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DESIGNING NATURAL AGONISTS FOR THYROID HORMONE RECEPTOR ALFA-1

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Keywords:

Hypothyroidism, Lipinski rule, Ttoxicity, Energy minimization, Molecular docking, Withanolide D

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ABSTRACT: Hypothyroidism is a common disorder of endocrine system in which the thyroid gland does not produce enough thyroid hormone. The most common cause of hypothyroidism is inflammation of the thyroid gland, which damages the gland's cells. It can affect growth, cellular processes and many other body functions. As more damage accumulates, the risk of Hypothyroidism increases. In order to make an impact on Hypothyroidism, we must understand the molecular processes and structure-function relationships of TSH permitted better understanding of the role of specific protein and carbohydrate domains in the synthesis, bioactivity, and clearance of this hormone. Thyroid hormone receptor alfa-1 (THRA1) is an endocrine nuclear receptor that mediates the transcriptional effects of thyroid hormone (triiodothyronine, T_3). It acts as an agonist *i.e.* it stimulates the production of thyroid hormone by triggering a response in the cell, which is selected as target protein. The primary goal of the project was to unearth natural chemical compounds that can be used as an agonist (increase thyroid hormone level in patients) for the treatment of hypothyroidism. The most effective compounds were isolated from Ashvagandha (*Withania somnifera*). A total of 14 molecules were selected. The molecules were screened based on the Lipinski's rule of 5. The screened molecules were subjected to toxicity analysis and those that passed the toxicity tests were analyzed for energy minimization and docking studies. From the docking studies analysis it was found that 5 compounds (Pseudotropine, Somniferinine, Withanolide -A, Withanolide - D and Withanine) were the lead compound for the hypothyroidism. Amongst these 5 Withanolide_D gave the best results.

INTRODUCTION: Hypothyroidism is a condition in which the thyroid gland does not make enough thyroid hormone. The most common cause of hypothyroidism is inflammation of the thyroid gland, which damages the gland's cells [1]. Hypothyroidism is the commonest clinical disorder of thyroid function [3].

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Hypothyroidism can caused by defect in the anatomy of the thyroid gland, abnormal development of the hypothylamus, disorders of the metabolism of the thyroid hormone, iodine deficiency, thyroid surgery, radiation treatment etc [4]. Among the various varieties of thyroid disorder the hypothyroidism is probably the most important, as it is requires an early diagnosis which is usually followed by appropriate therapy that can prevent the onset of brain damage. In a clinic based study from Mumbai out of 800 patients with thyroid diseases 79 % had hypothyroidism [5].

In accordance to the literature studies and the research carried out, the thyroid hormone receptor

alpha-1 (THRA1), a high affinity agonist receptor for triiodothyronine [7]. It is one of the several receptors for thyroid hormone that have been shown to mediate the biological activities of thyroid hormone [10]. It belongs to the nuclear hormone receptor family and its interaction with the thyroid hormone receptor group can be observed in the TR/RXR pathway [12]. It is also involved in the transcription factor activity. It acts as an agonist *i.e.* it stimulates the production of thyroid hormone by triggering a response in the cell. Some effects of thyroid hormones may be therapeutically useful in non-thyroid disorders if adverse effects can be minimized or eliminated. For instance, thyroid cancer is found to be less aggressive when THRA1 expression is increased [13]. From a series of homologous R(1)-substituted carboxylic acid derivatives, increasing chain length was found to have a profound effect on affinity and selectivity in a radio receptor binding assay for the human thyroid hormone [14]. The simulation of production of thyroid hormone is important to mediate the all biological activities of body. The primary goal of the project was to unearth natural chemical compounds that can be used as an agonist (increase thyroid hormone level in patients) for the treatment of hypothyroidism. The most effective compounds were isolated from Ashvagandha (*Withania somnifera*) [16]. A total of 14 molecules were selected.

With this detailed understanding of hypothyroidism and literature survey, the present study was carried out to understand the molecular features, structural analysis and role of THRA1 in hypothyroidism using bioinformatics approach. The use of bioinformatics methods allows, using all aspects of drug discovery, forming core of structure based drug design and has advantage of delivering drug more quickly and at economic cost. Structure based drug designing approaches involves the 3-D structure of protein on which docking studies of various individual small molecules have been carried in order to calculate their docking score and binding energy by utilizing a series of scoring functions. The virtual screening and molecular docking of the drug candidates on target protein could find out the best lead like compounds with further optimization of the compounds to designing the lead [17].

METHODS:

Collection of Compounds: The biochemical compounds found in the natural herb Ashvagandha (*Withania somnifera*) were collected through a literature survey [15]. *Withania somnifera* (Ashwagandha) is a plant used in medicine from the time of Ayurveda, the ancient system of Indian medicine. The dried roots of the plant are used in the treatment of thyroid disorders. A total of 14 compounds were collected.

Virtual Screening by Drug Likeliness Properties: These selected compounds were screened using Lipinski's rule of 5. Molecular descriptors and drug likeliness properties of these compounds were analysed using the Molinspiration server with based on Lipinski's Rules. Molinspiration server supports for calculation of important molecular properties such as LogP, molecular weight, number of rotatable bonds, number of hydrogen bond donors and acceptors. The compounds which passed the Lipinski's Rules were selected for toxicity analysis [18].

Toxicity Analysis: The pharmacokinetic properties such as Absorption, Distribution, Metabolism, Excretion and Toxicity of screened compounds were predicted using AdmetSAR database. AdmetSAR provides the latest and most comprehensive manually curated data for diverse chemicals associated with known ADMET profiles. admetSAR is a web based query tools incorporating a molecular build-in interface enable the database to be queried by SMILES and structural similarity search. The compounds which passed the toxicity test were selected for energy minimization [18].

Energy Minimization: The screened compounds were energy minimized using Marvin Sketch, a java based chemical editor for drawing chemical structures, queries and reactions. 10 conformers for each molecule were obtained and the one with the least energy was selected. Subsequently, these energy minimized molecules were used as ligands for docking against the selected receptor molecule [20].

Selection of the Receptor: In accordance to the literature studies and the research carried out, the thyroid hormone receptor alpha-1 (THRA1), a high

affinity agonist receptor for triiodothyronine [7], was selected as the target molecule. It is one of the several receptors for thyroid hormone that have been shown to mediate the biological activities of thyroid hormone [10]. It belongs to the nuclear hormone receptor family and its interaction with the thyroid hormone receptor group can be observed in the TR/RXR pathway. It is also involved in the transcription factor activity. Its structure was retrieved from Protein Data Bank (PDB), 1NAV being the PDB ID for the protein molecule.

Binding Site Prediction for THRA1: The binding site for THRA1 was predicted using CASTp server. Computed Atlas of Surface Topography of proteins (CASTp) provides an online resource for locating, delineating and measuring concave surface regions on three-dimensional structures of proteins. These include pockets located on protein surfaces and voids buried in the interior of proteins. And also it gives the binding site residues for targeted molecules [20].

THRA1 Protein Preparation: The protein (THRA1) required for the docking studies were retrieved from the Protein Data Bank at 2.3 Å root mean square deviations (RMSD) resolution which represents a three dimensional structure of target TSHA1. 1NAV being the PDB ID for the protein molecule. The coordinates of the crystallized THRA1 structure was complexed with water molecule. For molecular docking purpose water molecules were removed and hydrogen atoms were added [8].

Docking of Receptor with Ligand: Based on the drug likeliness properties and pharmacokinetic properties selected compounds were subjected for docking studies. To validate drug-target association, the molecular docking was performed on active compound with screened compounds in Autodock tool (version-4.0). The energy minimized ligands and the selected target protein was subjected to docking and compounds with the least binding affinity were sorted out [19].

RESULT AND DISCUSSION:

Collection of compounds: The biochemical compounds found in the natural herb Ashvagandha (*Withania somnifera*) were collected through a literature survey. The structure of molecules was downloaded in the SDF format from the chemical database, PubChem. A total 14 compounds were selected shown in **Table 1**.

TABLE 1: SHOWING NAME OF THE NATURAL COMPOUNDS AND PDB ID

Sr No.	Compound Name	PubChem-ID
1.	Anaferine	440934
2.	Anahygrine	12306778
3.	Beta-Sisterol	222284
4.	Chlorogenic acid	1794427
5.	Cysteine	5862
6.	Cuscohygrine	441070
7.	Pseudotropine	8424
8.	Scopoletin	5280460
9.	Somniferinine	101687980
10.	Tropanol	85947
11.	Witanolide	53477765
12.	Withanolide-A	11294368
13.	Withanolide-D	16167
14.	Withanine	21679027

Virtual Screening by Drug Likeliness Properties: Drug likeliness properties of compounds were predicted by Molinspiration server. It predicts polar surface area (LogP), number of hydrogen bond donors (nON) and number of hydrogen bond acceptors (nOHNH), number of rotatable bonds (nrotb) The molecular descriptors of 14 compounds were tested to Lipinski's rule of five. In that 10 compounds given in **Table 2** which showed drug likeness properties were selected for further analysis.

TABLE 2: SHOWING PREDICTED DRUG LIKELILESS PROPERTIES

Compound	LogP	MW	nON	Nohnh	Nrotb
Anaferine	1.38	224.35	6	2	2
Anahygrine	1.12	236.40	3	1	4
Pseudotropine	0.48	141.21	2	1	0
Scopoletin	1.33	192.17	4	1	1
Somniferinine	2.77	490.64	7	4	3
Tropanol	4.20	349.47	3	0	6
Cuscohygrine	0.86	224.303	3	0	4
Withanolide-A	4.15	470.61	6	2	0
Withanolide-D	4.15	470.61	6	2	2
Withanine	4.35	480.84	6	2	2

Toxicity Analysis: The pharmacokinetic properties such as Absorption, Distribution, Metabolism, Excretion and Toxicity of screened 10 compounds were predicted using AdmetSAR database. Blood-Brain Barrier (BBB) penetration, HIA (Human Intestinal Absorption), carcinogenicity and AMES toxicity were calculated. The predicted ADMET data were summarized in **Table 3** only 5 compounds were passed.

TABLE 3: SHOWING PREDICTED ADMET PROPERTIES

Compounds	Brain blood barrier	Human intestinal absortion	Carcinogenicity	AMES toxicity
Anaferine	BBB+	HIA+	No carcinogenicity	AMES toxicity
Anahygrine	BBB+	HIA+	No carcinogenicity	AMES toxicity
Pseudotropine	BBB+	HIA+	No carcinogenicity	No AMES toxicity
Scopoletin	BBB+	HIA+	Carcinogenicity	AMES toxicity
Somniferinine	BBB+	HIA+	No carcinogenicity	No AMES toxicity
Tropanol	BBB+	HIA+	No carcinogenicity	AMES toxicity
Cuscohygrine	BBB+	HIA+	carcinogenicity	No AMES toxicity
Withanolide-A	BBB+	HIA+	No carcinogenicity	No AMES toxicity
Withanolide-D	BBB+	HIA+	No carcinogenicity	No AMES toxicity
Withanine	BBB+	HIA+	No carcinogenicity	No AMES toxicity

Energy Minimization: The screened biochemical compounds were energy minimized using Marvin Sketch. Marvin Sketch, a java based chemical editor for drawing chemical structures. 10 conformers for each molecule were obtained and the one with the least energy was selected. Energy minimized compounds were shown in **Table 4**.

TABLE 4: SHOWING LIGAND ENERGY BEFORE AND AFTER ENERGY MINIMIZATION

Name of the ligand	Energy in Kcal/mol (before energy minimization)	Energy in Kcal/mol (after energy minimization)
Pseudotropine	80.07	76.34
Somniferinine	71.61	67.88
Withanolide_A	36.76	31.36
Withanolide_D	51.09	48.19
Withanine	52.12	48.28

Selection of the Receptor: In accordance to the literature studies and the research carried out, the thyroid hormone receptor alpha-1(THRA1), a high affinity agonist receptor for triiodothyronine [20], was selected as the target molecule. It is one of the several receptors for thyroid hormone that have been shown to mediate the biological activities of thyroid hormone [21]. It belongs to the nuclear hormone receptor family and its interaction with the thyroid hormone receptor group can be observed in the TR/RXR pathway. It is also involved in the transcription factor activity. Its structure was retrieved from Protein Data Bank (PDB), 1NAV being the PDB ID for the protein molecule.

Binding Site Prediction for THRA1: The binding site for THRA1 was predicted using CASTp server. Computed Atlas of Surface Topography of proteins (CASTp) provides an online resource for locating, delineating and measuring concave surface regions on three-dimensional structures of proteins. It predicts the total 32 active pockets and 41 residues present in active site shown in **Fig. 1.**

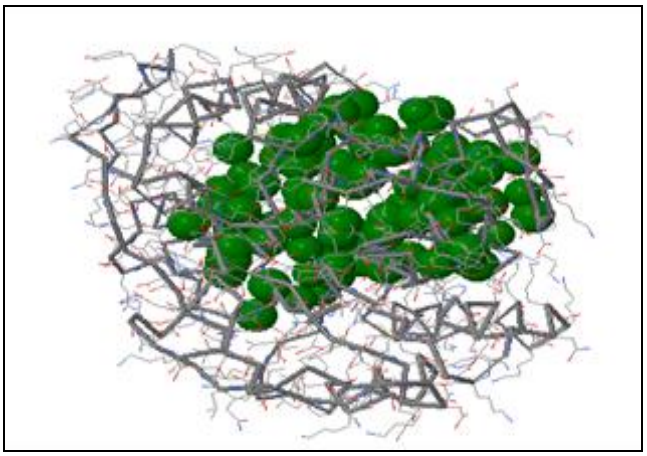

FIG. 1: SHOWING BINDING SITE FOR THRA1

Protein Preparation: The protein (THRA1) required for the docking studies were retrieved from the Protein Data Bank at 2.3 Å root mean

square deviations (RMSD) resolution which represents a three dimensional structure of target TSHA1. For molecular docking purpose water molecules were removed and hydrogen atoms were added using AutoDock tool shown in **Fig. 2**.

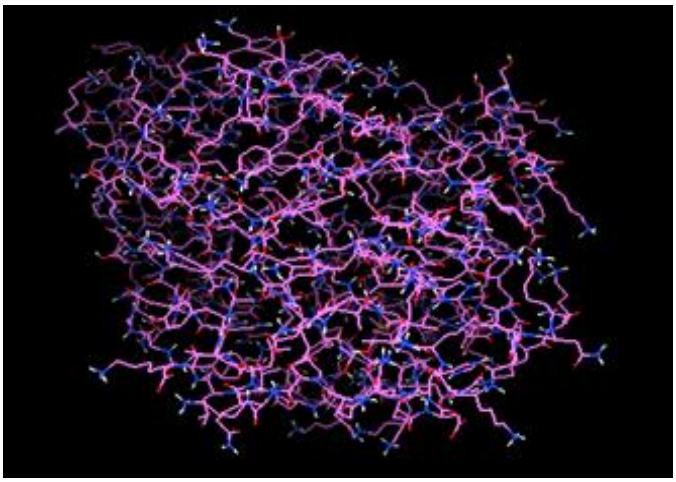

FIG. 2: VISUALIZATION OF THRA1 MOLECULE IN AUTODOCK

Docking of receptor with ligand: Docking analysis was carried out using Auto Dock Vina tool. Energy minimized 5 compounds (Pseudotropine, Somniferinine, Withanolide-A, Withanolide-D and Withanine) were subjected to docking studies. The receptor protein (THRA1) was kept rigid, while ligands were set flexible to rotate and explore most probable binding poses.

The binding energy and number of hydrogen bonds formed were shown in **Table 5**. Docking score or binding energy for the complex of THRA1 and ligand Withanolide_D shows the minimum binding energy (-7.40) and more number of hydrogen bonds (4) at Ser-161, Ly-163, His-183 and Asn-229 position and other interacting residues Leu-53, Asn-49, Tyr-51, Tyr-270, Phe-88, Glu-131, Asp-227, Glu-187 and Ser-186 shown in **Fig. 3**.

TABLE 5: SHOWING BINDING ENERGY SCORE AND NUMBER OF HYGROGEN BONDS FROM AUTODOCK VINA TOOL

Name of the ligand	Binding energy	Number of Hydrogen bonds
Pseudotropine	-5.11	2
Somniferinine	-6. 01	1
Withanolide-A	-7.02	2
Withanolide-D	-7.40	4
Withanine	-7.29	2

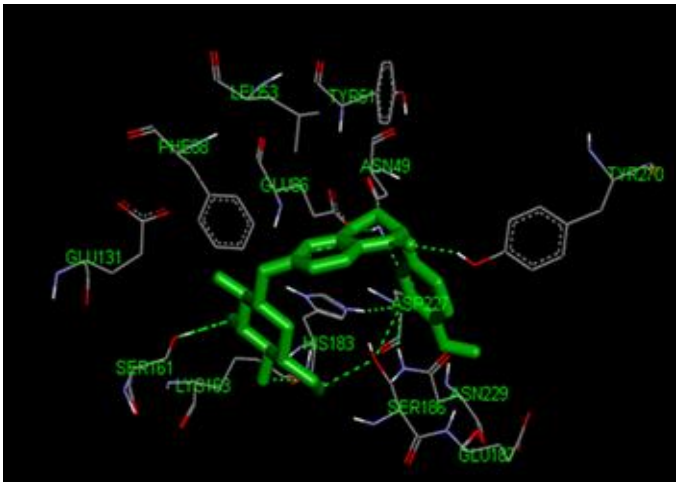

FIG. 3: SHOWING DOCKING BETWEEN THE THRA1 RECEPTOR AND WITHANOLIDE-D LIGAND

CONCLUSION: Hypothyroidism is the commonest clinical disorder of thyroid function. At present there is no natural potent drug available to treat hypothyroidism. Molecular docking is one of the powerful techniques for identifying biological significance and exploring new drugs by screening millions of compounds. Results of the current investigations clearly demonstrated that the well-established actions of screened natural compounds of Ashwgandha (Pseudotropine, Somniferinine, Withanolide-A, Withanolide-D and Withanine) can pave a new dimension for structure based drug designing and further detailed analysis can lead to a novel, natural treatment strategy for hypothyroidism.

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REFERENCES:

1. Dekkers OM, Berghout A, Muller AF, Smit JWA, Wiersinga WM, Peeters RP, van der Ploeg MJ and de Jong NW: Thyroid function disorders, Guidelines of the Netherlands Association of Internal Medicine, 2012; 123–134.
2. Dumont JE, Lamy F, Roger P and Maenhaut C: Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. Physiological Reviews, 2014; 72(3): 667-697.
3. Sunderesan S: Proceedings of symposium on elimination of IDD through universal access to iodized salt, 2015.
4. Braverman L and Utiger R: A text book of Introduction to hypothyroidism in Werner and Ingbar's The Thyroid: a fundamental and clinical text. Ninth Edition 2005.
5. Persani, L: Central hypothyroidism, The Journal of Clinical endocrinology and Metabolism, 2012.

6. Unnikrishnan, AG and Menon UV: Thyroid disorders in India, An epidemiological perspective, Indian J Endocrinol Metab, 2011; 15: S78-81.
7. International Union of Basic And Clinical Pharmacology: http://www.iuphardb.org/database/objectdisplayforward?objectid=588.
8. Online Mendelian Inheritance in Man (OMIM): MIM ID 190120, Thyroid Hormone Receptor Alpha 1.
9. PDB.Org: Structure of 1NAV.
10. Nuclear Receptor Signaling Atlas (NURSA): (www.nursa.org/molecule.cfm?moltype=receptor&molid=1a1&moldetail=pandp).
11. Uniprot KB: P10827 Thyroid Hormone Receptor Alpha.
12. Pathway Interaction Database, NCI Nature: Rxr_Vdr_ Pathway.
13. Sigmaaldrich: THRA-TR/RXR 13, Activation Pathway.
14. Onda M, Li D and Suzuki S: Expansion of Microsatellite in the Thyroid Hormone Receptor-Alpha1 Gene linked to increased receptor expression and less aggressive thyroid cancer, 2015; 8: 2870–2874.
15. Ye L, Li YL and Mellström K: Thyroid receptor ligands, agonist ligands selective for the thyroid receptor beta1, 2013; 46(9): 1580-8.
16. Jatwa R and Kar A: Amelioration of metformin induced hypothyroidism by *Withania somnifera* and *Bauhinia purpurea* extracts In Type 2 diabetics mice. Endocrine Research Unit, 2009; 23(8).
17. Singh G, Sharma PK, Dudhe R and Singh S: Biological Activities of *Withania somnifera*: Annals of Biological Research, 2010; 1(3): 56-63.
18. Shen J, Zhang W, Fang H, Perkins R, Tong W and Hong H: Homology modeling, molecular docking, and molecular dynamics simulations elucidated-fetoprotein binding modes, BMC Bioinformatics, 2013; 14: S6.
19. Paramashivam SK, Elayaperumal K, Natarajan BB, Ramamoorthy M, Balasubramanian S and Dhiraviam KN: *In silico* pharmacokinetic and molecular docking studies of small molecules derived from Indigofera as palathoides Vahl targeting receptor tyrosine kinases in Bioinformation, 2015; 11(2): 073-084.
20. Desai SA, Kumbhar SS, Katti VS, Choudhari PB and Bhatia MS: 3D QSAR and Pharmacophore modelling on chalcones as anti leishmanial agents potential trypanothione reductase Inhibitors, Journal of Applied Pharmaceutical Science, 2013; 3.
21. Wanjari P and Jayadeepa RM: A novel *In-silico* drug designing approach for identification of natural compounds for treatment of hypothyroid, Research Scientist, 2012.

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