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RESEARCH ARTICLE

COMPARATIVE ANALYSIS BETWEEN THE PROTEIN MCP-1 AND NEUROTROPHINS INVOLVED IN ALOPECIA AREATA

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ABSTRACT

Alopecia Areata is an autoimmune disease. It is an unpredictable disorder which can occur to a person of any age, it is commonly found in children and it also occurs in animals besides human beings. The two proteins are found to be involved in Alopecia Areata i.e. PDB ID 1SG1 of Neurotrophins family and PDB ID 1DOK of MCP-1 protein. Neurotrophins are the group of proteins, experimental data suggests that they may regulate cyclic activity of hair follicle and MCP-1 proteins are found to be present around the area having Alopecia Areata, as known from literature it increase the rate of hair follicle shedding by promoting more T-lymphocytes towards the point of growth of hair follicle and a comparison is being done between the two PDB ID 1SG1 of Neurotrophins family and 1DOK of protein MCP-1, along with the comparison between the various zinc analogs on basis of 99%, 95%, 90% similarity, for best docking results with both the protein's PDB ID's, for predicting a zinc analog which can be identified under the category of becoming a potential drug candidate in future. The best moldock scores were for zinc analog ZINC ID 22052012 for Biotin is -183.662 Kcal/mol, ZINC ID 13475552 for Capsaicin is -190.116 Kcal/mol and ZINC ID 09222049 for Epicatechin-3-gallate is -196.679 Kcal/mol. The two parameters for toxicity properties i.e. Oral rat LD50 and Mutagenicity were calculated, the Oral rat LD50 values for Biotin, Capsaicin and Epicatechin-3-gallate were 895.76, 3864.96 and 2178.79 mg/kg and the Mutagenicity values for Biotin, Capsaicin and Epicatechin-3-gallate were 0.17, 0.19 and 0.77 respectively. Thus finally the selected Zinc Analogs ZINC ID 22052012, ZINC ID 13475552, ZINC ID 09222049 is for three of the natural ligands i.e. Biotin, Capsaicin, Epicatechin-3-gallate respectively.

INTRODUCTION

Alopecia Areata is an unpredictable, recurring, non scarring, chronic inflammatory disorder of the hair and nails. Any hair-bearing area may be affected. The cause of Alopecia Areata is unknown although there is an overwhelming body of evidence implicating an autoimmune response mediated by T cells to an unknown hair-associated antigen (Jerry Shapiro and Shabnam Madani 2001). Alopecia Areata often results in very significant psychological distress, as the condition often afflicts children and teenagers (Amos Gilhar *et al.,* 2007). The prevalence rates of Alopecia Areata is 0.1% in the world population (Seok-Beom Hong *et al.,* 2005). Alopecia Areata can cause sudden and widespread scalp hair loss (Alopecia Areata totalis), or complete body hair loss (Alopecia Areata universalis), with resulting negative effects on the quality of life, psychosocial parameters, and psychoemotional symptoms (Amos Gilhar *et al.,* 2007). The primary symptom of Alopecia Areata is hair loss in small, round patches about the size of a quarter with smooth, hairless scalp under the patches. In many cases, the disease does not extend beyond a few bare patches. In some people, hair loss is more extensive. Although uncommon, the disease can progress to cause total loss of hair on the head or complete loss of hair on the head, face, and body. People with Alopecia Areata are generally otherwise in good health. The emotional aspects of living with hair loss can be challenging in a culture that views hair as a sign of youth and good health. Many people cope by learning as much as they can about the disease; speaking with others who are facing the same problem; and, if necessary, seeking counseling to help build a positive self-image. Most people with Alopecia Areata are well-adjusted, contented people living full lives. Hair loss is associated with a primarily CD41 perifollicular lymphocytic infiltrate, along with expression of both markers for immune system and ICAM-1 on the follicular epithelium. Alopecia Areata has HLA-(marker for immune system) associations, autoreactive T cells are present in the scalp infiltrate, but are not specific for Alopecia Areata. Circulating autoantibodies to follicular structures are present, but they are also reported in normal controls, and their role in disease pathogenesis is uncertain (Amos Gilhar *et al.,* 1998).

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MATERIALS AND METHODS

Retrieval of the target protein

A comparative study was done between two proteins involved in Alopecia areata i.e. PDB ID 1DOK of MCP-1 protein and PDB ID 1SG1 of Neurotrophins family.

Protein structures obtained from RCSB, PDB ID 1DOK of protein MCP-1 has 77residues of amino acids and PDB ID 1SG1 of Neurotrophins family has 120amino acids in length.The 3-dimensional structures of the proteins were studied with the help of visualization software RASMOL (http://www.rasmol.org/), the number of chains, groups, atoms and bonds along with the classification of the proteins were detailed out.

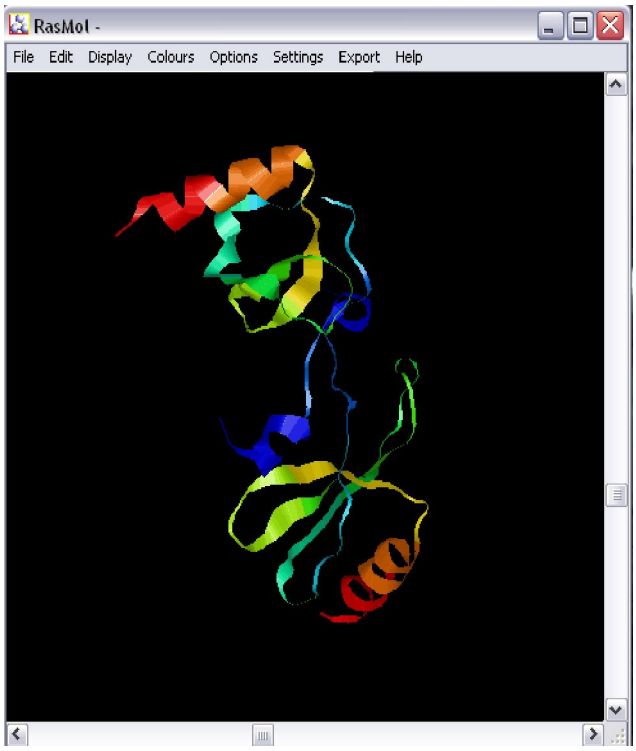


Fig.1. Image of PDB ID 1DOK of protein MCP-1.

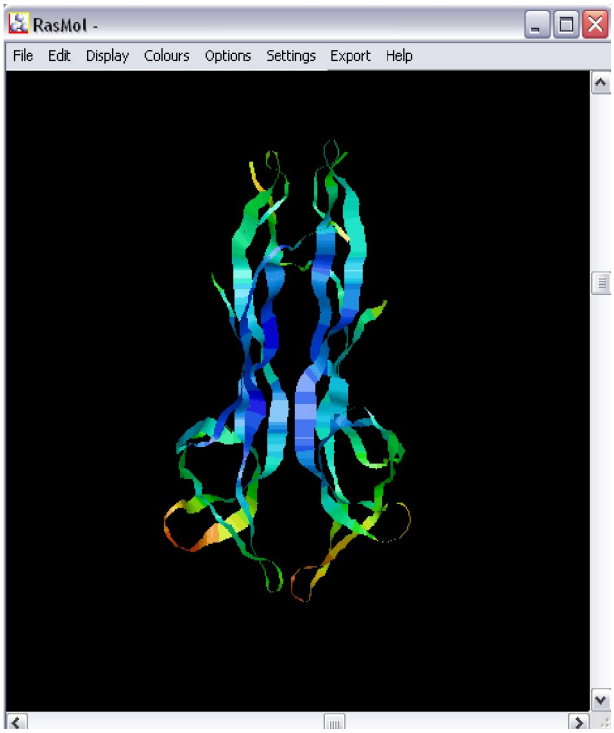
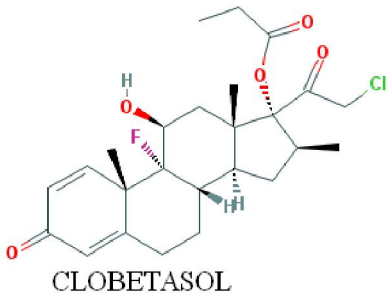


Fig.2. Image of PDB ID 1SG1 of Neurotrophins protein family

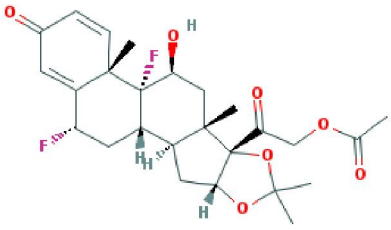
Virtual screening for inhibitors of the PDB ID 1DOK of protein MCP-1 and PDB ID 1SG1 of Neurotrophins family

To assess the virtual screening program, first of all the docking accuracy of MOLEGRO VIRTUAL DOCKER (http://www.clcbio.com/products/molegro-virtual-docker/) was evaluated for PDB ID 1DOK of protein MCP-1 and PDB ID

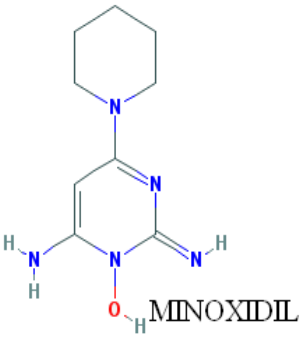
1SG1 of Neurotrophins family by docking the compounds extracted from PUBCHEM (http://www.ncbi.nlm.nih.gov/pccompound) and Drug Bank (http://www.drugbank.ca/), structures stored within pubchem and drug bank compounds are pre-clustered and cross-referenced by identity and similarity groups. The inhibitors were used



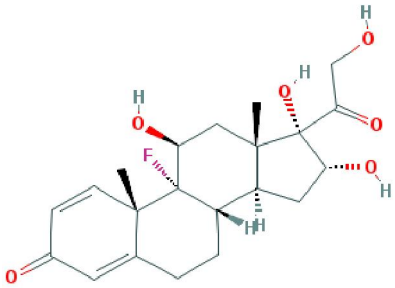
CLOBETASOL



FLUOCOCINONIDE



MINOXIDIL



TRIAMCONOLONE

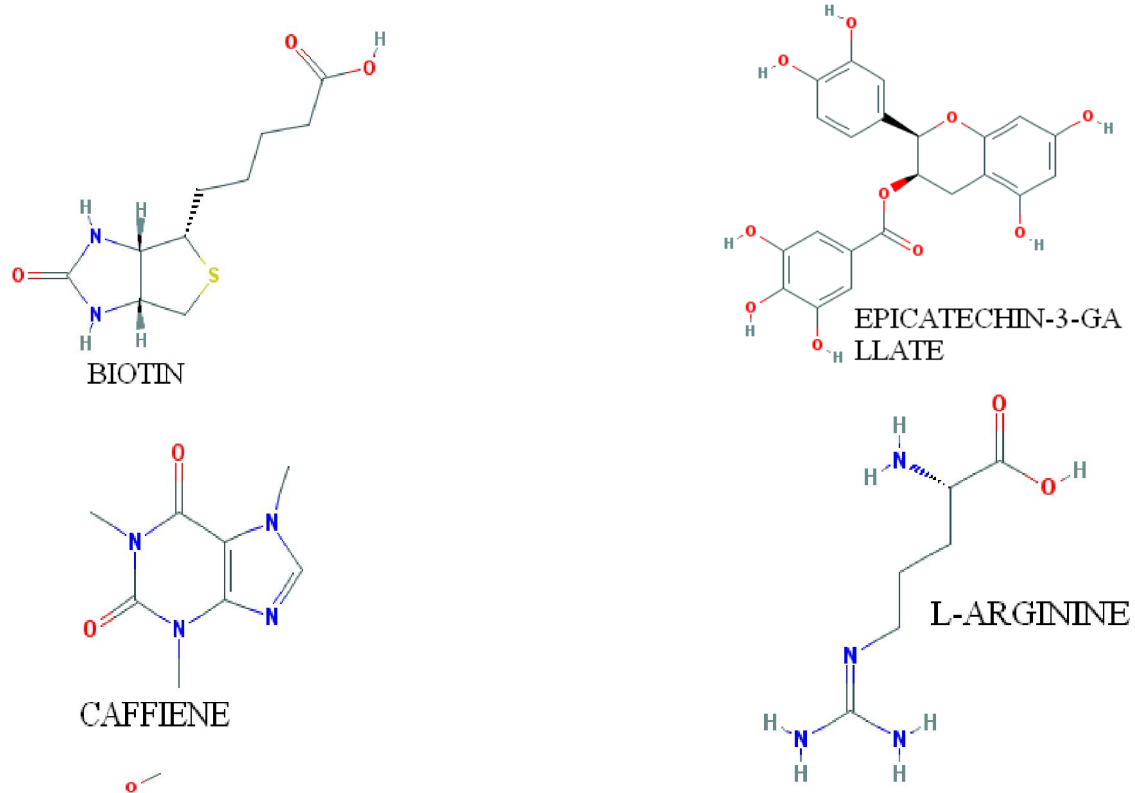


Fig. 3. Structure of the drugs and the natural compounds

(CLOBETASOL, FLUOCINONIDE, MINOXIDIL), compound still in clinical trials (TRIAMCINOLONE) and natural ligands (BIOTIN, CAFFEINE, CAPSAICIN, CURCUMIN, CYPROTERONE, EPICATECHIN-3-GALLATE, L-ARGININE) selected into the ligand binding site detected by POCKET FINDER (http://www.modelling.leeds.xac.uk/pocketfinder/help.html).

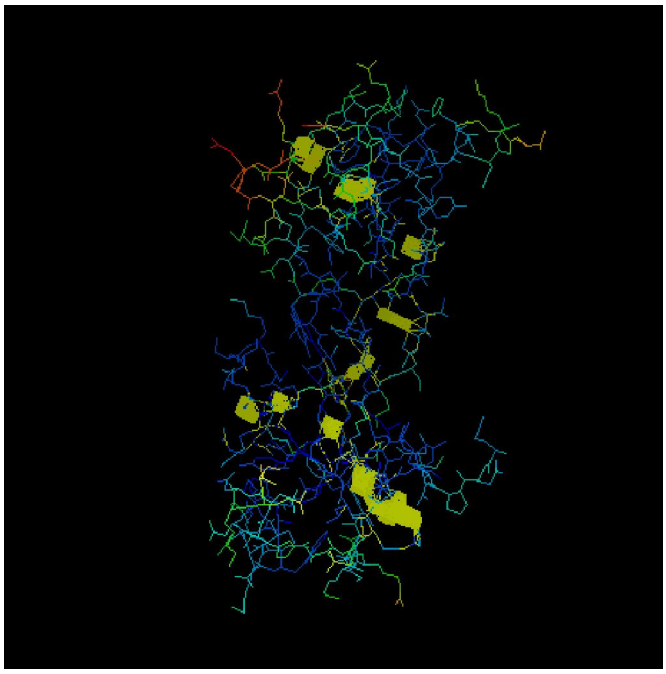


Fig. 4. Ligand binding site in protein MCP-1 PDB ID 1DOK

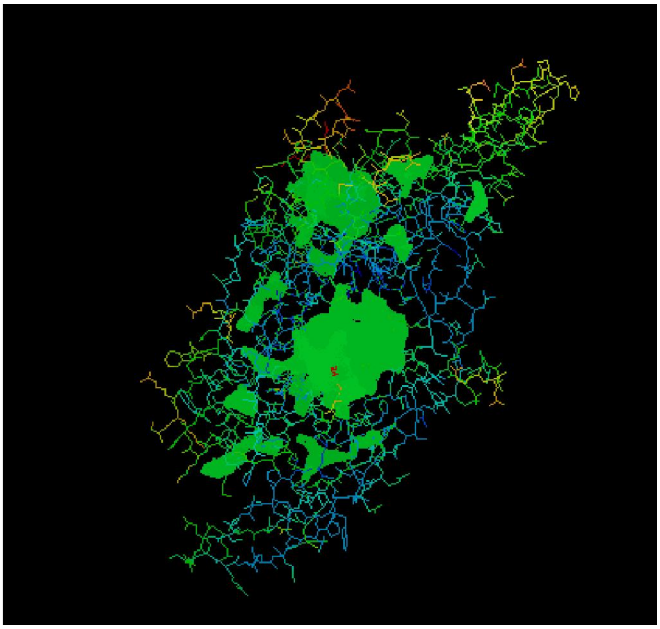


Fig. 5. Ligand binding site in Neurotrophins protein family PDB ID 1SG1

Three ligand binding sites were found in PDB ID 1DOK of protein MCP-1, out of which 1 was selected for further docking process, the selection was done on basis of largest volume of the cavity. Volume of the selected ligand binding site was 50.176 cubic angstoms and five binding sites were found in PDB ID 1SG1 of Neurotrophins family, out of which 2 ligand binding sites were selected for further docking, volume of the selected ligand binding sites were 137.216 cubic angstoms and 62.976 cubic angstoms.Some common amino acids found in Binding sites of PDB ID 1DOK of protein MCP-1 and PDB ID 1SG1 of Neurotrophins family were VAL, CYS, TYR, ILE, LEU, LYS, PHE and THR.MOLEGRO VIRTUAL DOCKER was then used to perform virtual screening on the PDB ID 1DOK and PDB ID 1SG1 of protein MCP-1 and Neurotrophins protein family using a screening set from the ZINC DATABASE (http://zinc.docking.org/) that contained 50 molecules for each of the inhibitors, the compounds in clinical trials and the natural ligands. Then the selection of the top-ranking 5% of compounds for PDB ID 1DOK and PDB ID 1SG1 of protein MCP-1 and Neurotrophins protein family after further analysis of the hit rate after Molegro virtual docker screening was done. Here, atomic environments were used to represent the two-dimensional compound structure for measurements of compound similarities and the protein-ligand interactions were used for the identification of docked positions.

Preparations of the target protein and screening set

Compound set were prepared from the ZINC DATABASE in January 2013 based on two criteria: firstly, compounds were selected according to their (99%, 95%, 90%) similarity with the drugs, compounds for clinical trials and natural ligands selected and secondly, excluding compounds with multiple components. Eventually a set of structures was obtained that consisted of 700 compounds. To reduce the complexity and running time of the computational program, the compounds were separated into number of different sets according to the extent of similarity on basis of percentage. Molegro Virtual Docker then docked each compound in the screening set against this binding cavity and ranked each compound by the docked energy of the docked conformation. The docked conformation of the selected compounds from ZINC DATABASE with the lowest scoring value was compared with docking values of the drugs, clinical trial compounds and the natural ligands selected and along with this the molecular recognition of both the protein was also investigated to determine the constraints of the ligand during the virtual screening.

RESULTS ANALYSIS OF DOCKING

Comparative analysis between pdb ID 1DOK of MCP-1 and pdb ID 1SG1 of neurotrophins protein family

Zinc analogs with lower docking scores were further compared for selection of compounds liable to be a potential drug candidate in future i.e. the comparison was done between the moldock scores of zinc analogs for both the receptors i.e. PDB ID 1DOK of protein MCP-1 and PDB ID 1SG1 of Neurotrophins family. Finally the 3 analogs were selected on basis of lower moldock score and also PDB ID 1SG1 of Neurotrophins family was considered to be the better receptor than PDB ID 1DOK of protein MCP-1, on basis of the comparative study done.

Toxicity Prediction

Extent of the toxic effects of the drugs and the natural ligands were predicted using the software T.E.S.T (Toxicity Estimation Software Tool) (http://www.epa.gov/nrmrl/std/qsar/qsar.html). The two properties of the drugs and the natural compounds were calculated i.e. Predicted Oral rat LD50 and the mutagenicity properties.

DISCUSSION

Recent progress in biology has brought about with it many protein structures for virtual screening as drug targets. However, without a previously validated target site on the targeted protein as a reference point, the number of lead candidates obtained from this type of screening is very large. Cellular toxicity further complicates biological activity assays as well. Here, in an attempt to devise a less resource-demanding screening process, we have focused on computational approaches that are solely based on the structures of a designated region of the target protein. Then, we performed virtual screening on a set of medical compounds because we recognized that using medical compounds could potentially minimize cellular toxicity. The drugs which are already being used as a remedy are having much higher toxic effects, the common side effects of these compounds are very serious like change in mental state, stammer while walking and in speech, rapid heart rate, blurred vision, muscle spasm, numbness, painful and swollen gums, etc. These adverse side effects is one of the main reasons which led us to work in for a more effective potential drug candidate with much lesser side effects, along with the comparison of the receptor with which it will result the best. From among the drugs, compounds in clinical trials and the natural ligands, the Zinc Analogs for natural ligands have comparatively shown much better results for docking with much lesser energy, therefore much higher stabilization of the receptor –ligand complex.

Table 1. Comparative analysis between PDB ID 1DOK of protein MCP-1 and PDB ID 1SG1 of Neurotrophins family

S.No	Inhibitors Name	Zinc Analogs (Id)	1DOK MolDock Score (-Kcal/mol)	1SGI MolDock Score (-Kcal/mol)	Compounds selected
1.	Clobetasol	ZINC56956114	-50.00	-125.729	
2.	Fluococinonide	ZINC36371106	-89.6239	-157.601	
3.	Biotin	ZINC22052012	-142.04	-183.662	ZINC22052012
4.	Caffeine	ZINC01069064	-70.907	-135.044	
5.	Capsaicin	ZINC13475552	-107.726	-190.116	ZINC13475552
6.	Curcumin	ZINC00899824	-109.086	-160.813	
7.	Cyproterone	ZINC21983606	-50.00	-147.22	
8.	Epicatechin-3-gallate	ZINC09222049	-125.179	-196.679	ZINC09222049
9.	L-arginine	ZINC54965112	-83.2949	-110.002	

ANALYSIS OF SELECTED COMPOUNDS

COMPOUND 1

- **INHIBITOR :-** Biotin
- **ZINC ID:-** ZINC ID 22052012
- **POPULAR NAME:-** N-Biotinylcaproylaminocaproic Acid

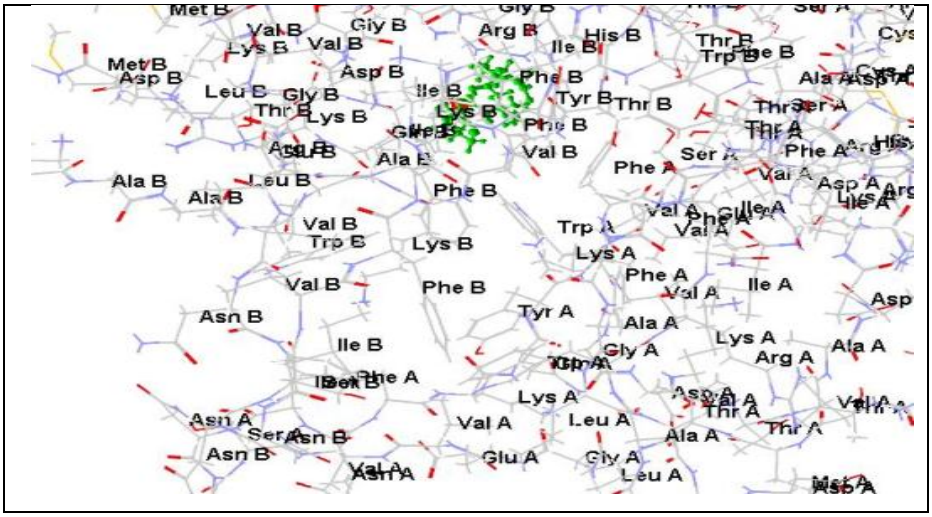


Fig. 6. Docking image of 1SG1 of Neurotrophins family and ZINC ID 22052012Moldock score -183.662Kcal/molAmino Acid residues at ligand binding site are Ile B, Lys B, Phe B, Val B

COMPOUND 2

- **INHIBITOR :-** Capsaicin
- **ZINC ID:-** ZINC ID 13475552
- **POPULAR NAME:-** Arvanil

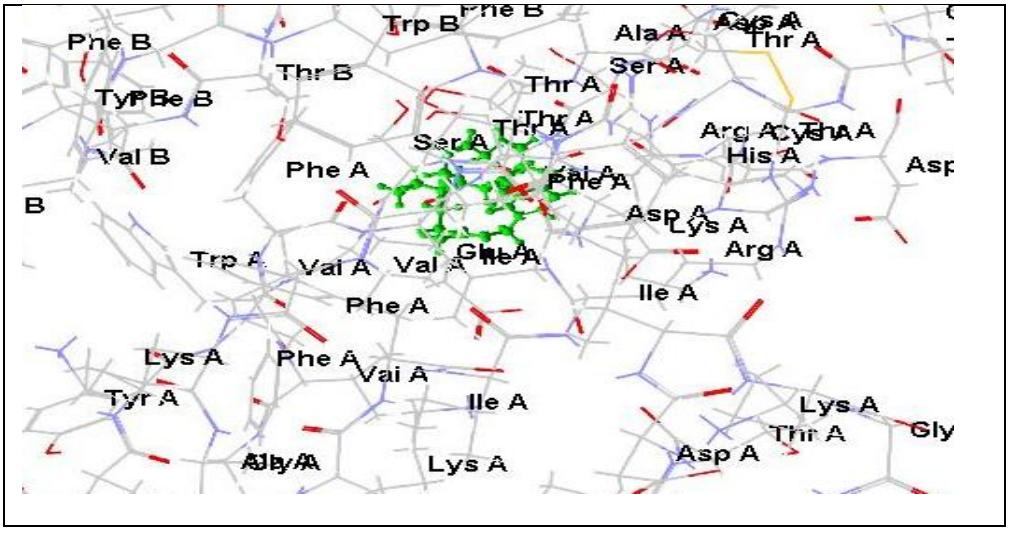


Fig. 7. Docking image of 1SG1 of Neurotrophins family and ZINC ID 13475552Moldock score -190.116 Kcal/molAmino Acid residues at ligand binding site are Glu A, Ile A, Phe A, Ser A, Thr A, Val A

COMPOUND 3

- **INHIBITOR :-** Epicatechin-3-gallate
- **ZINC ID:-** ZINC ID 09222049
- **POPULAR NAME:-** Catechin pentaacetate

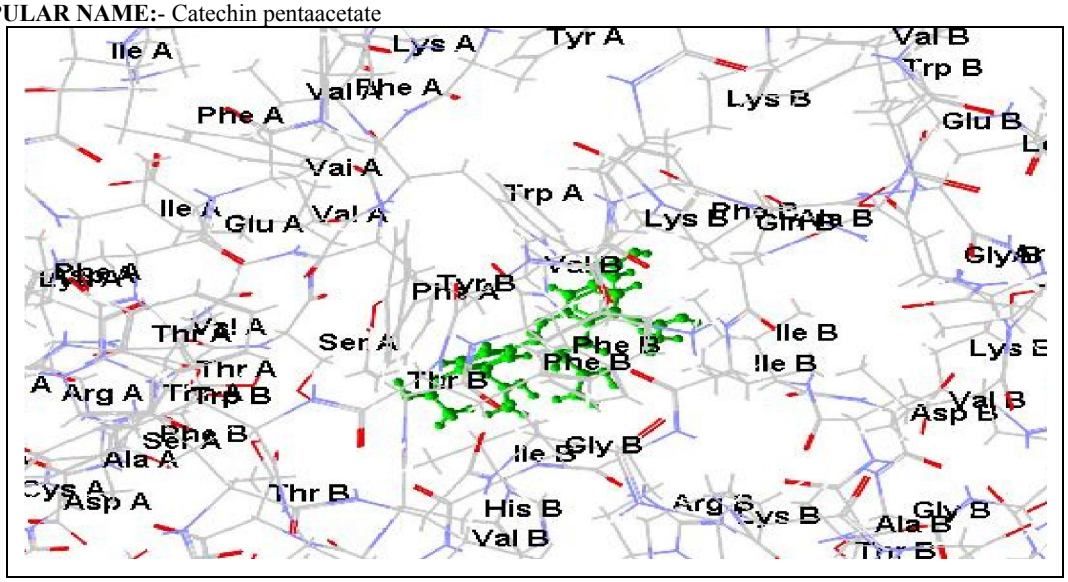
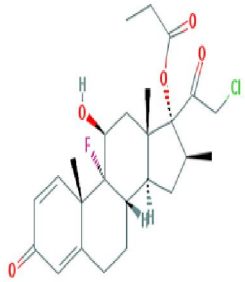
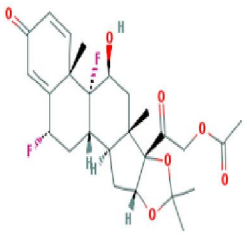
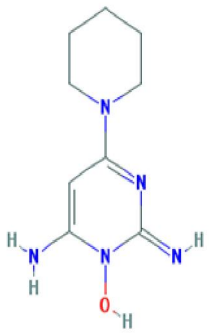
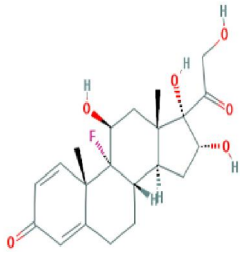
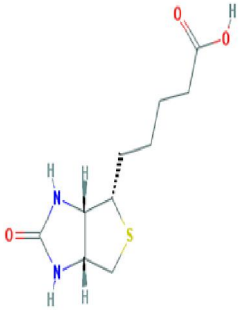
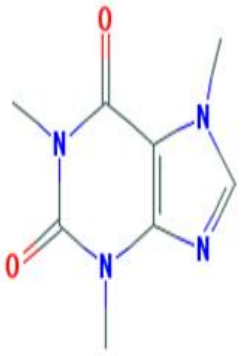
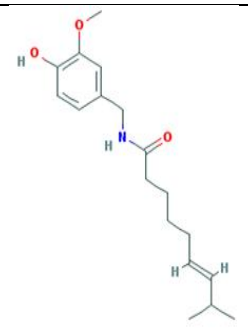
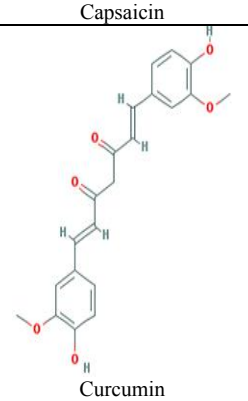


Fig. 8. Docking image of 1SG1 of Neurotrophins family and ZINC ID 09222049Moldock score -196.679 Kcal/molAmino Acid residues at ligand binding site are Phe B, Thr A, Val B

Table 2. Predicted oral rat LD50 for drugs and natural ligands

S.No	Molecule	Prediction results			Individual predictions	
		End point	Experimental value	Predicted value	Method	Predicted value - Log10 (mol/kg)
1.	 Clobetasol	Oral rat LD_{50} - Log10(mol/kg)	N/A	2.89	Hierarchical clustering	2.91
					FDA	3.25
		Oral rat LD_{50} mg/kg	N/A	532.23	Nearest neighbor	2.50
2.	 Fluocinonicide	Oral rat LD_{50} - Log10(mol/kg)	4.55	3.18	Hierarchical clustering	4.49
		Oral rat LD_{50} mg/kg	14.00	324.94	FDA	2.55
					Nearest neighbor	2.50
3.	 Minoxidil	Oral rat LD_{50} - Log10(mol/kg)	N/A	2.59	Hierarchical clustering	N/A
		Oral rat LD_{50} mg/kg	N/A	533.24	FDA	2.60
					Nearest neighbor	2.59

4.	 Triamcinolone	Oral rat LD_{50} - Log10(mol/kg)	N/A	N/A	Hierarchical clustering	N/A
		Oral rat LD_{50} mg/kg	N/A	N/A	FDA	N/A
					Nearest neighbor	3.37
5.	 Biotin	Oral rat LD_{50} - Log10(mol/kg)	N/A	2.44	Hierarchical clustering	2.87
		Oral rat LD_{50} mg/kg	N/A	895.76	FDA	2.54
					Nearest neighbor	1.90
6.	 Caffiene	Oral rat LD_{50} - Log10(mol/kg)	3.00	2.84	Hierarchical clustering	2.93
		Oral rat LD_{50} mg/kg	192.00	278.14	FDA	2.65
					Nearest neighbor	2.95
7.	 Capsaicin	Oral rat LD_{50} - Log10(mol/kg)	N/A	1.90	Hierarchical clustering	1.82
		Oral rat LD_{50} mg/kg	N/A	3864.96	FDA	1.92
					Nearest neighbor	1.95
8.	 Curcumin	Oral rat LD_{50} - Log10(mol/kg)	N/A	2.56	Hierarchical clustering	2.63
		Oral rat LD_{50} mg/kg	N/A	1015.00	FDA	2.45
					Nearest neighbor	2.60

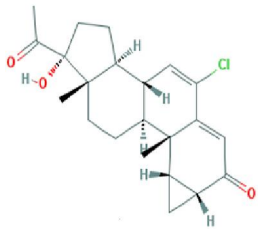
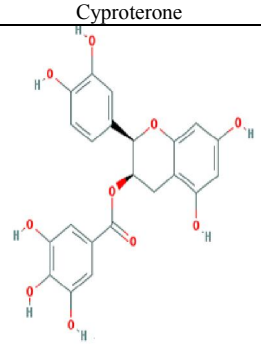
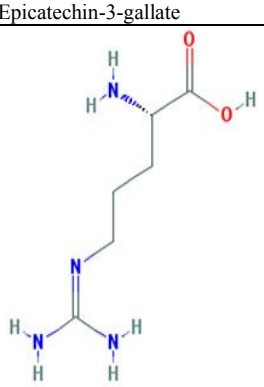
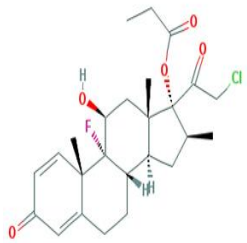
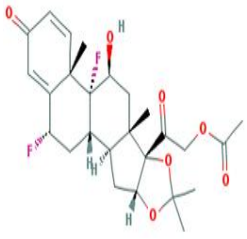
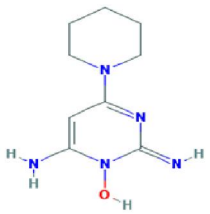
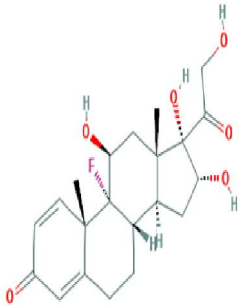
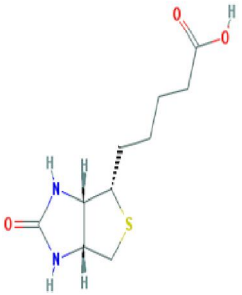
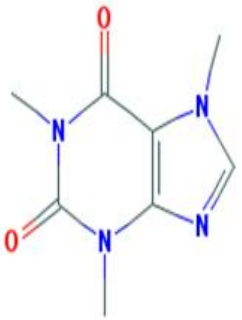
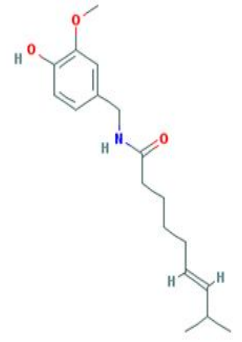
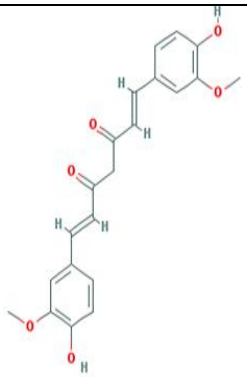
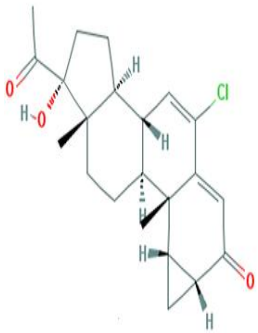
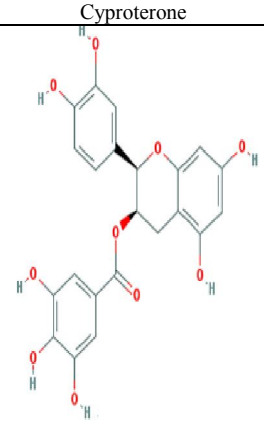
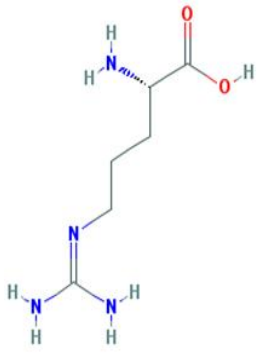
9.	 Cyproterone	Oral rat LD_{50} - Log10(mol/kg)	N/A	3.09	Hierarchical clustering	N/A
		Oral rat LD_{50} mg/kg	N/A	303.86	FDA	3.77
					Nearest neighbor	2.41
10.	 Epicatechin-3-gallate	Oral rat LD_{50} - Log10(mol/kg)	N/A	2.31	Hierarchical clustering	2.41
		Oral rat LD_{50} mg/kg	N/A	2178.79	FDA	2.27
					Nearest neighbor	2.25
11.	 L-arginine	Oral rat LD_{50} - Log10(mol/kg)	N/A	2.21	Hierarchical clustering	1.77
		Oral rat LD_{50} mg/kg	N/A	1077.86	FDA	1.57
					Nearest neighbor	3.28

Table 3. Mutagenicity properties for drugs and natural ligands

S.No	Molecule	Prediction results			Individual predictions	
		End point	Experimental value	Predicted value	Method	Predicted value -Log10 (mol/kg)
1.	 Clobetasol	Mutagenicity value	N/A	0.04	Hierarchical clustering	0.32
		Mutagenicity result	N/A	Mutagenicity Negative	FDA	-0.20
					Nearest neighbor	0.00
2.	 Fluocinonicide	Mutagenicity value	N/A	0.08	Hierarchical clustering	0.08
		Mutagenicity result	N/A	Mutagenicity Negative	FDA	0.16
					Nearest neighbor	0.00

3.	 Minoxidil	Mutagenicity value	N/A	0.51	Hierarchical clustering	0.71
		Mutagenicity result	N/A	Mutagenicity Positive	FDA	0.51
					Nearest neighbor	0.67
4.	 Triamcinolone	Mutagenicity value	N/A	N/A	Hierarchical clustering	N/A
		Mutagenicity result	N/A	N/A	FDA	N/A
					Nearest neighbor	0.33
5.	 Biotin	Mutagenicity value	0.00	0.17	Hierarchical clustering	0.13
		Mutagenicity result	Mutagenicity Negative	Mutagenicity Negative	FDA	0.37
					Nearest neighbor	0.00
6.	 Caffiene	Mutagenicity value	0.00	0.11	Hierarchical clustering	0.05
		Mutagenicity result	Mutagenicity Negative	Mutagenicity Negative	FDA	-0.05
					Nearest neighbor	0.33
7.	 Capsaicin	Mutagenicity value	N/A	0.19	Hierarchical clustering	0.22
		Mutagenicity result	N/A	Mutagenicity Negative	FDA	0.02
					Nearest neighbor	0.33

8.	 Curcumin	Mutagenicity value	0.00	0.09	Hierarchical clustering	0.13
		Mutagenicity result	Mutagenicity Negative	Mutagenicity Negative	FDA	0.12
					Nearest neighbor	0.00
9.	 Cyproterone	Mutagenicity value	N/A	0.09	Hierarchical clustering	0.14
		Mutagenicity result	N/A	Mutagenicity Negative	FDA	0.13
					Nearest neighbor	0.00
10.	 Epicatechin-gallate	Mutagenicity value	N/A	0.77	Hierarchical clustering	0.79
		Mutagenicity result	N/A	Mutagenicity Positive	FDA	0.51
					Nearest neighbor	1.00
11.	 L-arginine	Mutagenicity value	N/A	0.37	Hierarchical clustering	0.25
		Mutagenicity result	N/A	Mutagenicity Negative	FDA	0.18
					Nearest neighbor	0.67

The Moldock scores of zinc analog ZINC ID 22052012 for Biotin is -183.662 Kcal/mol, ZINC ID 13475552 for Capsaicin is -190.116 Kcal/mol and ZINC ID 09222049 for Epicatechin-3-gallate is -196.679 Kcal/mol. The two parameters for toxicity properties i.e. Oral rat LD50 and Mutagenicity were calculated, the Oral rat LD50 values for Biotin, Capsaicin and Epicatechin-3- gallate were 895.76, 3864.96 and 2178.79 mg/kg and the Mutagenicity values for Biotin, Capsaicin and Epicatechin-3-gallate were 0.17, 0.19 and 0.77 respectively.Thus finally the selected Zinc Analogs ZINC ID 22052012, ZINC ID 13475552, ZINC ID 09222049 is for three of the natural ligands i.e. Biotin, Capsaicin, Epicatechin-3-gallate respectively.

Conclusion

Docking was performed (X=4.12, Y=53.95, Z=17.68) at the binding site of PDB ID 1SG1 of Neurotrophins family and (X=27.14, Y=46.98, Z=21.33) at the binding site of PDB ID 1DOK of protein MCP-1.Among known inhibitors Biotin, Capsaicin, Epicatechin-3-gallate has shown quite good docking scores for PDB ID 1SG1 of Neurotrophins family as well as PDB ID 1DOK of protein MCP-1. Inhibitors of lower docking score were used for similarity search from ZINC database. Obtained molecules were further screened to find more potent inhibitor, after the comparison between the docking results of both the receptor molecules, among all the screened analogsZINC ID 22052012 for Biotin, ZINC ID 13475552 for Capsaicin, ZINC ID 09222049 for Epicatechin-3-gallate have given best docking scores.The docking result of the zinc analogs was found to be better for PDB ID 1SG1 of Neurotropins family as compared to the docking result zinc analogs for PDB ID 1DOK of protein MCP-1. Based on the obtained data these molecules could be used for development of potent inhibitor of PDB ID 1SG1 of Neurotrophins family. Thus the receptor 1SG1 (PDB ID of Neurotrophins family) is considered to be more active participant in Alopecia Areata, therefore it can positively be used for development of potent inhibitor for treatment of Alopecia Areata.

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