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IN-SILICO STRUCTURE-ACTIVITY RELATIONSHIP AND MOLECULAR DOCKING STUDY OF NEROLIDOL AND ITS MODIFIED ANALOGUE AGAINST THE *TRYPANOSOMABRUCEI*HEXOKINASE

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Abstract

Trypanosoma brucei is a species of parasitic kinetoplastid belonging to the genus *Trypanosoma*. The parasite is the cause of a vector-borne disease of vertebrate animals, including humans, carried by genera of tsetse fly in sub-Saharan Africa. In humans T. brucei causes African trypanosomiasis, or sleeping sickness. Nerolidol is a terpene that is also known as peruviol. It is found in a number of plants, including cannabis sativa. Nerolidol has two isomers and is a sesquiterpene, which means it is more stable than other terpenes. Molecular docking study was carried out on nerolidol and its modified analogue against *Trypanosoma brucei* hexokinase using the Autodock Vina software. Extensive structure activity relationship study was also carried out on these molecules. The physiochemical analysis, lipophilicity, solubility, pharmacokinetics and Lipinski drug likeness of nerolidol and its monosubstituted analogue was evaluated. The modification was done by substituting one of the methyl group (CH_3) attachment of nerolidol with a COOH functional group. The scoring function (empirical binding free energy) and hydrogen bond formation was used to estimate the inhibitory effect of the protein-ligand complex. The binding energy of nerolidol was -5.9kcal/mol while that of its COOH analogue was -6.1Kcal/mol. The low free binding energy value (more negative value) displayed by the COOH analogue means it show a better activity than nerolidol. The two ligands showed the ability to cross the blood brain barrier (BBB). This also shows that they are both neuroactive. This result indicated that the COOH analogue may be better antiparasitic agent than nerolidol. Synthesis and pre-clinical studies of this monosubstituted derivative against the *Trypanosoma brucei* hexokinase is therefore recommended in order to confirm its potential as a better antiparasitic agent.

Keywords: *Docking; Trypanosome brucei Hexokinase; Pharmacokinetics; Blood Brain Barrier*

Introduction

Trypanosomiasis or trypanosomosis is the name of several diseases in vertebrates caused by parasitic protozoan trypanosomes of the genus *Trypanosoma* [1]. In humans this includes African trypanosomiasis and Chagas disease. A number of other diseases occur in other animals. Approximately 30,000 people in 36 countries of sub-Saharan Africa have African trypanosomiasis, which is caused by either *Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodesiense* [2]. Chagas disease causes 21,000 deaths per year mainly in Latin America [3]. The tsetse fly bite erupts into a red chancre sore and within a few weeks, the person can experience fever, swollen lymph glands, blood in urine, aching muscles and joints, headaches and irritability. In the first phase, the patient has only intermittent bouts of fever with lymphadenopathy together with other non-specific signs and symptoms [4]. The second stage of the disease is marked by involvement of the central nervous system with extensive neurological effects like changes in personality, alteration of the biological clock (the circadian rhythm), confusion, slurred speech, seizures and difficulty in walking and talking. These problems can develop over many years and if not treated, the person dies. It is common to the African continent [5].

Trypanosoma brucei hexokinase is the first enzyme of the glycolytic pathway, and it catalyzes glucose phosphorylation. The enzyme has a low sequence homology (36%) with human hexokinase which enhances the chances of developing specifc inhibitors. The human enzyme is also twice as large owing to possible gene duplication [6]. *Trypanosoma brucei* hexokinase has also been shown to have relatively low specifcity towards sugars and is not regulated by glucose 6-phosphate and glucose 1, 6 -bisphosphate, like the human hexokinase. In addition, studies have shown that the enzyme also exhibits low specificity towards nucleotides including ATP, Uridine Triphosphate (UTP) and Cytidine Triphosphate (CTP) [7]. According to these authors, the lack of specifcity may render the nucleotide-binding pocket of this enzyme an interesting target for drug development. The glucose-binding pocket offers an interesting target as well. Moreover, RNAi studies have shown that the *Trypanosoma brucei* hexokinase is essential for the parasite's survival [8].

Nerolidol, also known as peruviol and penetrol, is a naturally occurring sesquiterpene found in the essential oils of many types of plants and flowers [9]. There are two isomers of nerolidol, cis and trans, which differ in the geometry about the central double bond. Nerolidol is present in neroli, ginger, jasmine, lavender, tea tree, Cannabis sativa, and lemon grass, and is a dominant scent compound in Brassavolanodosa [10]. Additionally, it is known for various biological activities which include antioxidant, anti fungal, anticancer, and antimicrobial activity [11].

In this study, an *In-Silico* Structure-Activity Relationship and molecular docking study was directed at investigating the inhibitory effect of nerolidol and its modified analogue on the structure and function of *Trypanosoma brucei* hexokinase, by predicting the binding energies and various pharmacokinetics parameters necessary for computational drug design.

Materials and Methods

Sequence Retrieval

The *Trypanosoma brucei* hexokinase amino acid sequence was retrieved from the National Center for Biotechnology Information (NCBI) Database. The NCBI houses database series with relevance to biotechnology and biomedicine and is also a useful resource for bioinformatics services, analysis and tools. Major databases comprise the GenBank which is for sequences of DNA and PubMed which is a bibliographic repository for literatures regarding biomedicine. Other databases include the NCBI and the Epigenomics database [12]. *Trypanosoma brucei* hexokinase was assigned the accession number of CAC69958.1.

Physiological–biochemical characterization

The physicochemical characterization,molecular weight, isoelectric point (pI), total number of negative and positive residues, aliphatic index, extinction coefficient, instability index, and grand average hydropathicity (GRAVY) of the *Trypanosoma brucei* hexokinase were predicted utilizing the ExpasyProtparam server [13].

Protein 3D Structure

The crystallized 3D structure of the *Trypanosoma brucei* hexokinase was not available for downloads from the Protein Data Bank (PDB) repository. Therefore a homology model of the 3D structure was generated using the SWISS-MODEL structural bioinformatics server [14].

Ligand Preparation

The 2D structure of nerolidol and its modified analogue were designed using the MarvinSketch software [15]. Both designed structures were downloaded and saved as mrv files in preparation for docking.

File Conversion

Saved mrv files from the ligand preparation process were converted into SMILES strings (Simplified Molecular Input-Line-Entry System) using the Open Babel Open Source Chemistry Toolbox. Open Babel, a chemical toolbox is designed to speak many of the languages of chemical data. It's an open and collaborative project allowing anyone to make searches, conversions, analysis, or storage data from molecular modeling, chemistry, solid-state materials, biochemistry, or related areas [16].

Ligand Minimization and Visualization

Nerolidol and its modified analogue were minimized using the UCSF Chimera software. UCSF Chimera is an extensible program for analyzing and interactively visualizing molecular structures and related data which include supramolecular assemblies, density maps, alignment of sequences, results from molecular docking, trajectories and conformational ensembles [17].

Molecular docking

Molecular docking was performed using AutoDock Vina Software [18]. Physicochemical, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of Nerolidol and its analogue were determined using SwissADME Server [19].

Results and Discussion

Structure of 6-Gingerol

Figure 1 and 2 shows the 2D structures of nerolidol and its COOH derivative as designed by the MarvinSketch software. Modifications that resulted into the derivative of nerolidol was made through the substitution of the CH3 group attachment to the carbon-2 (C2) of the compound with the COOH functional group.

The 3D Structure of Nerolidol

The 3D structure of nerolidol was generated though the input of the canonical SMILES obtained from the OpenBabel conversion of the 2D structure of the compound into the Chimera visualizer. The "Build Structure" function of the Chimera visualizer was enacted to generate a 3D structure which was saved as a Mol2 file. The saved Mol2 file was viewed using the Pymol visualizer and this same software was used in labeling and viewing each atom making up the compound.

The nerolidol modification as reported in figure 2 was made by substituting one of the CH3 functional group attachment of the compound with another functional group (COOH). This CH3 group is clearly depicted in the nerolidol 3D structure in figure 3. The carbon component of the functional group is labeled C1 while the H components are labeled H1, H2, H3

Molecular Docking

Selected docking results obtained from the use of the AutoDock Vina docking software were displayed in figure 4 and 5. The displayed pictures show the differential poses and binding orientation of nerolidol and its COOH analogue respectively to the *Trypanosoma brucei* hexokinase. At the left portion of the displayed picture is the table depicting the series of possible binding scores that can be obtained based on the differential binding poses of the experimental compounds. The best binding score for each docking processes were selected for the purpose of this study.

The docking poses of the experimental compounds showed that they bind in a very similar pattern with the active site of *Trypanosoma brucei* hexokinase, as is evident from the superposition of nerolidol and its COOH analogue in Figures 4 and 5. The calculated free energy of binding of the nerolidol and its COOH analogue were -5.9, and -6.1Kcal/mol. This confirms that the structural modification implemented in this study is

significantly related to their activity [20, 21]. Also, this proved the reliability of the docking results [22].

In Silico Pharmacokinetics

Lipinski's rule of five [23] which can also be referred to as the Pfizer's rule of five is a rule described for the evaluation of drug likeness or for the determination of biological and pharmacological activities in specific compounds for the purpose of evaluating physical and chemical properties in determining likely orally active drugs for administration. The rule states that, orally active drugs in general must not violate more than one of the following criteria: The hydrogen bond donors must not be more than 5 (the summation of nitrogen–hydrogen and oxygen–hydrogen bonds), hydrogen bond acceptors must not exceed 10 (all nitrogen or oxygen atoms), the molecular mass of the compound must be less than 500 Da, an octanol-water partition coefficient (log P) must not exceed 5 [24]. Nerolidol and its COOH analogue violated none of the Lipinski's rule and therefore are likely to be drugs.

The solubility of a compound in water could improve its biotransformation and elimination as a drug [24]. Nerolidol and its COOH analogue were soluble in water (Figure 6 and 7). The molecular weight of both experimental compounds were less than 500g/mol, showing that they can be considered as drug-like [25]. A compound can also be considered drug-like if it is characterized by high lipophilicity (less than 5) [26] and this is expressed as Log Po/w. The lipophilicity values of nerolidol and its modified analogue were less than 5 and are most likely to be drugs.

High penetration is needed for most of the drugs targeting the central nervous system (CNS), whereas blood brain barrier (BBB) penetration should be minimized for non-CNS drugs to avoid undesired side-effects [27]. Pharmacokinetically, the gastrointestinal drug absorption of both experimental compounds were high and both has the ability to cross the blood brain barrier (BBB). This shows that these compounds are neuroactive agents.

For synthetic accessibility, values of 5 to10 means that the drug could be synthesized [24]. Nerolidol and its COOH analogue showed values less than 4. This means that the compounds can easily be synthesized in the laboratory.

Physiological and Biochemical Characterization of the Trypanosoma brucei Hexokinase

The Isoelectric point calculation indicated that the *Trypanosoma brucei* hexokinase is basic in nature (pI = 9.04). Instability index measures the stability of a protein in a test tube [28]. *Trypanosoma brucei* hexokinase had an instability index of 39.34 and a half-life of 30 hours in mammalian reticulocyte. This enzyme is therefore regarded as stable as with an instability index value less than 40.

Since there is a good correlation between aliphatic index and thermostability of globular proteins [29], it can predict thermostability. The *Trypanosoma brucei* hexokinase has a high aliphatic index of 91.91 indicating its increased stability at high temperatures.

The Grand average hydropathicity (GRAVY) predicts interaction of a protein with water [30], with lower values indicating better interaction. Hsp90 proteins had very low values, indicating good interaction with water. This was supported by hydrophobicity calculations indicating hydrophilic behavior.

Conclusion

We carried out an *In-Silico* Structure Activity Relationship and molecular docking study on *Trypanosoma brucei* hexokinase, using nerolidol and its structural analogue as the experimental compounds. The results obtained indicated that the COOH analogue may have a better functional activity having shown a high binding energy value and exhibited a higher level of specificity and affinity with the target enzyme. These compounds also have been seen to be neuroactive drugs as they possess the ability to penetrate the blood brain barrier of the central nervous system (CNS).

Synthesis and pre-clinical studies of the monosubstituted derivative of nerolidol is therefore recommended.

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Figure 1: The two dimensional (2D) structure of Nerolidol as designed using the MarvinSketch software.

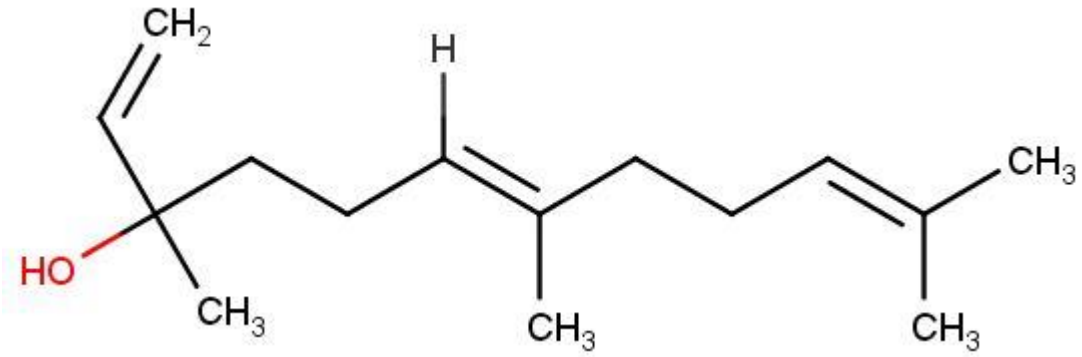


Figure 2: The two dimensional (2D) structure of COOH derivative of Nerolidol as designed using the MarvinSketch software.

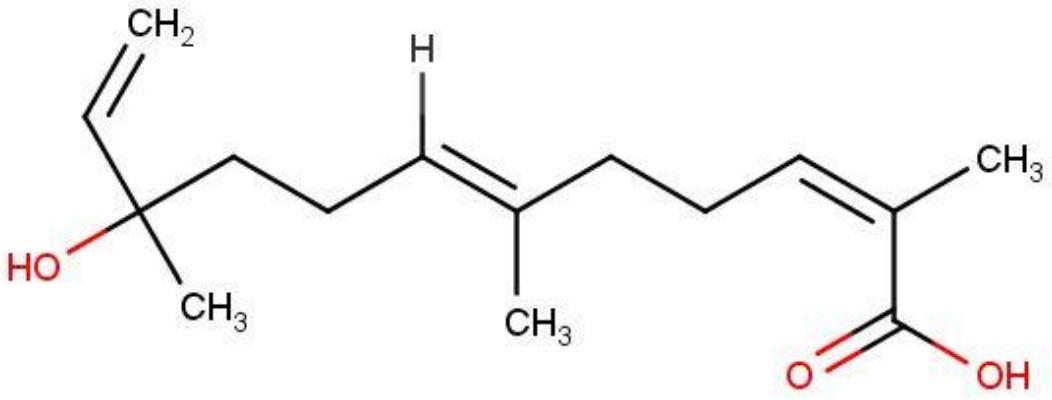


Figure 3: 3D structure of Nerolidol showing all labeled atoms

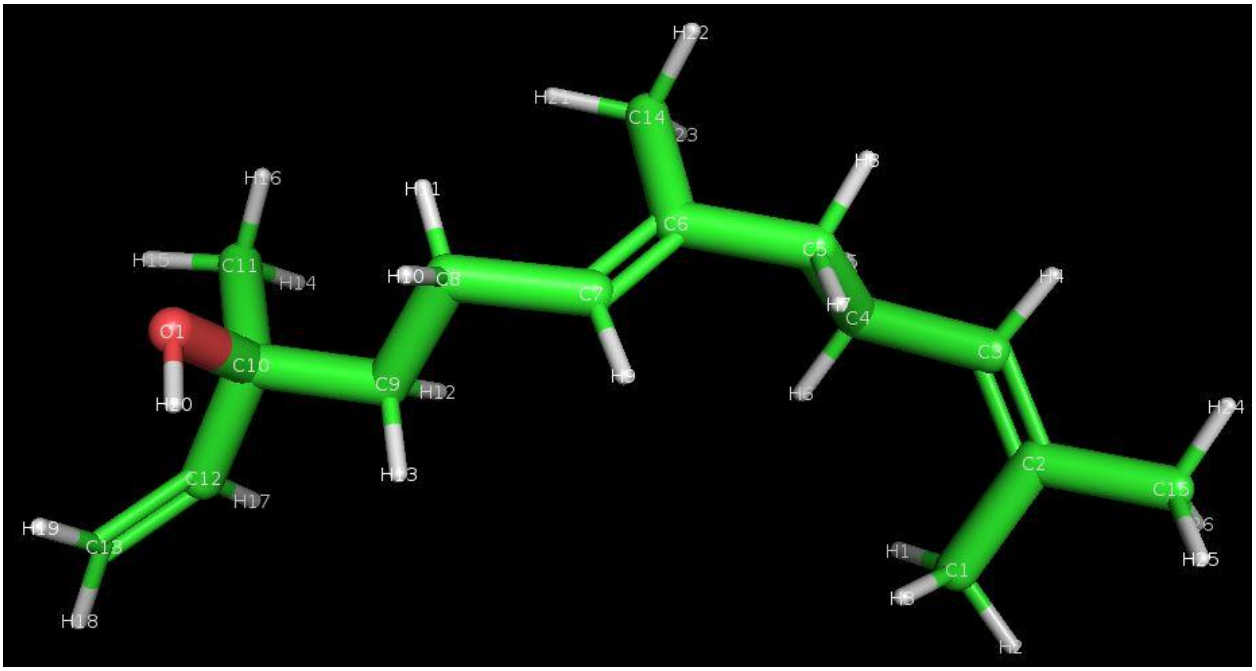


Figure 4: Predicted molecular docking score and binding pose between *Trypanosoma brucei* Hexokinaseand Nerolidol

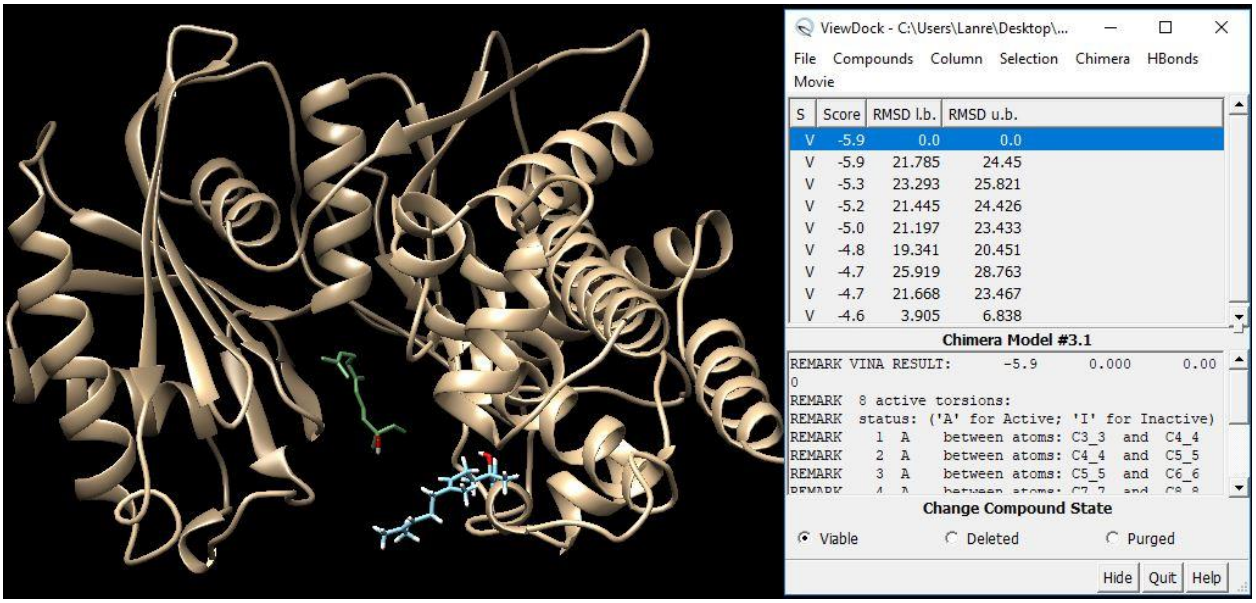


Figure 5: Predicted molecular docking score and binding pose between *Trypanosoma brucei* Hexokinaseand the COOH Analogue of Nerolidol

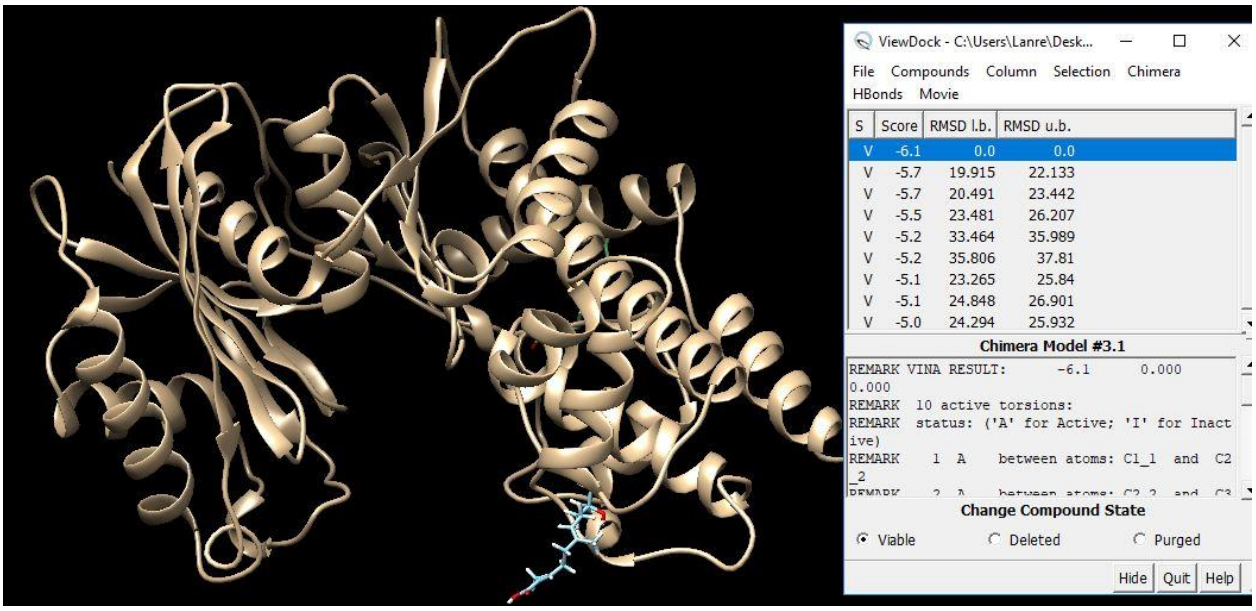
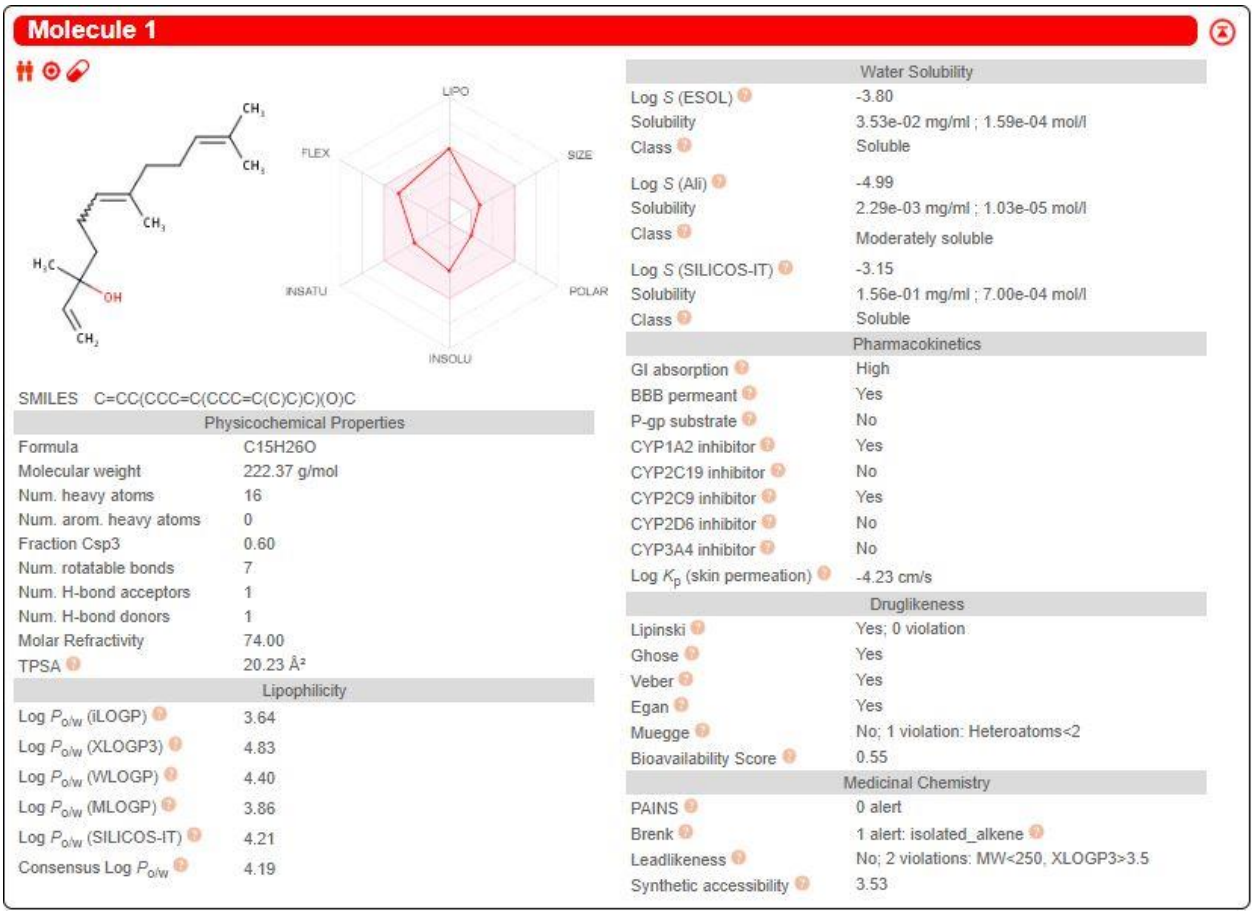


Figure 6: *In-Silico* Pharmacokinetics and Drug likeness Prediction for Nerolidol



Molecule 1

SMILES C=CC(CCC=C(CCC=C(C)C)C)(O)C

Physicochemical Properties	
Formula	C15H26O
Molecular weight	222.37 g/mol
Num. heavy atoms	16
Num. arom. heavy atoms	0
Fraction Csp3	0.60
Num. rotatable bonds	7
Num. H-bond acceptors	1
Num. H-bond donors	1
Molar Refractivity	74.00
TPSA	20.23 Å²

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	3.64
Log $P_{o/w}$ (XLOGP3)	4.83
Log $P_{o/w}$ (WLOGP)	4.40
Log $P_{o/w}$ (MLOGP)	3.86
Log $P_{o/w}$ (SILICOS-IT)	4.21
Consensus Log $P_{o/w}$	4.19

Water Solubility	
Log *S* (ESOL)	-3.80
Solubility	3.53e-02 mg/ml ; 1.59e-04 mol/l
Class	Soluble
Log *S* (Ali)	-4.99
Solubility	2.29e-03 mg/ml ; 1.03e-05 mol/l
Class	Moderately soluble
Log *S* (SILICOS-IT)	-3.15
Solubility	1.56e-01 mg/ml ; 7.00e-04 mol/l
Class	Soluble

Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-4.23 cm/s

Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	No; 1 violation: Heteroatoms<2
Bioavailability Score	0.55

Medicinal Chemistry	
PAINS	0 alert
Brenk	1 alert: isolated_alkene
Leadlikeness	No; 2 violations: MW<250, XLOGP3>3.5
Synthetic accessibility	3.53

Figure 7: *In-Silico* Pharmacokinetics and Drug likeness Prediction for the COOH Analogue of Nerolidol