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Research Article

ESTABLISHING NOVEL DRUG LEADS FOR AMYOTROPHIC LATERAL SCLEROSIS USING *IN-SILICO* METHOD.

Puneet A.S[1, 2*], Vinay R.S[1, 2], Preenon Bagchi[2], Mahesh. M

[1.] Univeresity Visveswaraya college of Engineering, K.R.Circle, Bangalore, 560001, Karnataka, India..

[2.] Azyme Biosciences Pvt.Ltd, Bangalore, 560069, Karnataka, India.

*Correspondence author Email:1991.puneet@gmail.com,puneet@sarvasumana.in

ABSTRACT

Amyotrophic Lateral Sclerosis is a progressive neurodegenerative disease causing muscle spasticity, muscle wasting which leads to difficulty in speaking, swallowing and breathing. Mutations that occurring in superoxide dismutase [Cu-Zn] enzyme encoded in the SOD1 gene, Angiogenin protein encoded in the ANG gene, SIGMAR1_HUMAN mutated gene and Valosin-containing protein enzyme encoded in VCP gene have been implicated as factors thatcause ALS. Drug ligands found in natural herbs such as *7-hydroxycoumarin* ligand interacted with ANG1 gene.*Kavain* ligand interacted with SGMR1_HUMAN gene, *7-hydroxycoumarin, Methysticin, Scopoletin* ligands interacted with SOD1 gene. These ligands can be tested for their efficacy in *in-vitro* receptor ligand binding assay studies.

Keywords— Amyotrophic lateral Sclerosis, virtual screening, ADME screening, Drug Designing, Gene Mutations

INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS), also called as “Lou Gehrig’s disease” and Charcot disease which affects nerve cells in the brain and the spinal cord [1]. Amyotrophic Lateral Sclerosis causes disorders such as muscle spasticity, rapidly progressive weakness due to muscle wasting. This results in difficulty speaking, swallowing and breathing. Research infers that the disease starts usually around the age of 60 except in cases that are directly inherited from a person’s parents [2, 3, 4].

Lateral Sclerosis is caused by multi-gene mutations such as SOD1, C9ORF72, TARDBP, FUS,

VAPB, VCP, UBQLN2, ALS2, SETX, OPTN, ANG, and SPG11 cause Amyreophic Lateral Sclerosis [1].

An improved understanding of ALS genetics should lead to better trial designs, insights into common molecular pathways. [1]

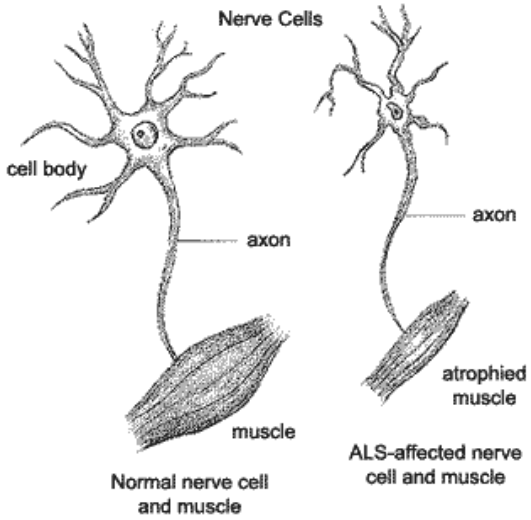

Fig. 1.1 Nerve cells and Muscle of normal person and ALS Affected person

Mutation is genes such as superoxide dismutase (SOD1) [1, 3], Angiogenin(ANG) [1, 2], Valosin Containing protein (VCP) [1, 3] and SIGMAR1 [4] genes are associated with causing ALS are taken in this study.

The use of Natural Herbs and oils containing several medicinal phytochemicals are taken into account into this study to identify novel, potential lead drug molecules for Amyotrophic Lateral Sclerosis (ALS) and tested *in-sillico* in this study.

SOD1: Mutations in the gene that produces Cu/Zn superoxide dismutase (SOD1) enzyme were associated with approximately 20% of familial ALS. This enzyme is a powerful antioxidant that protects the body from damage caused from

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SOD1: Mutations in the gene that produces Cu/Zn superoxide dismutase (SOD1) enzyme were associated with approximately 20% of familial ALS. This enzyme is a powerful antioxidant that protects the body from damage caused from *superoxide* a toxic free radical generated in the mitochondria. Studies involving transgenic mice have yielded several theories about the role of SOD1 in mutant SOD1 familial amyotrophic lateral sclerosis [1, 3].

ANG: This gene provides instructions for making a protein called angiogenin, which promotes the formation of new blood vessels from pre-existing blood vessels through a process called angiogenesis. Research suggests that several ANG mutations [1, 2] that may increase the risk of developing ALS and most mutations reduce the activity of angiogenin a reduction in its ability to promote the production of rRNA and subsequently the production of proteins have negative effect on cells. It is noted that people who have ANG gene mutations also develop frontotemporal dementia (FTD).

VCP: VCP protein is essential for maturation of ubiquitin –containing autophagosomes, and mutant mediated through its effect on TDP-43 protein, a major constituent of ubiquitin inclusions that neuropathologically characterize ALS, It is estimated that the phenotype IBMPFD to include motor neuron degeneration suggests that VCP mutations may account for 1%-2% of familial ALS [1, 3].

SIGMAR1: SIGMAR gene sequencing revealed a mutation affecting a highly conserved amino acid located in the transmembrane domain of the encoded protein, sigma-1 receptor [4]. The mutated protein showed an aberrant subcellular distribution NSC34 cells.

In this work the 3D structure of the selected proteins for docking are generated using homology modelling with the help of MODELLER software [9]. Suitable ligands are selected from compounds found in natural herbs, docking these ligands to receptor proteins to reverse or lessen the side effects are tabulated using SMILES [10] and ADME studies is carried out, to identify novel drug leads for ALS.

METHODOLOGY

A. Identification of genes related to Amytrophic lateral Sclerosis

The amino acid sequences of the receptor proteins SOD1, SIGMAR1, ANG and VCP were retrieved from the National Center for Biotechnology Information (NCBI) [6]. Using Basic Local Alignment and Search Tool (BLAST) [7] search engine against Protein Data Bank (PDB) [8] and the homologous templates for Amyotrophic Lateral Sclerosis (ALS) was selected. Using these homologous templates the 3D structure of the receptor was generated using MODELLER [9] software.

B. Establishing the 3D structure of the gene receptor using homology modelling.

The homologous templates were used to construct the 3D structure of the receptor proteins Angiogenin (ANG) [1,2], SIGMAR1 [4], VCP [1, 3] and SOD1 [1, 3] with the help of MODELLER [9] software using python programs.

Modeller generated models were verified using Ramachandran Plot [8, 12] to find the number of residues in core, allowed and disallowed region and the Best 3D structure model is selected.

C. Identifying Novel Drug Ligands and Ligand Preparation.

Medicinal herbs for mood stabilization, anti-depression and neurological disorders [13, 16] are taken into consideration and their compounds are selected for treatment of Amyotrophic lateral sclerosis. These compounds are used as ligands for the receptors.

The compounds' 3D structure are downloaded from PUBCHEM database which is maintained by NCBI, the downloaded compounds are converted into pdb format using CHIMERA tool [17].

D. Virtual Screening.

The compounds which are selected from the medicinal herbs are used as the drug ligands for the receptor genes. 3D structure were retrieved for these compounds and the 3D structure of the gene receptor using homology modelling are docked together with the help of HEX server[11].

E. Computer Aided Drug Designing.

The docked complex are evaluated using PYMOL software [14]. These docked results are viewed and analyzed. The table is constructed for these results with the interacting amino acids, number of interactions and their energy.

F. ADME Screening.

ADME is an Acronym for Absorption, Distribution, Metabolism and Excretion. It is the widely used method for checking the selected drug ligands.

MOLINSPIRATION [15] server was used which took the SMILES information and calculated ADME properties.

RESULTS AND DISCUSSION

Journal of Medicinal Chemistry and Drug Discovery

A. Identification of genes that cause Amyotrophic lateral sclerosis.

The receptor Proteins were identified and selected from National centre for Biotechnology Information.

Table 3. 1: Proteins with NCBI Accession number

Protein	NCBI Accession Number
ANG1	P03950.1
SGMR1_HUMAN	Q99720.1
SOD1	P00441.2
VCP	P55072.4

B. Establishing the 3D structure of the gene receptor using homology modelling.

3D structure of the ANG, VCP, SGMR1, SOD1 was modelled using the MODELLER [9] software. Python programs were used to sequence the 3D structure based on *homology modelling*. Homology modelling means similar study, that is proteins with similar structure or similar structural motif as of the query protein.

Selected templates were retrieved from the protein data bank, here BLAST result is used as an input to the MODELLER software [9]. The top template (best result) for each protein is selected for further consideration. The quality of the 3D structure of the proteins is judged based on statistical significance. Ramachandran Plot [8, 12] is used to select the output of each protein candidate and to select the best among the 3D models.

Journal of Medicinal Chemistry and Drug Discovery

Table 3.2: Ramachandran Plot Scores for 3D models.

Gene	Residues in Core Region	Residues in allowed Region	Residues in generously allowed Region	Residues in Disallowed Region
ANG1_1	116(93.9%)	7(5.6%)	1(0.8%)	0(0.0%)
SGMR1	170(87.6%)	19(9.8%)	3(1.5%)	2(1.0%)
SOD1	111(91.0%)	10(8.2%)	1(0.8%)	0(0.0%)
VCP	584(83.5%)	97(13.9%)	10(1.4%)	8(1.1%)

Fig 3.1: Angiogenin (ANG) gene

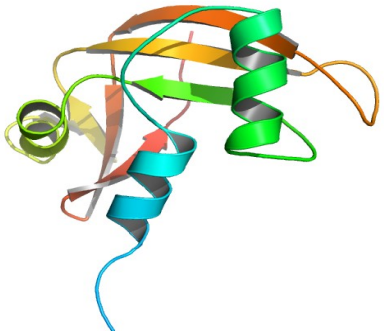
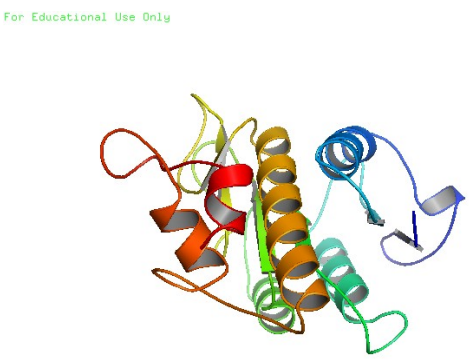


Fig 3.2: SGMR1_HUMAN gene



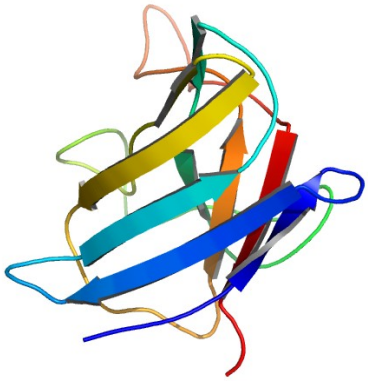


Fig 3.3: SOD1 gene

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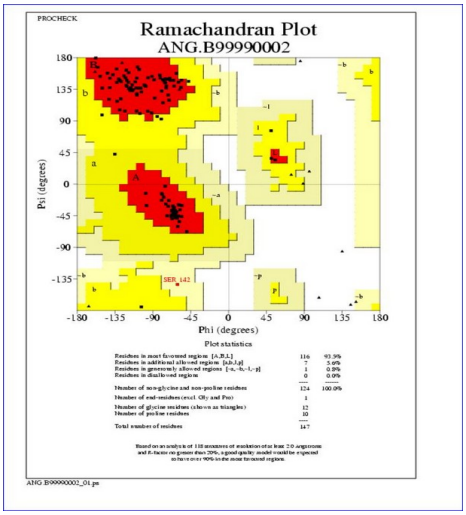
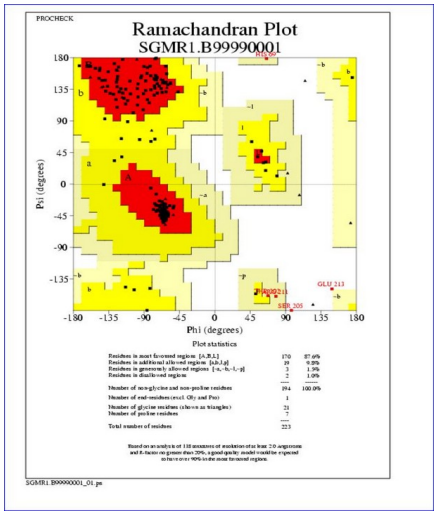
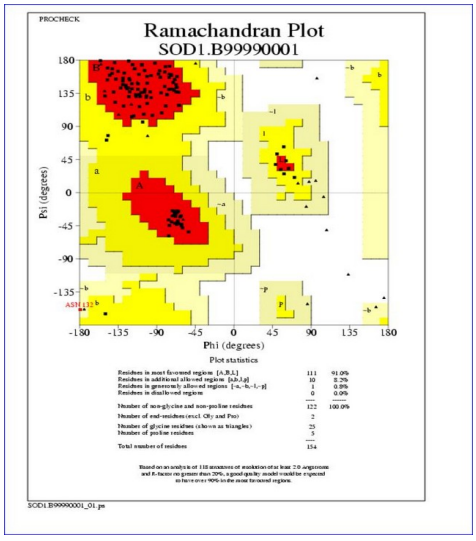
Ramachandran plot:



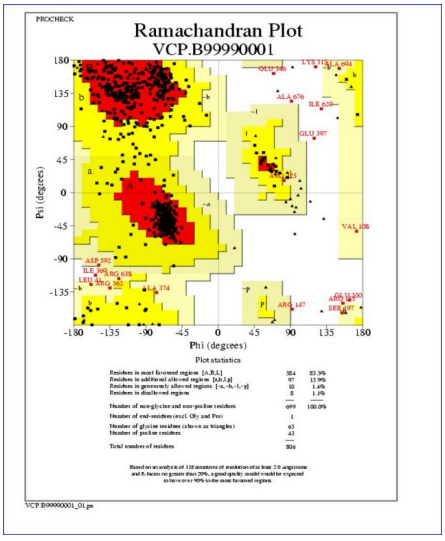
Angiogenin



SGMR1_HUMAN



SOD1



VCP

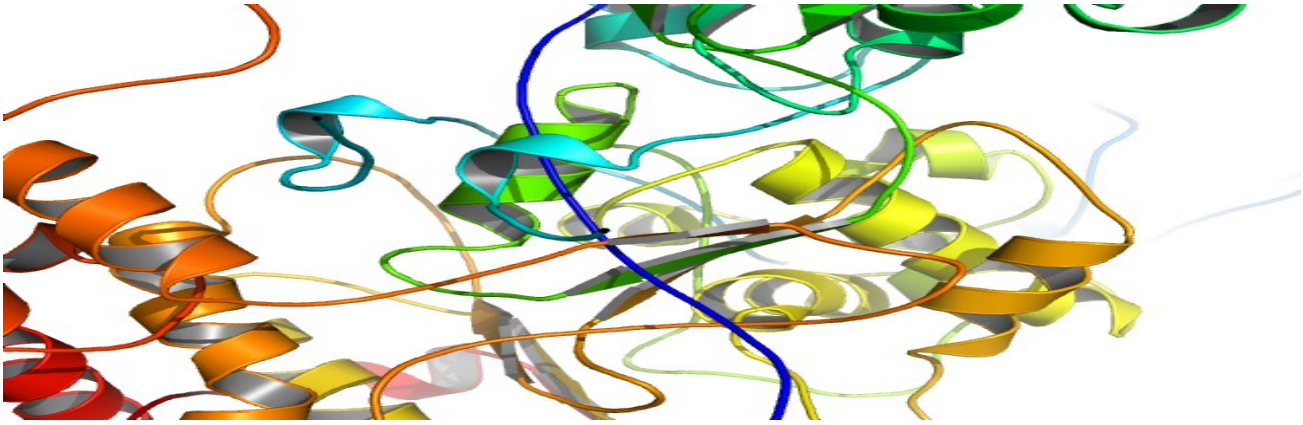


Fig 3.4: VCP gene

Table 3.3: Ramachandran Plot scores generated for 3D structures

ANG.B99990001	110(88.7%)	12(9.7%)	1(0.8%)	1(0.8%)
ANG.B99990002	**116(93.9%)**	**7(5.6%)**	**1(0.8%)**	**0(0.0%)**
ANG.B99990003	112(90.3%)	8(6.5%)	3(2.4%)	1(0.8%)
ANG.B99990004	110(88.7%)	12(9.7%)	2(1.6%)	0(0.0%)
ANG.B99990005	112(90.3%)	11(8.9%)	1(0.8%)	0(0.0%)
SGMR1.B99990001	**170(87.6%)**	**19(9.8%)**	**3(1.5%)**	**2(1.0%)**
SGMR1.B99990002	168(86.6%)	21(10.8%)	4(2.1%)	1(0.5%)
SGMR1.B99990003	167(86.1%)	20(10.3%)	7(3.6%)	0(0.0%)
SGMR1.B99990004	164(84.9%)	22(11.3%)	5(2.6%)	3(1.5%)
SGMR1.B99990005	167(86.1%)	18(9.3%)	7(3.6%)	2(1.0%)
SOD1.B99990001	**111(91.0%)**	**10(8.2%)**	**1(0.8%)**	**0(0.0%)**
SOD1.B99990002	107(87.7%)	12(9.8%)	2(1.6%)	1(0.8%)
SOD1.B99990003	106(86.9%)	13(10.7%)	3(2.5%)	0(0.0%)
SOD1.B99990004	107(87.7%)	15(12.3%)	0(0.0%)	0(0.0%)
SOD1.B99990005	105(86.1%)	16(13.1%)	1(0.8%)	0(0.0%)
VCP.B99990001	**584(83.5%)**	**97(13.9%)**	**10(1.4%)**	**8(1.1%)**
VCP.B99990002	581(83.1%)	87(12.4%)	23(3.3%)	8(1.1%)
VCP.B99990003	582(83.3%)	91(13.0%)	19(2.7%)	7(1.0%)
VCP.B99990004	575(82.3%)	95(13.6%)	19(2.7%)	10(1.4%)
VCP.B99990005	570(81.5%)	95(13.6%)	22(3.1%)	12(1.7%)

Table 3.4: Medicinal Herbs and their Chemical Compounds

Sl No.	Herbs Scientific Name	Herbs Common Name	Compounds of the Herb
1	Piper Methysticum	KAVA	Desmethoxyyangonin, Yangonin, Kavain, Methysticin
2	Hypericum perforatum	St. John's Wort	Gallocatechol, Isoquercetin, hyperforin
3	Passiflora caerulea	Blue passion flower	7-hydroxycoumarin, Scopoletin
4	Eschscholzia californica	California Poppy	Allocryptopine, Cryptopine

C. Identifying novel ligands targeting the gene receptor.

Medicinal Herbs contain several natural compounds which have healing abilities to the human body and treat the nerves.

Herbs such as St. John's wort [13], California Poppy [16] etc., contain natural compounds that calm the nerves and can help reduce the symptoms inhibited by an ALS patient. This information is collected from online resources and the drug compounds are retrieved from PUBCHEM [10] database. The 3D structure of the compounds are retrieved from PUBCHEM, the 3D structure file of novel drug ligands is downloaded and converted to PDB format using CHIMERA software tool.

D. Computer Aided Drug Designing.

Each established 3D structure of gene receptor is docked with each of the compounds. Docking [11] is carried out which binds two individual structures and form a single structure, In this paper we have docked receptor protein (large molecule) which was developed using homology modelling with drug ligands (small molecule) the chemical structure of the drug ligands were retrieved from PUBCHEM [10].

The selected ligands are docked with the gene receptors using HEX server [11]. The docked Protein compounds are evaluated by observing there docking energy, the number of interactions between the ligand and receptor gene. We infer by checking the compounds are docked properly. The compounds with the least energy and best interactions are selected for ADME studies.

E. ADME SCREENING:

ADME is an acronym for absorption, distribution, metabolism and excretion. MOLINSPIRATION [15] is an web tool which is easy to use, it calculates the molecular properties such as logP, polar surface area, number of hydrogen bond donors, acceptors and prediction of Bioactivity score for the drug targets.

Table 3.5: Principle Description calculated in MOLINSPIRATION module

Compund Name	miLogP (a)	TPSA (b)	natoms (c)	MW (d)	nON	nOHNH	nrotb (e)	volume	nviolations (f)
7-hydroxycoumarin	1.511	50.439	12	162.144	3	1	0	136.605	0
Gallocatechol	1.077	130.602	22	306.27	7	6	1	252.159	1
Isoquercetin	-0.364	210.503	33	464.379	12	8	4	372.206	2
Hyperforin	8.392	71.441	39	536.797	4	1	11	559.152	2
Kavain	2.161	35.539	17	230.263	3	0	3	215.179	0
Methysticin	2.051	54.007	20	274.272	5	0	3	239.108	0
Scopoletin	1.329	59.673	14	192.17	4	1	1	162.15	0

[a] Octanol-water partition coefficient 'LogP'

[b] Total Molecular Polar Surface Area (TPSA)

[c] Number of atoms (natoms)

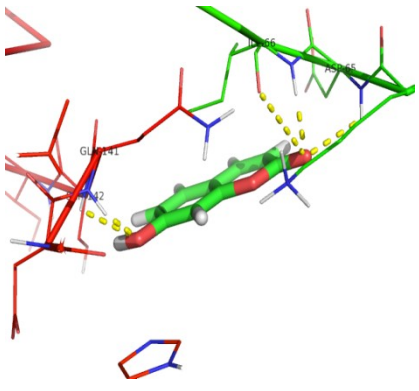
[d] Molecular Weight (MW)

[e] Number of rotatable bonds (nrotb)

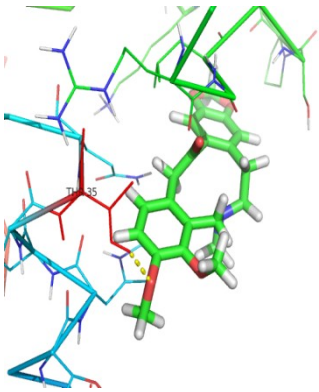
7-hydroxycoumarin, Kavain, Methysticin, Scopoletin are the selected ligands for further ADME screening.

Table 3.6: Drug Ligands that Dock with Receptor Genes

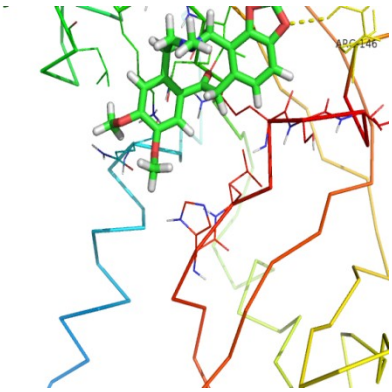
Receptor Gene	Drug compounds
ANG1	7-hydroxycoumarin, Gallocatechol, Isoquercetin, Hyperforin.
SGMR1_HUMAN	Kavain, Gallocatechol, Isoquercetin
superoxide dismutase (SOD1)	7-hydroxycoumarin, Methysticin, Hyperforin, Scopoletin
VCP	NIL



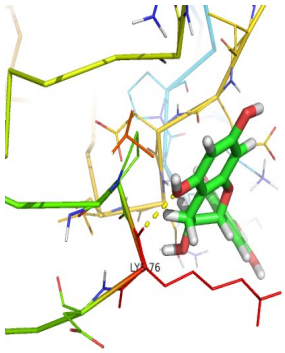
7-hydroxycoumarin-ANG



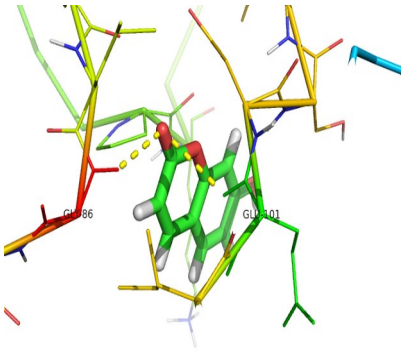
Allocryptopine-ANG



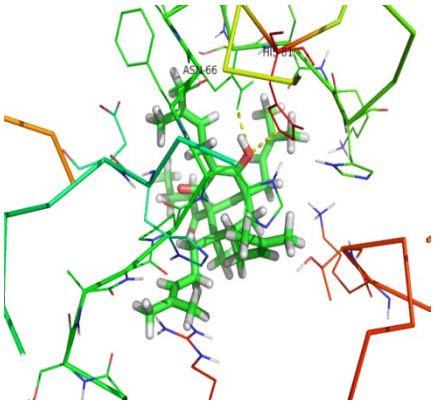
Cryptopine –ANG



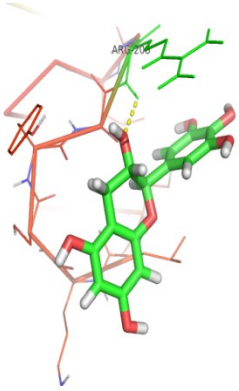
Gallocathechol-SOD1



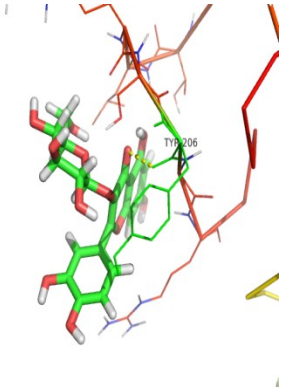
7-hydroxycoumarin-SOD1



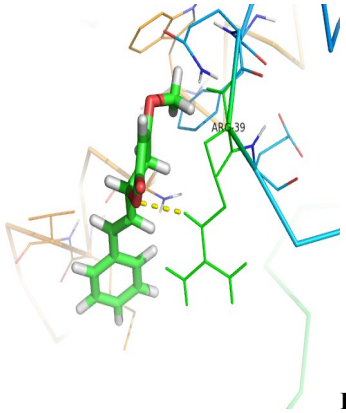
Hyperforin-SOD1



Gallocathecol-SGMR1



Isoquercetin-SGMR1



avain-SGMR1

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Fig: 3.4 Docked Drug ligands to Receptors

Table 3.7: Docked Results

Sl no	Name of the Protein	Compound Name	Energy (kcal/mol)	No. Of Interactions	Interacting amino acids	docking
1	**Angiogenin**	**7-hydroxycoumarin_ANG_2**	**-1.669809e+02**	**1** **1** **2** **1**	**SER142** **GLN141** **ILE66** **ASP 65**	**YES√**
2	Angiogenin	Scopoletin_ANG2	-1.758243e+02	1	LYS-106	YES
3	Angiogenin	desmethoxyyangonin_ANG2	-2.103483e+02	1	CYS-131	YES
4	Angiogenin	Kavain_ANG2	-2.100294e+02	1 1	SER-52 GLN-43*	YES
5	Angiogenin	yangonin_ANG2	-2.311557e+02	1	GLU-91	YES
6	Angiogenin	Methysticin_ANG2	-2.170065e+02	1	GLN-43*	YES
7	**Angiogenin**	**Gallocatechol_ANG2**	**-2.259313e+02**	**1** **1** **1** **1**	**GLN-36** **HIS-32** **LEU-59** **PRO-62**	**YES**
8	**Angiogenin**	**Isoquercetin_ANG2**	**-2.677328e+02**	**1** **1** **1**	**GLU-132** **SER-28** **ARG-29**	**YES**
9	**Angiogenin**	**Hyperforin_ANG2**	**-2.919551e+02**	**1** **1** **1**	**HIS-138** **SER-142** **ILE-66***	**YES**
10	Angiogenin	Allocryptopine_ANG2	-2.582697e+02	1	THR-35	YES
11	Angiogenin	Cryptopine_ANG2	-2.418186e+02	1	ARG-146*	YES
12	SGMR1_HUMAN	7-hydroxycoumarin_SGMR1	-1.726919e+02	-	-	NO
13	SGMR1_HUMAN	desmethoxyyangonin_SGMR1	-2.305799e+02	-	-	NO
14	SGMR1_HUMAN	Scopoletin_SGMR1	-1.837375e+02	-	-	NO
15	SGMR1_HUMAN	yangonin_SGMR1	-2.463440e+02	-	-	NO
16	**SGMR1_HUMAN**	**Kavain_SGMR1**	**-2.209939e+02**	**1**	**ARG-39**	**YES**
17	SGMR1_HUMAN	Methysticin_SGMR1	-2.395386e+02	-	-	NO
18	**SGMR1_HUMAN**	**Gallocatechol_SGMR1**	**-2.502860e+02**	**1**	**ARG-208**	**YES**

CONCLUSIONS

As per the results of virtual screening 7-hydroxycoumarin, Kavain, Methysticin, Scopoletin with least docking scores and most interactions were selected for further ADME studies. It was seen in studies that *7-hydroxycoumarin* ligand interacted with ANG1 gene. *Kavain* ligand interacted with SGMR1_HUMAN gene, *7-hydroxycoumarin, Methysticin, Scopoletin* ligands interacted with SOD1gene. We conclude that these ligands could be selected as novel ligands for Amyotrophic Lateral Sclerosis *in-sillico*. Further these ligands can be tested for their efficacy in treating Amyotrophic lateral Sclerosis Disorder by *in-vitro* receptor ligand binding assay studies.

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