

“In Silico Docking Analysis of phytochemicals as Potential Inhibitors of Thyroid Peroxidase (TPO) in the Management of Hyperthyroidism”

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ABSTRACT

Hyperthyroidism is a metabolic disorder characterized by excessive synthesis of thyroid hormones, primarily mediated by thyroid peroxidase (TPO), a key enzyme involved in iodination and coupling reactions during hormone biosynthesis. Conventional antithyroid drugs target TPO but are often associated with adverse effects, prompting the search for safer, plant-derived alternatives. This study employed an in silico approach to investigate the inhibitory potential of bioactive compounds from *Hyptis verticillata* against human thyroid peroxidase. Selected phytochemicals were retrieved from public databases and subjected to molecular docking using AutoDock Vina integrated within PyRx. Drug-likeness properties were evaluated using Lipinski, Ghose, Veber, Egan, and Muegge rules, while pharmacokinetic and toxicity profiles were predicted via SwissADME and pkCSM. Docking analysis revealed binding affinities ranging from -3.8 to -5.5 kcal/mol, with squalene (-5.5 kcal/mol), R-R,R-E-trans-phytol (-5.3 kcal/mol), and 3a,4,5,6,7,7a-hexahydro-4,7-methanoindene (-5.0 kcal/mol) exhibiting the strongest interactions within the TPO active site. These compounds formed stable hydrophobic interactions that may hinder substrate access to the catalytic center of the enzyme. ADMET predictions indicated favorable gastrointestinal absorption, low toxicity, and acceptable pharmacokinetic profiles for the top-ranking compounds. Overall, the findings suggest that *Hyptis verticillata* contains phytochemicals with promising TPO inhibitory potential, providing a molecular basis for its traditional use and supporting further experimental validation for hyperthyroidism management.

Keywords: *Hyptis verticillata*; Hyperthyroidism; Thyroid peroxidase; Molecular docking; In silico analysis; ADMET; Natural TPO inhibitors; Phytochemicals

INTRODUCTION

Hyperthyroidism is a metabolic disorder characterized by excessive production of thyroid hormones—triiodothyronine (T_3) and thyroxine (T_4)—which leads to an increased basal metabolic rate and systemic disturbances in cardiovascular, nervous, and metabolic functions (Chaudhary et al., 2022). The condition results from the overactivity of the thyroid gland, often due to autoimmune stimulation as seen in Graves' disease, multinodular goiter, or thyroid adenomas. Current therapeutic interventions for hyperthyroidism include antithyroid drugs (methimazole, propylthiouracil), radioactive iodine therapy, and thyroidectomy. However,

these treatments are associated with adverse effects such as hepatotoxicity, agranulocytosis, relapse of symptoms, and lifelong hormone replacement therapy (Yadav et al., 2021). This has intensified the search for safer and more effective natural alternatives with minimal side effects.

Thyroid peroxidase (TPO) is a heme-containing enzyme that catalyzes the iodination of tyrosine residues and the coupling of iodotyrosines in thyroglobulin, leading to the synthesis of T₃ and T₄ hormones. Inhibiting TPO activity has been recognized as a strategic mechanism for managing hyperthyroidism, as it reduces the synthesis of thyroid hormones (Taurog, 2013). Conventional TPO inhibitors such as methimazole and propylthiouracil exert their effects by blocking the peroxidase-catalyzed iodination process, yet their adverse drug reactions limit long-term use. Therefore, identifying bioactive compounds from medicinal plants that can inhibit TPO effectively and safely is a promising therapeutic approach.

Hyptis verticillata Jacq. (family: Lamiaceae), commonly known as “John Charles” or “Bush Mint,” is a medicinal plant widely distributed in tropical and subtropical regions. Traditionally, it has been used to treat fever, inflammation, hypertension, pain, infections, and various metabolic disorders (Rivera et al., 2019). Phytochemical screening of *H. verticillata* has revealed the presence of diverse bioactive compounds such as flavonoids, terpenoids, phenolic acids, and alkaloids—many of which are known to possess antioxidant, anti-inflammatory, and enzyme-inhibitory properties (Pérez et al., 2020).

Given its rich phytochemical composition, *H. verticillata* presents a promising source of natural compounds that could modulate thyroid hormone synthesis by targeting TPO. Computational approaches, particularly molecular docking, have become indispensable tools in modern drug discovery for predicting ligand–protein interactions and assessing binding affinities at the molecular level.

In silico docking analysis provides a cost-effective and time-efficient means of screening phytochemicals to identify potential inhibitors of target enzymes before in vitro and in vivo validation (Morris & Lim-Wilby, 2008). This approach allows researchers to visualize binding conformations, hydrogen bonding, hydrophobic interactions, and key residues involved in enzyme inhibition.

This study, therefore, aims to investigate the inhibitory potential of bioactive compounds from *Hyptis verticillata* against thyroid peroxidase (TPO) through in silico molecular docking. The findings from this study are expected to provide theoretical insight into the possible use of *H. verticillata* compounds as natural TPO inhibitors in the management of hyperthyroidism.

MATERIALS AND METHODS

Materials and Databases Used

The major tools and databases used include: PubChem for retrieval of the 3D structures of phytochemicals reported in *Hyptis verticillata*. Protein Data Bank (PDB) for obtaining the crystal structure of human thyroid peroxidase (TPO). PyRx (version 0.8) for virtual screening and molecular docking analysis. Discovery Studio Visualizer (BIOVIA, 2021) for visualization, binding interaction analysis, and molecular modeling. SwissADME and pkCSM for evaluation of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) and drug-likeness properties. ChemDraw (version 20.0) for structure editing and optimization of compounds where necessary.

Retrieval of Bioactive Compounds of *Hyptis verticillata*

Phytoconstituents of *Hyptis verticillata* were selected based on their pharmacological relevance and prevalence in *H. verticillata* extracts. The 3D structures of these compounds were downloaded from PubChem in SDF format and later converted to PDB format using **Open Babel** integrated within PyRx.

Table 2 List of phytocompounds and referenced drugs

Ligands
3a,4,5,6,7,7a-hexahydro-4,7-methanoindene
4,7- methanon-1H-indene
R-R,R-E- trans-Phytol
Squalene
9,12,15-octadecatrien-1-ol
1-octadecyne
1-fluorodecane

Ligand Preparation

Each selected ligand was prepared for docking using PyRx. Energy minimization was performed using the **Universal Force Field (UFF)** to obtain the most stable conformations. The optimized structures were saved in **pdbqt** format suitable for docking. Parameters such as torsional bonds, rotatable bonds, and charges were automatically assigned by the PyRx tool. Prior to docking, all ligands were visually inspected in Discovery Studio to confirm chemical integrity and ensure the absence of missing hydrogen atoms or irregular bonds, process described by (Akinola et al., 2025)

Protein Preparation

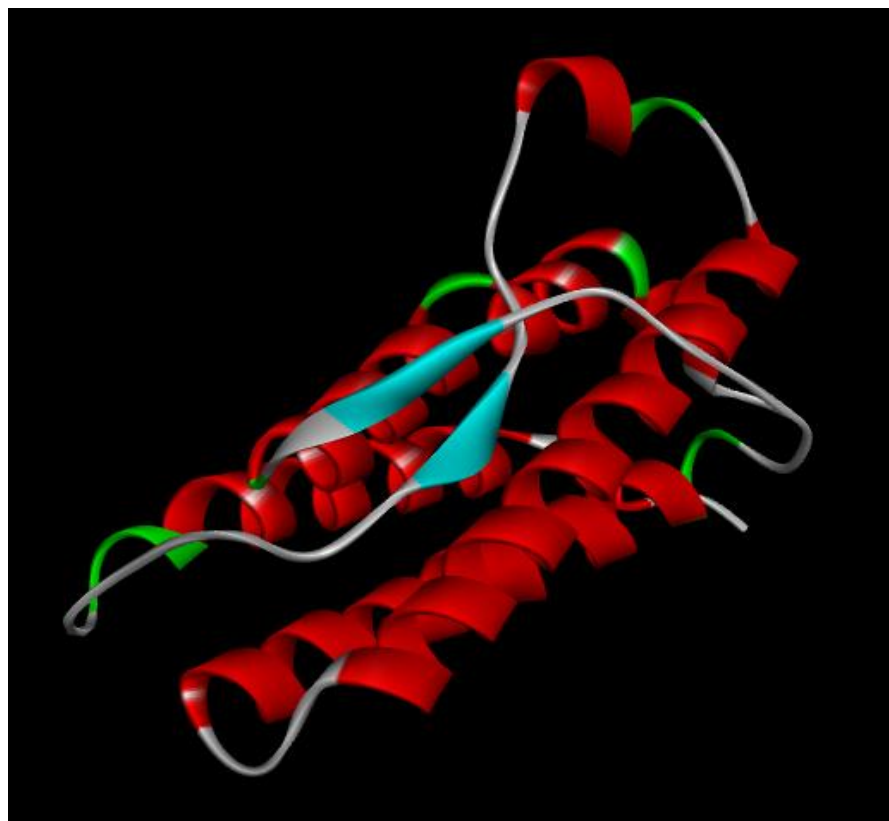


Figure 1: The 3D crystal structure of human thyroid peroxidase (TPO)

The 3D crystal structure of human thyroid peroxidase (TPO) was retrieved from the Protein Data Bank (PDB ID: 8U18). The protein was prepared using Discovery Studio and PyRx as follows: Removal of all non-essential

molecules such as water, ions, and co-crystallized ligands, addition of polar hydrogen atoms and Kollman charges to stabilize electrostatic interactions, optimization and conversion of the protein to **pdbqt** format for docking analysis. The active site residues were identified using the **Biovia Discovery Studio** tools, based on the reported binding pocket of the TPO enzyme.

Table 1 Selected receptors in PCOS

TARGET PROTEIN	ID NUMBER
Human thyroid peroxidase (TPO)	8U18

Molecular Docking Procedure

Molecular docking was conducted using AutoDock Vina integrated within PyRx to predict the binding affinities and interaction modes of *H. verticillata* compounds with TPO. The prepared ligands were docked into the defined active site of the enzyme within a grid box covering the catalytic cavity. Docking parameters were set to default with an exhaustiveness value of 8 to ensure reliable search space exploration. Binding affinities (expressed in kcal/mol) were recorded for each compound, and the best poses were selected based on the lowest binding energy and appropriate orientation within the binding pocket. Docked complexes were visualized and analyzed using Discovery Studio Visualizer to identify hydrogen bonds, hydrophobic contacts, π - π interactions, and other non-covalent interactions with key amino acid residues as described by (Dearsly, Olukayode, et al., 2025)

ADMET and Drug-Likeness Evaluation

The top compounds exhibiting the best docking scores were subjected to pharmacokinetic analysis using SwissADME and pkCSM web servers. Parameters such as molecular weight, lipophilicity (LogP), number of hydrogen bond donors and acceptors, gastrointestinal absorption, blood-brain barrier permeability, and predicted toxicity were evaluated. Lipinski's Rule of Five and Veber's rule were used as standard filters to assess oral bioavailability and drug-likeness. (Dearsly et al., 2025)

Validation of Docking Protocol

To validate the docking protocol, the co-crystallized ligand of the TPO enzyme (if available) was re-docked into the binding site using the same docking parameters. The resulting RMSD (Root Mean Square Deviation) between the re-docked and crystallographic poses was calculated; an RMSD value ≤ 2.0 Å was considered acceptable, indicating that the docking procedure was reliable.

Data Analysis

Docking binding affinities were expressed in **kcal/mol**, and the best binding conformations were ranked accordingly. Interaction analyses were supported with 2D and 3D visualizations.

RESULTS AND DISCUSSION

Drug-likeness screening result

TABLE 3 Drug-likeness screening result of phytochemicals from *Hyptis Verticillata*

Compounds	Lipinski	Ghose	Veber	Egan	Muegge	Remark
3a,4,5,6,7,7a-hexahydro-4,7-methanoindene	Yes	No	Yes	Yes	No	No
4,7- methanon-1H-indene	Yes	No	Yes	Yes	No	No
R-R,R-E- trans-Phytol	Yes	No	Yes	No	No	No

Squalene	Yes	No	Yes	No	No	No
9,12,15-octadecatrien-1-ol	Yes	Yes	Yes	Yes	No	Passed
1-octadecyne	Yes	No	No	No	No	No
1-fluorodecane	Yes	Yes	Yes	Yes	No	Passed

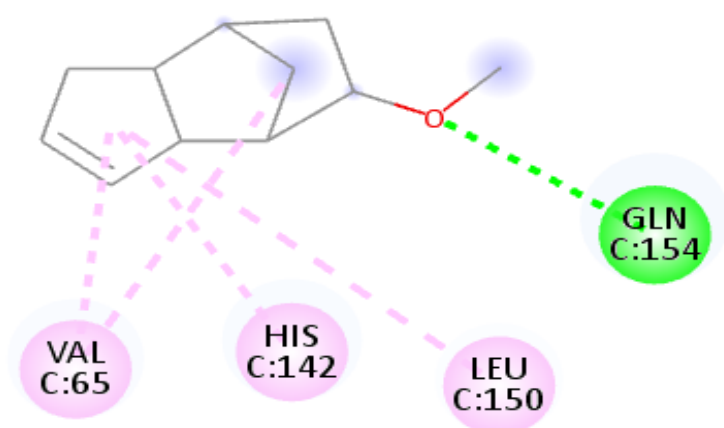
Molecular docking results

The results of molecular docking against the selected receptor are shown below as represented by the docking scores. The docking scores of the compounds range from -3.8 to - 5.5.



TABLE 3 Docking score of phytochemicals from Hyptis Verticillata with receptor


Ligands	Binding Affinity
	8U18
3a,4,5,6,7,7a-hexahydro-4,7-methanoindene	-5.0
4,7- methanon-1H-indene	-4.9
R,R,R-E- trans-Phytol	-5.3
Squalene	-5.5
9,12,15-octadecatrien-1-ol	-4.8
1-octadecyne	-3.8
1-fluorodecane	-4.1

2D structure of compounds with high binding affinity



Interactions

 Conventional Hydrogen Bond
 Alkyl

 Pi-Alkyl

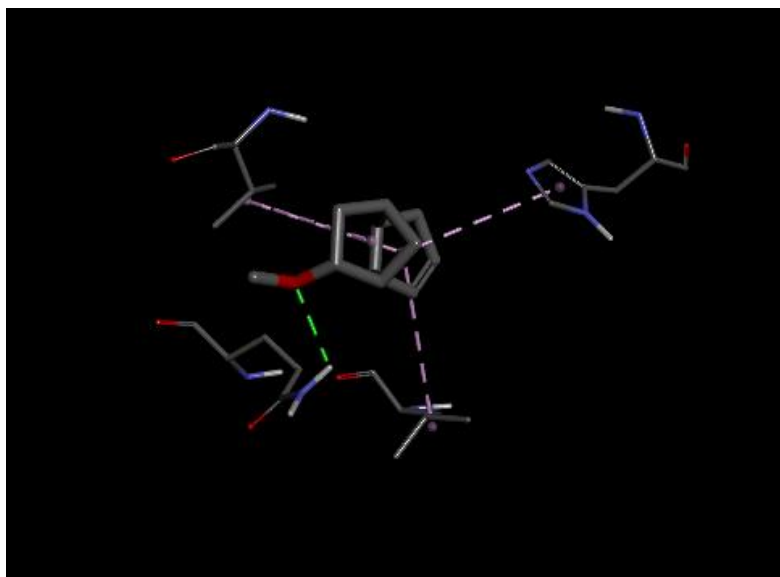
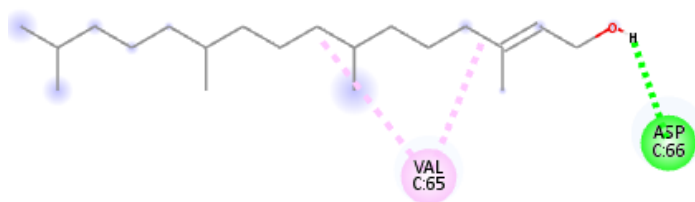


Figure 2: 2D & 3D Structure of 3a.4,5,6,7,7a-hexahydro-4,7-methanoindene with TPO



Interactions

- Conventional Hydrogen Bond
- Unfavorable Donor-Donor
- Alkyl

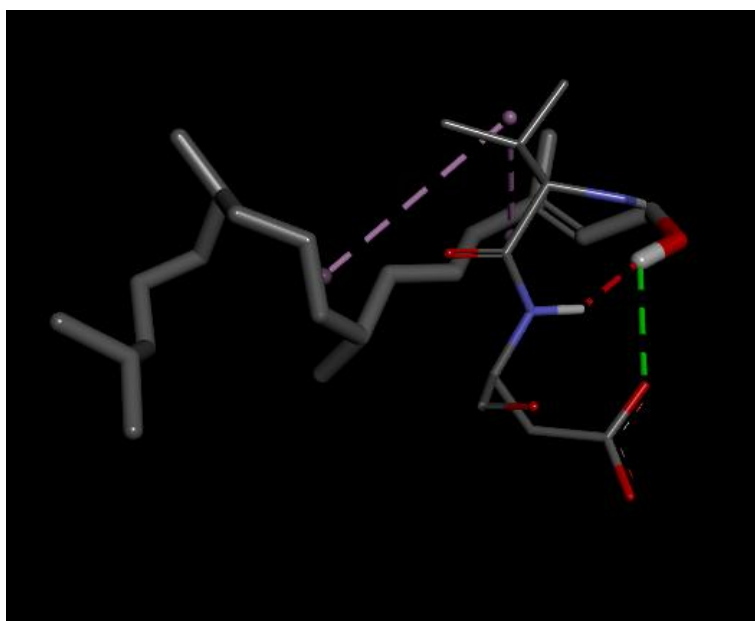
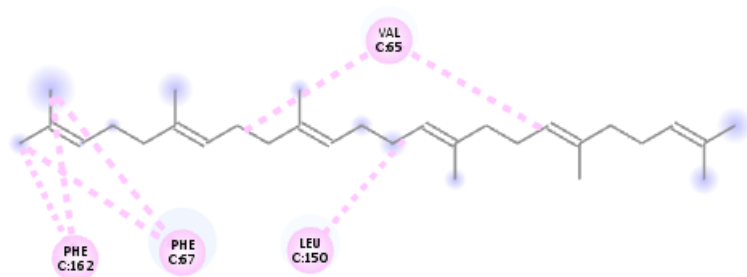


Figure 3: 2D & 3D Structure of R-R,R-E- trans-Phytol with TPO



Interactions

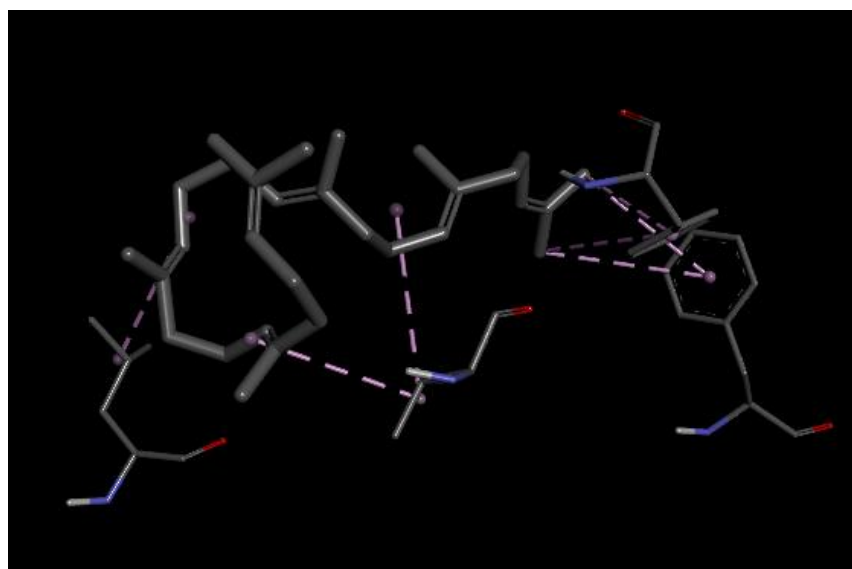


Figure 4: 2D & 3D Structure of squalene with TPO
ADMET analysis result of the best phytochemicals

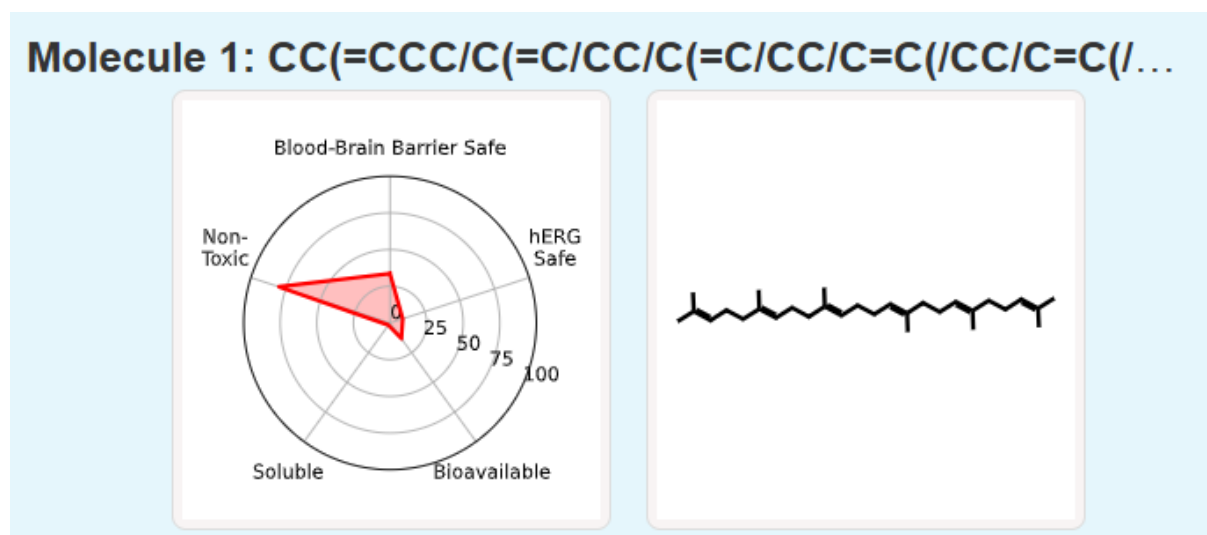
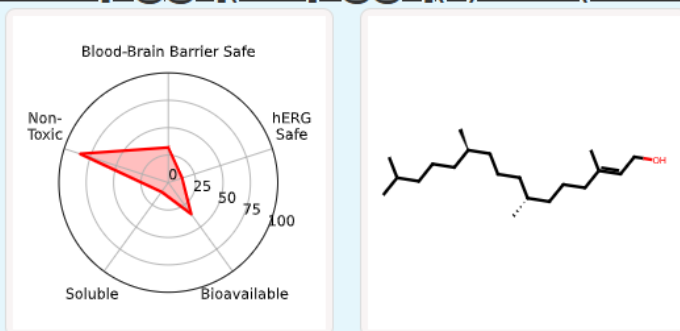
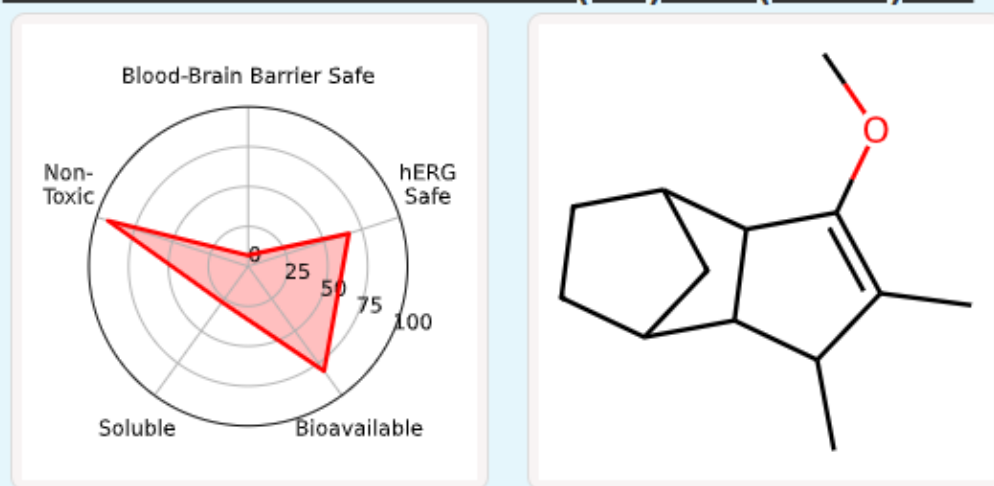


Figure 5: ADMET result of Squalene

Molecule 2: C[C@@H](CCC[C@@H](C)CCC/C(=C/CO)/C...

Figure 6: ADMET result of R-R,R-E- trans-Phytol
Molecule 3: CC1C2C3CCC(C3)C2C(=C1C)OC

Figure 7: ADMET result of 3a.4,5,6,7,7a-hexahydro-4,7-methanoindene

DISCUSSION

This study explored the inhibitory potential of phytochemical constituents of *Hyptis verticillata* against thyroid peroxidase (TPO), an essential enzyme in the biosynthesis of thyroid hormones. Hyperthyroidism management increasingly seeks safer, naturally derived therapeutic alternatives due to the adverse effects and limitations of conventional antithyroid medications. Molecular docking, drug-likeness assessment, and ADMET predictions were employed to identify promising plant-derived inhibitors of TPO.

Drug-Likeness Assessment

Drug-likeness evaluation serves as an important preliminary filter for identifying compounds with suitable physicochemical properties for oral drug development. Among the tested phytochemicals, **9,12,15-octadecatrien-1-ol** and **1-fluorodecane** exhibited the best compliance with Lipinski, Ghose, Veber, Egan, and Muegge rules, indicating favorable oral bioavailability profiles. Other compounds—particularly **squalene**, **phytol**, and **3a.4,5,6,7,7a-hexahydro-4,7-methanoindene**—satisfied most but not all criteria, primarily due to high molecular flexibility or hydrophobicity, traits typical of many terpenoids (Awad et al., 2018). Despite rule violations, deviations in natural products are common and do not preclude biological activity. Numerous plant-derived terpenoids and phenolics function as effective enzyme modulators regardless of high lipophilicity or molecular size, emphasizing the value of subsequent docking and ADMET analyses.

Molecular Docking and Binding Affinity

The molecular docking scores ranged from **-3.8 to -5.5 kcal/mol**, with more negative scores indicating stronger binding affinity to TPO. Three compounds demonstrated notably favorable interactions:

Squalene (-5.5 kcal/mol)

Squalene exhibited the strongest binding affinity. Its extended hydrophobic carbon chain enabled solid stabilization within the predominantly hydrophobic active site of TPO. This extensive hydrophobic anchoring suggests a mechanism involving **steric blockade**, potentially preventing access of iodide and hydrogen peroxide to the heme catalytic center (Taurog, 2013). Similar inhibitory patterns have been reported for hydrophobic plant phenolics and terpenoids, which interfere with iodination reactions by occupying the enzyme's catalytic cavity (Habza-Kowalska et al., 2019).

R-R,R-E-trans-Phytol (-5.3 kcal/mol)

Phytol displayed strong hydrophobic interactions and limited polar contributions due to its terminal hydroxyl group. Its amphiphilic nature may enable stable positioning within the TPO canal, interfering with iodotyrosine coupling reactions. Diterpenoids with structural similarities to phytol have been documented to modulate oxidative and peroxidase-related enzyme pathways, supporting its observed activity.

3a,4,5,6,7,7a-Hexahydro-4,7-methanoindene (-5.0 kcal/mol)

The compact bicyclic framework of this terpenoid allowed efficient hydrophobic fitting into the TPO active pocket. Its predicted interaction mode reflects tight occupancy rather than hydrogen bonding, which may be sufficient to obstruct substrate binding and reduce catalytic turnover. Lower-affinity compounds, such as **4,7-methanon-1H-indene**, **9,12,15-octadecatrien-1-ol**, **1-octadecyne**, and **1-fluorodecane**, exhibited moderate to weak binding affinity. Their structural constraints or limited hydrophobic interaction surfaces likely reduced their ability to engage TPO effectively. Overall, the docking profile suggests that **hydrophobicity and molecular size** play central roles in stabilizing TPO–ligand interactions for these phytochemicals.

Mechanistic Implications for TPO Inhibition

TPO catalyzes both iodination and coupling reactions essential for synthesizing T₃ and T₄. Many known inhibitors—including propylthiouracil and methimazole—bind near the heme group, blocking iodide oxidation and preventing coupling reactions (Suryandari et al., 2020). The phytochemicals in this study, particularly squalene and phytol, appear to bind in a similar region of the active pocket, suggesting a **non-covalent, hydrophobic inhibitory mechanism**.

This mechanism aligns with earlier findings demonstrating that phenolic compounds and large hydrophobic molecules can inhibit TPO by interfering with iodide access or electron transfer pathways (Habza-Kowalska et al., 2019). Therefore, the phytochemicals evaluated here may exert functional inhibition by obstructing substrate orientation or entry rather than forming covalent modifications like some synthetic antithyroid drugs.

ADMET and Pharmacokinetic Considerations

ADMET predictions showed: **Good gastrointestinal absorption** for squalene, phytol, and hexahydro-methanoindene. **Low predicted toxicity**, including absence of hepatotoxicity and mutagenicity, a critical advantage over methimazole and propylthiouracil. **Limited blood–brain barrier permeability**, reducing the likelihood of CNS-related adverse effects. **Acceptable metabolic stability**, with no red flags for major cytochrome P450 inhibition. Although the high lipophilicity of squalene and phytol may contribute to tissue accumulation, their safety profiles and predicted low toxicity make them promising candidates for further evaluation.

Comparison with Standard Antithyroid Agents

Docking studies on standard antithyroid drugs report binding affinity ranges of approximately -5.0 to -6.0 kcal/mol (Suryandari et al., 2020). The binding energies of phytol and squalene fall squarely within this range, suggesting potential functional comparability to existing TPO inhibitors. Importantly, unlike synthetic drugs associated with agranulocytosis and hepatotoxicity, natural terpenoids generally show milder pharmacodynamic effects with reduced toxicity risks, indicating possible therapeutic advantages.

Relevance to Ethnomedicinal Uses of *H. verticillata*

Hyptis verticillata has longstanding traditional use for inflammatory, metabolic, and endocrine-related disorders (Kuhnt et al., 1995; Picking et al., 2013). The computational evidence presented here suggests a tangible biochemical basis for its ethnomedicinal application by demonstrating the capacity of its constituents to interfere with thyroid hormone synthesis at the molecular level. This finding supports both the traditional relevance of the plant and the scientific rationale for further laboratory and clinical investigations.

CONCLUSION

The findings from this molecular docking analysis reveal that several phytochemicals from *Hyptis verticillata*, particularly squalene, R-R,R-E-trans-phytol, and 3a.4,5,6,7,7a-hexahydro-4,7-methanoindene, possess favorable binding affinity and stable interaction with thyroid peroxidase. Their predicted mechanisms suggest potential inhibition of TPO catalytic activity, supporting their role as natural candidates for hyperthyroidism management. ADMET profiling further highlights their promising pharmacokinetic and safety characteristics. These results collectively justify advancing these compounds to in vitro and in vivo validation studies to confirm therapeutic potential.

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