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Identification of Bioactive Lead Compound against Central Hypothyroidism – An *Insilico* Approach

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Abstract : Computational approaches are intended to provide system level insight into complex biological processes that underlie serious diseases. In this work, an attempt has been made to utilize *in-silico* docking procedures to study a clinically important and common disease – Central hypothyroidism(CH). Currently, there is a significant burden of thyroid disease around the world; the most crucial part is people are unaware of the condition and mostly women are 5-8 times more likely than men to have thyroid problem. Although several treatment procedures are available *i.e.,* Synthetic and Ayurveda methods; most of these drugs create various side effects and thus forcing the patients to continue the medication throughout their life. Taking this gaps into consideration, this study has focused on several bioactive components from plant that has the ability to treat CH. Through virtual screening and docking procedure, β-glucan, a group of β-D-glucose polysaccharides present in oats *(Avena sativa)* was identified as lead compound. Additionally, it was confirmed from various literature survey that they pose anti-oxidant properties and thus have a unique ability to be used as nutraceutical in treating the symptoms of CH.
Keywords : Ayurveda, Central hypothyroidism, Molecular docking, Nutraceuticals.

Introduction:

Hypothyroidism or under-active thyroid, is a common disorder of endocrine system in which the thyroid gland does not produce enough thyroid hormone[1]. Hypothyroidism is believed to be common health issue in India as it is worldwide[2]. It is known to affect all people especially adult women and children. About 10% of Indian population is affected by hypothyroidism and the critical part is that many are unaware of their condition[3]. In India, hypothyroidism was usually categorized under the cluster of Iodine Deficient Disorders (IDD), which is not the only cause. It is also caused by inappropriate level of Thyroid Stimulating Hormone (TSH) which results in lower production of T3 and T4 hormones which ultimately leads to retardation of physical and mental growth.

The thyroid gland is the only source of thyroid hormone in the body; the process requires iodine and the aminoacid tyrosine. Hypophysiotropicthyrotropin releasing hormone synthesizing neurons resides in hypothalamic paraventricular nucleus (PVN), are the central regulators of hypothalamic-pituitary-thyroid (HPT) axis[4,5,6].The TRH synthesis and release from neurons are primarily under negative feedback regulation by thyroid hormone. When there is inadequate iodine in diet, or absence of negative feedback mechanism, it results in hypothyroidism.

Hypothyroidism is usually classified into two: one is primary hypothyroidism and the other is secondary hypothyroidism or central hypothyroidism(CH). In case of primary hypothyroidism, the level of TSH and T4 are normal whereas in central hypothyroidism there will be oscillations in the level of TSH and T4.

There are various causes of CH which includes mainly inadequate amount of iodine in diet, medications, radiation therapy, genetic mutations[7].These causes leads to symptoms like fatigue, headache, asthenia, edema, weight gain, constipation, generalized lethargy[8,9].To treat central hypothyroidism, it is better to reduce most of these symptoms. Thyroid gland will function appropriately and the production of T3 and T4 will be produced in adequate amount when the symptoms like High cholesterol level, iodine deficiencies are reduced.

The objective of our work is to treat these symptoms. Till date, hypothyroidism has been treated by synthetic and ayurvedic drugs. Synthetic drugs are the supplements of Levothyroxine sodium[10].Ayurvedic treatment uses kanchanaraguggulu and shigrupatrakwatha. Though these medications treat these symptoms they have side effects like improper body weight, changes in menstrual cycle, hairloss, depression, allergic reactions and headache[11].Also in these approaches there are other complications like improper body maintenance, expensive medications and patients are made to take these drugs for their lifetime. An alternative method, Molecular Docking technique has been used in this work to overcome the side effects associated with current treatment. The term molecular docking refers to a key tool in structural molecular biology and computer assisted drug design. It aims at identifying the binding site of the target/protein and predicting the protein-ligand interaction[12].

The molecular cloning and functional expression of the TSH receptor or TR has led to rapid advances in understanding the structure and function of molecule. Knowledge of its genomic structure provides information on the evolutionary origin of TSH receptor as well as on the functional organization of its extracellular domain, which is responsible for ligand binding[13]. TR is a heterodimeric 28 kDa, a glycoprotein hormone released from the anteromedial pituitary and is a regulator of thyroid function[14].Its synthesis is controlled by the hypothalamic neuropeptide. The two peptide subunits, α and β of TSH are non-covalently linked and co-translationally glycosylated[15]. The TSH molecule present in humans consists ofan α-subunit with two oligosaccharide chains and a β-subunit with one oligosaccharide chain. Each subunit contains a terminal sialic acid and a sulphate residue that confer TSH binding and biological activity[16].

In this study we have chosen the bioactive metabolites of plants which has anti-inflammatory, anti-cholesterol and anti-oxidant properties as ligands which are capable of binding with the TSH receptor.

Experiment:

Protein preparation:

The three dimensional crystal structure of Thyrotropin Stimulating Hormone (TSH) receptor was obtained from Protein DataBank(PDB) (http://www.rcsb.org/pdb/). The ID of the target protein in PDB is 3G04.The protein preparation was carried out by the "Protein Preparation "module in the Molegro Virtual Dockertool. In this preparation it assigns missing bonds, flexible torsions, bondorders, charges to the protein structure and make it ready for the docking process.

Ligand preparation:

In this study we have chosen the active metabolites of plants which has anti-inflammatory, anti-cholesterol and anti-oxidant properties as ligands. They are alpha linolenic acid[28],linoleic acid from fish oil[17,18,20], avenanthramide- a, b and c[22,23,24,26],beta-glucan,ferulic acid from oats[23,24],bacopasaponsin-c, bacopaside-1, jujubogenin from neer brahmi[21],punicalagin from pomegranate[27], lauricacid, caprylicacid, capric acid from coconut oil[29,30],turmerone from turmeric[19,25].The structure of these ligands were retrieved from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and Chemspider database (http://www.chemspider.com/).Using the tool Open Babel these compounds were converted into sdf format for using it comfortably in Molegro Virtual Docker. The compounds were then prepared using "Prepare Molecules" module to obtain structures with necessary charges, bonds, flexible torsions and finally the energy minimized structures.

Molecular Docking:

The molecular docking was carried out using Molegro Virtual Docker(MVD) and to get a good potential binding sites 5 cavities were identified using "Detect cavities" module in which the parameter was set to molecular surface with expanded van der Waals and others to default settings. The docking was then carried out which works on the basis of MolDock Simplex Evolution search algorithm. The SE search performs the local search on the poses generated Nelder-Mead search algorithm which was extended to consider the individuals in the population into account. It was set to a maximum of 10 runs and made to constrain the poses to cavity. The maximum iterations were set at 1500 with the maximum population size of 50which is higher than the number of degrees of freedom. The energy threshold was assigned to 100 at each step below which the poses are added to the population and with simple evolution size of maximum 300steps.The energy minimization was carried out after docking process. The bond flexibility of the ligands was fixed while the side-chain flexibility of amino acids present in the binding cavity were assigned with the tolerance of 1.00 and strength of 1.00.The Root-mean-square deviation threshold was set to 1.00 for multiple cluster poses. The Re-ranking scoring function is much better scoring function at determining the best pose even though it is computationally expensive than the MolDock scoring function. The protein-ligand interactions were tested and on the basis of MolDock and Re-rank scores the best interaction is selected.

Result and Discussion:

Treatment of CH symptoms rather than treating the disease is the main objective of the work considering the fact that the patients are forced to continue the medication throughout the life. Initially, ligands screening was done in common for both synthetic and bioactive compounds. Later, considering the vital effect of synthetic compounds, this study was focused on the screening of bioactive compounds. In general, a molecular docking is one of the optimization problems in which the objective is to find the best ligand binding site with least energy. Considering the fact, that there is several docking software – the difference lay in the sampling and the scoring functions employed. In this study, MVD is used because of its ability to automatically find the potential binding sites using cavity detection algorithm. Docking was carried out to obtain protein-ligand interaction with TSH receptor and 15 virtually screened ligand. The results were sorted and analyzed based on the MolDock scores and Re-rank scores and the poses with best scores are displayed in the Table 1.

Table 1: Mol Dock and Re-rank scores of 15 selected ligands against TSH receptor.

S.No	Compound	Mol Dock Score (Kcal/mol)	Re-Rank score (Kcal/mol)	H-Bond (Kcal/mol)
1.	Alpha linolenic acid	-127.049	-97.8058	0
2.	Avenanthramide-a	-130.332	-103.503	-5.77825
3.	Avenanthramide-b	-134.412	-108.356	-6.02508
4.	Avenanthramide-c	-130.812	-114.264	-8.42708
5.	bacopasaponsin-c	-142.465	-91.3405	-21.5983
6.	bacopaside-1	-103.928	-106.643	-12.9075
7.	**β-glucan**	**-196.225**	**-133.231**	**-15.5509**
8.	Capric acid	-93.1483	-75.2662	-2.6243
9.	Caprylic acid	-83.6693	-68.691	-4.14237
10.	Ferulic acid	-93.1258	-76.115	-7.29363
11.	Jujubogenin	-101.639	-41.1831	-3.94729
12.	Linoleic acid	-129.028	-95.6222	-3.14011
13.	Punicalagin	-133.427	-100.43	-4.99535
14.	tumerone	-100.719	-83.811	-1.34727
15.	Lauric acid	-103.316	-82.8252	-4.9834

From the table it is evident that β-glucan has the highest MolDockscore(-196.225),Re-rank score(-133.231) and the interaction score (-189.578Kcal/mol)(Figure 1).Based on the ligand with highest affinity further analysis was carried. The ligand is bound within one of the five cavities predicted (average cavity volume=821.248Å^3).The interaction strength between the ligand and the protein largely depends on the number of H-bonds and the energy required to form the H-bond. From the data obtained, the number of H-bonds pose by β-glucan is 10; whereas the other ligand - bacopaside-1pose 20 H-bond interactions with the protein,however it was neglected due to low MolDock (-99.7849),Re-rank(-25.0897) and interaction(-120.547Kcal/mol) scores indicating a poor potential in interacting with the target protein compared to β-glucan.

Analyzing the binding nature of the β-glucan with the target protein it was observed amino acid *viz.*, Gly-42,Gln-39,Glu-83,Lys-42,Gly-41, Ser-168,Asn-170 andLys-166.The interaction between O(8) and Gln-39 is having the minimal distance of 2.09Å with 1.81 Kcal/mol(Figure-2). The total E-pair energy is -192.72 Kcal/mol which is the energy pairwise steric and H-bond energy between ligand and receptor atom. The E-intra energy is -18.5633 Kcal/mol which is internal energy between ligand atoms. From the available interaction data, it is clearly evident that β-glucan has the best binding affinity towards protein with least energy than other ligands.

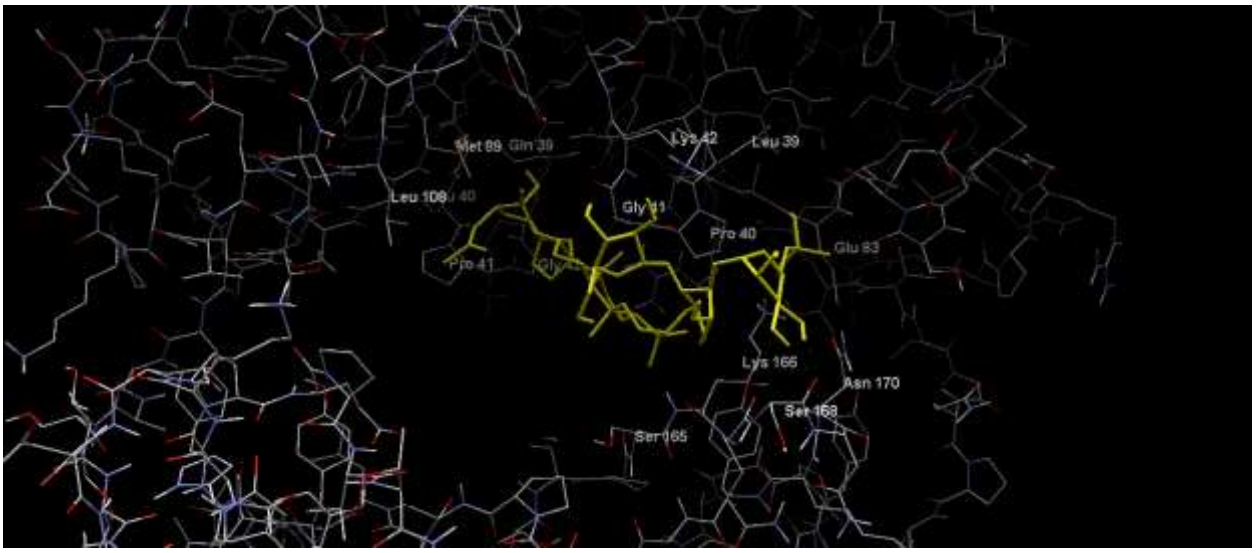

Figure 1:Docking view of ligand β-glucanand TSH receptor.

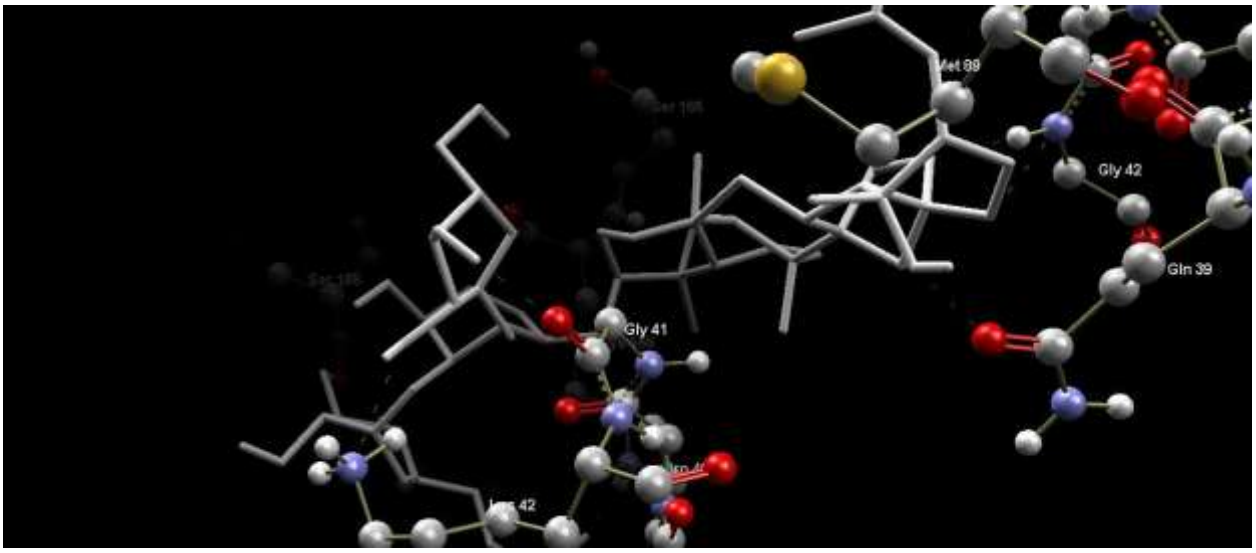

Figure 2:H-Bond interaction between β-glucanand Gly 41 of target protein present in the active site.

Conclusion:

A major hindrance in drug discovery is to find novel compounds as starting points in lead optimization. *In-silico* methods of screening the identified compounds that have potential affinity towards target molecule is a powerful technique. There is a rapid growth in development of software that virtually screen compounds based on protein-ligand interaction. The docking analysis carried out as mentioned above suggests β-glucanas the lead ligand capable of binding with the TSH receptor with least energy and it is expected to reduce the symptoms of Central Hypothyroidism as it can be utilized as nutraceutical having anti-oxidant property.

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