

A Novel *In-Silico* Drug Designing Approach for Identification of Natural Compounds for Treatment of Hypothyroid

Pankhuri Wanjari ¹⁺ and R.M Jayadeepa ²

¹ B.Tech undergraduate, Dept. of Bioinformatics, National Institute of Technology, Bhopal 462001-INDIA

² Research Scientist, Department Of Bioinformatics, IOCB, Bangalore 560071-INDIA

Abstract. Hypothyroidism is an endocrine disorder characterized by abnormally low thyroid hormone production, resulting in the deficiency of thyroid hormone. Hypothyroid can be treated by giving synthetic drug intake of L-thyroxin (T4) and L-triiodothyronine (T3) as a replacement or supplementary therapy in patients. Some of the drugs used for this purpose are Liotrix, Thyrolar and many more. The main objective of this research work is to find natural compounds that can help to stimulate thyroid hormone production. The molecules were screened based on the Lipinski's rule of 5. A total of 115 molecules were selected. 1NAV, a thyroid receptor alpha in complex with an agonist selective for thyroid receptor beta, was selected as the target protein. The molecules were subjected to docking analysis with 1NAV. The most effective compounds were isolated from *Ashvagandha (Withania Somnifera)*, *Astragalus (Huang Qi)*, *Gotukola (Centella Asiatica)*, *Triphala*, *Punarnava*, *Bauhinia Purpurea*, *Watercress* and *Dandelions (Taraxacum Officinale)*. The compounds were subjected to toxicity analysis and those that passed the toxicity tests were analyzed for binding site. From the active site analysis it was found that amino acids like Asp, Gly, Lys, Val, Asn, Gly, and Arg; which were present on site 7, showed the most prominent site. In silico docking results showed that 5 compounds viz Withaferin A, Calycosin-7-O-Beta-D-Glucoside, Withanolide D, Rotenone and Quercetin were the lead compound for the disease. Amongst these 5, Withaferin A and Calycosin-7-O-Beta-D-Glucoside gave the best results.

Keywords: hypothyroidism, natural molecules, Lipinski rule, toxicity, active site, Withaferin A, Calycosin-7-O-Beta-D-Glucoside

1. 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. The purpose of the treatment is to replace the undersupplied thyroid hormone. Levothyroxine is the most commonly used medication [1] [2]. Insufficient amount of thyroid hormone leads to increase in TSH level which may cause the thyroid gland to enlarge and form goiter [3]. Natural remedy includes use of many herbs, primarily *Withania Somnifera*(*Ashvagandha*)[4][8],*Bauhinia Purpurea*[5], *Astragalus*[9][10], *Punarnava*[11][12], *Triphala*[11], *Gotukola*[13] and many more[14]. Thyroid hormone receptor alpha 1NAV was selected as the target protein [15]. It is an endocrine nuclear receptor that mediates the transcriptional effects of thyroid hormone (triiodothyronine, T3) [16]. 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 [7] 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

⁺ Corresponding author. Tel: +919424313163

E-mail address: wanjaripankhuri9@gmail.com

receptors alpha(1) and beta(1) [6]. The thyroid hormone receptor (THRA) interacts with thyroid hormone receptor group. This can be observed in the TR/RXR pathway [17] [18].

2. Method

2.1. Collection of Compounds

The biochemical compounds found in the natural herbs were collected through a literature survey. These compounds were screened using Lipinski's rule of 5. The structure of molecules that concurred with the Lipinski rule was downloaded in the SDF format from the chemical database, PubChem. A total of 115 molecules were collected.

2.2. Energy Minimization

The screened biochemical 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.

“See Fig. 1.a, Fig. 1.b, Fig. 1.c.”

2.3. 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 [19] [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.

2.4. Docking of Receptor with Ligand

The energy minimized ligands and the selected target protein was subjected to docking using Auto Dock Vina and compounds (approx. 10 to 15) with the least binding affinity were sorted out. These compounds were docked with the target protein using HEX (see table 1). The binding site was located using Q-site finder (see table 2). The site common to maximum number of compounds was put forward as the binding site of our lead compound. The site of the lead compounds and the binding site of the commercial drug, Liotrix, was compared “see Fig. 2.a, Fig. 2.b and Fig. 3”

2.5. Toxicity Analysis

Toxicity analysis was carried out for the ligand molecules using OpenTox. Toxicity findings for some compounds were not available in OpenTox. In such a situation, QSIRIS Property Explorer was used to determine drug likeliness of the compound. Human health effects such as carcinogenicity, tendency to cause irritation and corrosiveness to skin and eyes and mutagenicity were evaluated and the molecules which passed the toxicity test were selected for further analysis (see table 3).

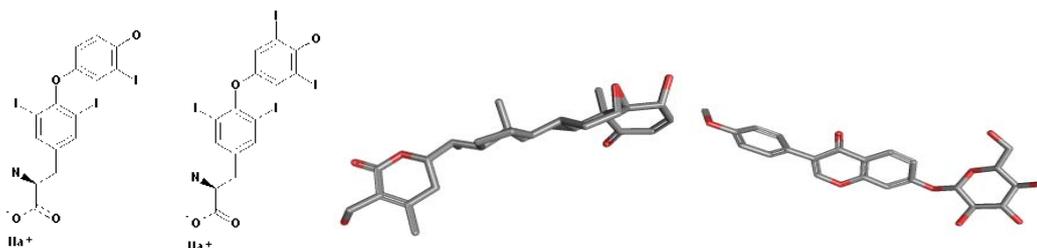


Fig. 1: (a) 2D Structure of Liotrix (b) 3D Structure of Withaferin A 3D (c) Structure of Calycosin-7-O-Beta-D-Glucoside



Fig. 2: (a) Binding Site of Withaferin A (b) Binding Site of Calycosin-7-O-Beta-D-Glucoside.

The **green** color shows the **Active Site 7**, which was found to be the most prominent site. Amino acids like Asp, Gly, Lys, Val, Asn, Arg were present on this site.

3. Result

From an assemblage of 115 biochemical compounds, top 5 molecules were obtained after docking and toxicity analysis. These were *Withaferin A*, *Calycosin-7-O-Beta-D-Glucoside*, *Withanolide D*, *Rotenone* and *Quercetin*. From the active site analysis it was observed that amino acids like Asp, Gly, Lys, Val, Asn, Gly, Arg; which were present on site 7, showed the most prominent binding site. The commercial drug Liotrix was taken as the reference drug. It was docked against the target protein 1NAV using HEX. It bound to site 7 with a docking score (E Total) of -204.65. *Withaferin A* (from *Ashvagandha*) showed the highest docking score (E Total) of -184.25 followed by *Calycosin-7-O-Beta-D-Glucoside* (from *Astragalus*) with a score of -174.11. Both of these compounds showed high drug likeliness and are not carcinogenic. *Withaferin A* may cause some irritation. *Calycosin-7-O-Beta-D-Glucoside* showed no such irritating effects (see table 3). Both of these compounds proved to be the best lead compounds for the treatment of hypothyroid.

4. Discussion

The primary goal of the research was to unearth natural chemical compounds that can be used as an agonist (increase thyroid hormone level in patients) for the treatment of hypothyroid. Natural compounds isolated from the hypothyroid treating herbs were screened based on Lipinski's rule of 5. A total of 115 molecules were obtained. These molecules were subjected to docking analysis with the target protein 1NAV using Auto Dock Vina and HEX. Molecules with the highest binding affinity and E Total were selected and made to undergo toxicity analysis. The selected molecules, that passed the toxicity test, were analyzed for binding site. From the active site analysis, using Q-Site Finder, it was observed that amino acids like Asp, Gly, Lys, Val, Asn, Gly, Arg; which were present on site 7, showed the most prominent binding site. The 5 natural compounds that bound to site 7 were *Withaferin A*, *Calycosin-7-O-Beta-D-Glucoside*, *Withanolide D*, *Rotenone* and *Quercetin*. Amongst these, *Withaferin A* and *Calycosin-7-O-Beta-D-Glucoside* gave the best results.

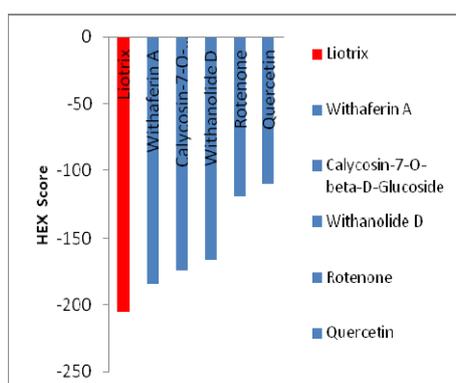


Fig 3: Comparison of Commercial Drugs vs. Natural Compounds

NOTE: *Astragalus (Calycosin-7-O-Beta-D-Glucoside)* is usually recommended for hyperthyroid and is sometimes cited as being barred for use in the treatment of hypothyroid [14] [22]. However, the results obtained in this research contradict the above statement showing results in favor of *Astragalus*.

5. Conclusion

In the *In Silico* docking results, 5 compounds viz. Withaferin A, Calycosin-7-O-Beta-D-Glucoside, Withanolide D, Rotenone and Quercetin were established as the lead compounds for the treatment of hypothyroid. Amongst these five, Withaferin A and Calycosin-7-O-Beta-D-Glucoside gave the best results. Most of the chemical and physical properties, such as hydrogen bond-acceptor, hydrogen bond-donor, heavy atom count etc. of the drug Liotrix and the lead compound Calycosin-7-O-Beta-D-Glucoside were analogous.

6. Acknowledgement

I would like to thank my guide for her gratifying encouragement and supervision throughout the project. I thank my parents from the bottom of my heart for their blessings and support without which it would not have been possible for me to do this project. Above all, thank you God for each and everything you blessed me with.

7. References

- [1] US National library of medicine, Pubmed health: Hypothyroidism(ncbi.nlm.nih.gov/pubmedhealth/PMH0001393/)
- [2] MedicineNet.Com:Hypothyroidism(Medicinenet.Com/Hypothyroidism/Article.Htm)
- [3] Endocrineweb.Com: Hypothyroidism Too Little Thyroid Hormone (Endocrineweb.Com/Conditions/Thyroid/Hypothyroidism-Too-Little-Thyroid-Hormone)
- [4] G.Singh*, P.K.Sharma, R.Dudhe and S.Singh. Biological Activities of Withania Somnifera: *Dept. Of Pharmaceutical Technology, Meerut Institute Of Engineering And Technology, Meerut, U.P, India*.Scholars research library. Annals of Biological Research, 2010, 1 (3) : 56-63, ISSN 0976-1233 CODEN(USA)
- [5] Jatwa.R, Kar.A; Amelioration of Metformin Induced Hypothyroidism by Withania Somnifera and Bauhinia Purpurea Extracts In Type 2 Diabetics Mice. *Endocrine Research Unit, School Of Life Sciences, Devi Ahilya University, Indore (M.P) India 452001*: John Wiley & Sons, Ltd. Phytotherapy Research, Volume 23, Issue 8, 2009
- [6] Ye L, Li YL, Mellström K, et al. Thyroid Receptor Ligands. 1. Agonist Ligands Selective For the Thyroid Receptor Beta1: Source: *Karo Bio AB, Novum, Huddinge S-141 57, Sweden*: Journal of Medicinal Chemistry, 2003 Apr 24; 46(9):1580-8. PMID: 12699376
- [7] Onda M, Li D, Suzuki S, et al. Expansion of Microsatellite in the Thyroid Hormone Receptor-Alpha1 Gene Linked to Increased Receptor Expression and Less Aggressive Thyroid Cancer, *The Burnham Institute, La Jolla, California 92037, USA*: Clinical cancer research, Vol. 8, 2870–2874, September 2002, PMID: 12231529
- [8] Panda S, Kar. A. Changes in Thyroid Hormone Concentrations after Administration of Ashwagandha Root Extract to Adult Male Mice, *School Of Life Sciences, D.A. University, Indore, India*: Journal of Pharmacy and Pharmacology, 1998 Sep;50(9):1065-8. PMID: 9811169
- [9] Chen MD, Kuang AK, Chen JL. Influence of Yang Restoring Herb Medicines upon Metabolism of Thyroid Hormone in Normal Rats and a Drug Administration Schedule. 1989 Feb; 9(2):93-5, 70. PMID: 2500264
- [10] Naturalpetrx.Com/herb_info.php?ingredient_id=1
- [11] Ajita Patel. Hypothyroidism, a Comparative Study of Allopathic and Ayurvedic Management of the Disease, *California College Of Ayurveda*
- [12] Ryan English. Hypothyroidism, an Ayurvedic Approach (ezinearticles.com/?Hypothyroidism---An-Ayurvedic-Approach&id=705474)
- [13] Foundationsofherbalism.Com: Medicinal Herb Walk/ Gotukola
- [14] Ohlone Herbal Center: Thyroid Gland, Anatomy and Physiology.

- [15] PDB.Org: Structure Of 1NAV
- [16] Nuclear Receptor Signaling Atlas (NURSA):
(www.nursa.org/molecule.cfm?moltype=receptor&molid=1a1&moldetail=pandp)
- [17] Pathway Interaction Database, NCI Nature: Rxr_Vdr_Pathway.
- [18] Sigmaaldrich: THRA-TR/RXR Activation Pathway.
- [19] International Union Of Basic And Clinical Pharmacology:
<http://www.iuphardb.org/database/objectdisplayforward?objectid=588>
- [20] Online Mendelian Inheritance in Man (OMIM): MIM ID 190120, Thyroid Hormone Receptor Alpha 1.
- [21] UNIPROT KB: P10827 Thyroid Hormone Receptor Alpha.
- [22] Livestrong.com: Herbs to avoid with hypothyroidism.

List of tables:

Table 1: Scores from Auto dock Vina and HEX

S.No	Name Of The Ligand	Binding Affinity (Auto Dock Vina Score)	E Total (Hex Score)
1	Withaferin A	-7.3	-184.25
2	Calycosin-7-O-Beta-D-Glucoside	-6.7	-174.11
3	Withanolide D	-7.8	-166.55
4	Rotenone	-7.0	-119.33
5	Quercetin	-6.7	-110.24

Table2: Binding Site Analysis Using Q-Site Finder.

S.No	Compound Name	Active Site
1	Withaferin A	SITE 7
2	Calycosin-7-O-Beta-D-Glucoside	SITE 7
3	Withanolide D	SITE 7
4	Rotenone	SITE 7
5	Quercetin	SITE 7

Table 3: Toxicity Analysis Using OpenTox

Compound Name	Carcinogenicity	Skin/Eye Irritation & Corrosiveness And Mutagenicity
Withaferin A	NO	May cause skin/eye irritation. Medium risk of mutagenicity
Calycosin-7-O-beta-D-glucoside	NO	NO
Withanolide D	NO	Highly mutagenic and irritant
Rotenone	medium risk of tumerogenic and reproductive effect	Highly irritant. Not corrosive to skin. (for eye irritation: not lesion r34, r35, r36 or r41)
Quercetin	medium risk of tumerogenic and reproductive effect	Irritating and corrosive to skin. Highly mutagenic.