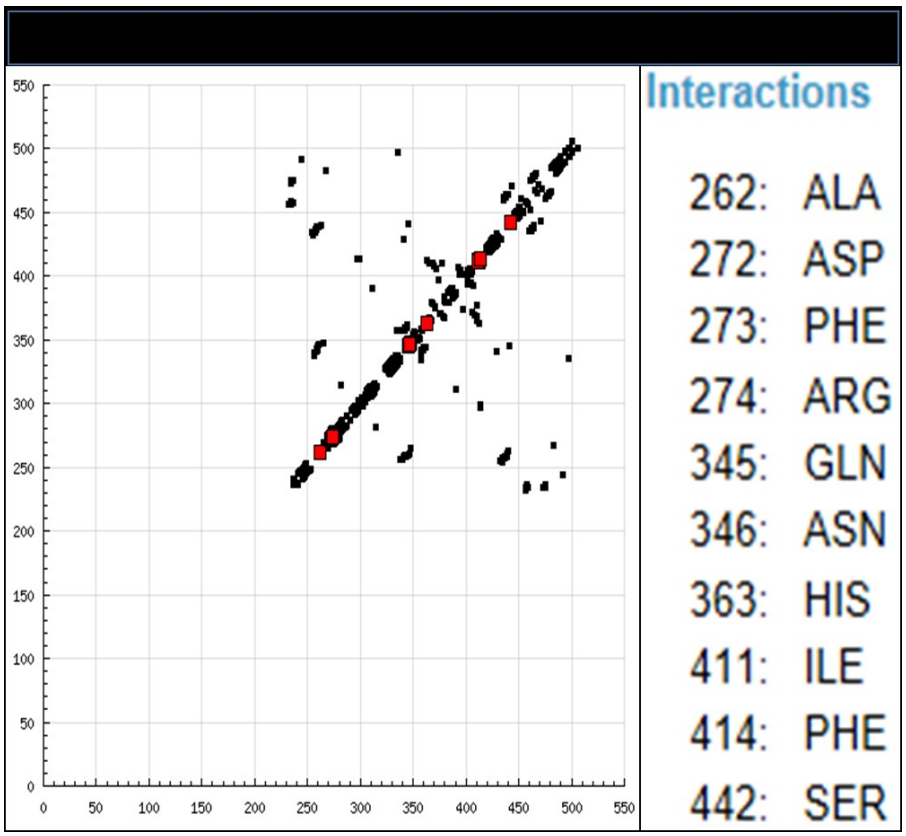

Figure 4.33: HBPLOT for Docking(DOcking Server)

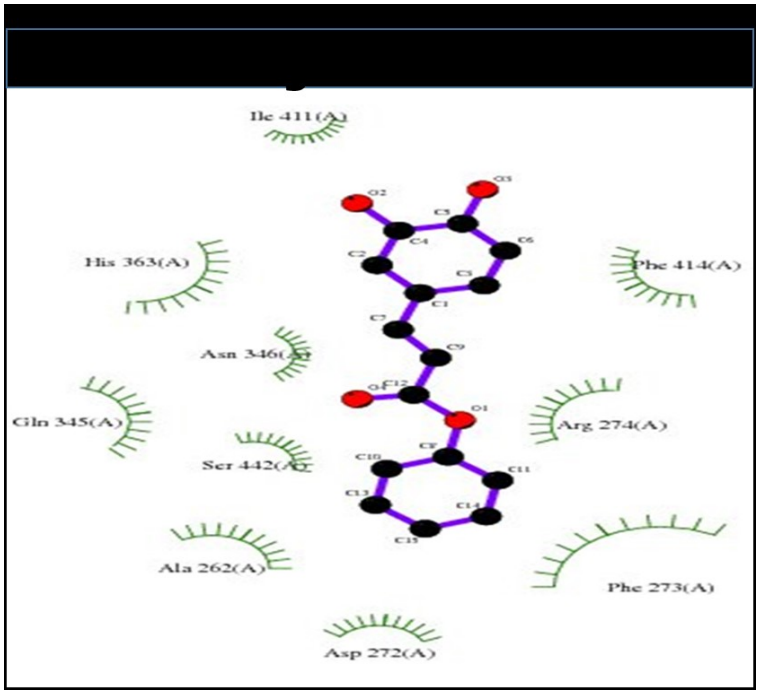

Figure 4.34: 2DPLOT for Docking(DOcking Server)

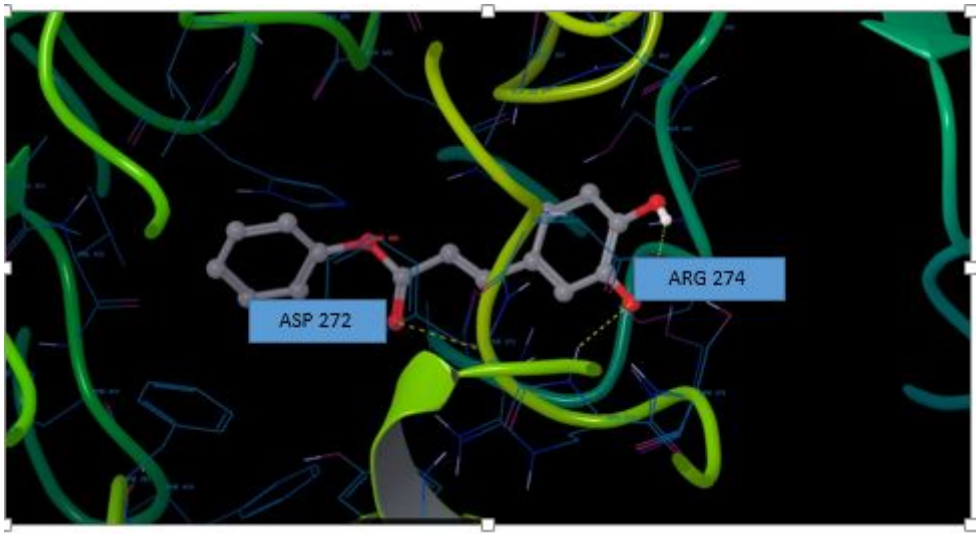

Figure 4.35: Docked complex of SIRT1 and CAP(Schrodinger)

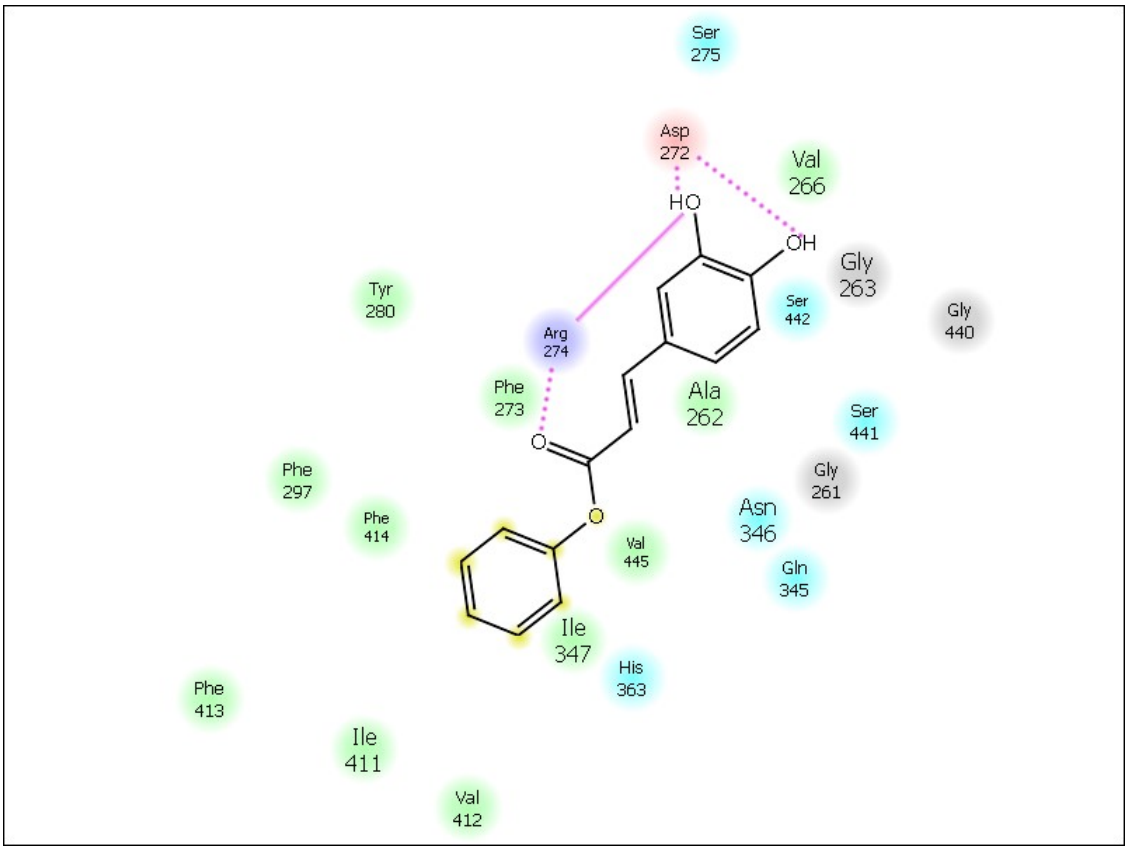

Figure 4.36: 2DPLOT of Docked complex of SIRT1 and CAP(Schrodinger)

Chapter 5

DISCUSSION

Discussion

There are many experimental studies available for methods and validation of drug discovery in the area of anti-ageing. But no computational attempts were tried in this direction. Chemical-chemical, chemical-protein interaction information was taken as the initial data and application of clustering algorithm was applied for the same which is a new and effective approach for drug discovery. Non drug likeliness of drugs were predicted using SmartFilter and only three drugs failed all filters which is the measure of non druglikeliness. From ADME-Druglikeliness result it is clear that out of 13, only one drug CID5910817 or Phenyl caffeate has good drug like properties. Further toxicity and application domain were checked for these this drug and it showed that all that it could be used for anti-aging purpose. DFT results show the greater stability and bioactivity of the proposed drug by having less energy gap between HOMO and LUMO orbitals in comparison to Resveratrol(Control drug). Calculated values of energy parameters indicates that all these drugs are electronically and geometrically stable to be used as anti-aging drugs. Docking results suggest the better binding affinity of CAP with SIRT1 than Resveratrol and hence better capacity to treat age related disorders and Wrinkles a skin problem. With this work we propose that Phenyl caffeate(CAP) has all the anti aging properties and its working pathway is proposed in fig **??**. CAPc could be used for pre clinical and clinical trials. Using Kegg pathway for Longevity nd docking and Dft result as a reference we can get an idea about working mechanism of CAP drug to act as an anti-aging drug and increase the longevity n humans. Hence it leaves the area open for further experimental research to check this proposed pathway. Those drugs which fail druglikeliness test in ADME prediction can also be explored further with chemoinformatic studies to be made anti-aging drugs. This study may provide a new direction to the medicinal field for discovering novel anti-aging drugs. After clinical research these drugs may help to solve the problem of aging and other age related diseases.

Chapter 6

CONCLUSION

CONCLUSION

Caffeic acid phenyl(CAP) is a phenol derivative of caffeic acid. These phenolic compounds are phytochemicals which are synthesized by higher plants and provides protection against free radical attack. They have antioxidant, antimicrobial and cytotoxic properties. CAP act as STACs and hence by activating SIRT1 it can increase longevity in humans. Other possible mechanism of action is shown in figure 6.1.[42]

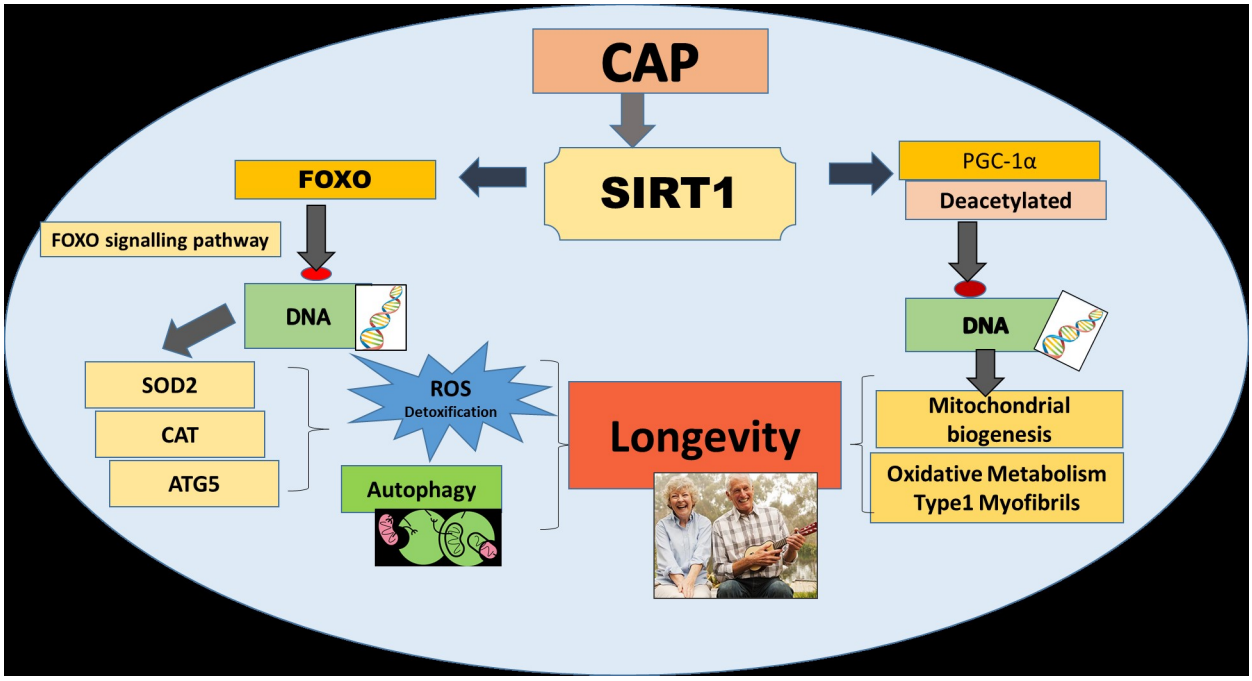

Figure 6.1: Proposed mechanism for Sirt1 activation by CAP

Chapter 7

FUTURE PROSPECTS

FUTURE PROSPECTS

Molecular dynamics simulation study can be performed to evaluate the stability of docked complex(Caffeic acid phenyl and SIRT1) through 15 ns MDS. Simulation would provide the exact binding interaction of the docking complex with system embedded with water molecules, temperature and pressure.

Cell viability test and western blotting could also be performed to confirm the anti aging properties of Phenyl caffeate.

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