

Research Article

Evaluation of Carvacrol and its Receptor (Ubiquinone-c-reductase) as a Potential Anti-malarial Drug

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A B S T R A C T

In recent times natural products are exploiting for its medicinal properties due to its minimum side-effects. Carvacrol which is one of the monoterpene's isolated from the essential oil of *Oreganum vulgare* possesses various antimicrobial, anti-oxidant and anti-cancerous activities. Therefore, in this present study carvacrol was selected to study its anti-malarial activity. A comparative study of commercially available drugs (Malarone) and carvacrol was conducted to understand the putative mechanism of action of carvacrol and its potential as a natural anti-malarial drug. Way2drug tool was used to predict the biological activity of the compound using the PASS Online server to predict the target protein of carvacrol. Further, Ubiquinone-c-reductase was selected for molecular docking with carvacrol because Malarone cures malaria by inhibiting the activity of enzyme. Ubiquinone-c-reductase is the key enzyme of the electron transport chain. It resulted that the amino acids positioned at 264-267 in chain 'A' of ubiquinone-c-reductase, were efficiently targeted by carvacrol with a minimum energy value of -5.4 Kcal/ mol. The present study resulted that carvacrol behaves as a natural blocker for ub-c-reductase may serve as a possible mechanism to develop carvacrol as a potential herbal anti-malarial drug.

Keywords: Carvacrol, *Oreganum vulgare*, Malaria and Malarone

Introduction

Malaria is one of the infectious diseases affecting humans caused by parasite namely *Plasmodium* type. Out of all types, *Plasmodium falciparum* is one of the major parasites causing deaths due to malaria. Female *Anopheles* mosquito acts as a vector for the parasite to human blood. Further, from the blood, parasite reaches to liver for its maturation and reproduction. After replica, the parasite enters in erythrocyte, de-differentiates,

replicates the DNA and thereby infects RBCs. *P. falciparum* itself shows less activity of the Electron Transport Chain (ETC) pathway thereby used the host ETC pathway of erythrocyte for synthesizing pyrimidine and transfer of an electron from cytochrome c reductase to cytochrome c.⁷ Various studies have demonstrated that inhibitors of ETC pathway can be targeted to design antimalarial drug.⁸ The prevalent antimalarial drug commercialized is Malarone, a combination of atovaquone and proguanil used to treat and cure malaria.¹ Atovaquone is the analog of ubiquinone

and thereby inhibits the cytochrome-c-reductase activity in the intra-erythrocyte parasitic ETC pathway.⁴ It disturbs the membrane potential of mitochondria, further causes collapsing, damaging and death of cells. It has been studied that atovaquone is responsible for the parasite death due to the indirect inhibition of dihydroorotate dehydrogenase, which requires the ETC pathway and is essential to pyrimidine biosynthesis.¹¹ Proguanil is used in combination with atovaquone as another antimalarial drug. It gets converted to its active form cycloguanil, as it enters inside the liver (Tablets). Cycloguanil inhibits the activity of the enzyme dihydrofolate reductase, which catalyzes the conversion of dihydrofolate to Tetrahydrofolate (THF).²

However, some findings report adverse effects of using Malarone such as coughing, diarrhea, nausea, vomiting or headache. These days, natural products are preferred as they have more advantages with minimum adverse effects. In this study antimalarial activity of carvacrol is reported. Carvacrol is one of the secondary metabolites extracted from the essential oil of *Oreganum vulgare*. It is synthesized by the Mevalonic acid pathway, which occurs in the cytosol of plant cells. Acetyl-CoA is a precursor of this pathway and the products are Isopentenyl Pyrophosphate (IPP) and Dimethylallyl Pyrophosphate (DMAPP).⁵ DMAPP undergoes condensation with IPP to synthesize Geranyl diphosphate (GPP) in the presence of enzyme prenyltransferase. These GPP undergo cyclization catalyzed by terpene synthase in order to yield terpenoid compound.⁶ The main enzyme involved in the synthesis of carvacrol in *Oreganum vulgare* is terpene synthase.⁶ This study is focused on predicting the potential target of carvacrol to inhibit the growth of *Plasmodium falciparum* using the Way2drug tool. This bioinformatics tool helps in identifying the most probable new lead molecule with specific biological activity. It predicts biological activities based on the molecular structure of the compound.¹³ Probable value $P=1/n \sum P_a/(P_a+P_i)$ is used to predict the activity of any compound and significant P value showed the compound with the highest biological potential. A comparative analysis has been done among carvacrol and constituents of commercialized antimalarial drug Malarone to evaluate the applicability of carvacrol as an effective drug.

To study the molecular binding of carvacrol with the potential receptor, molecular docking is done using the AutoDock standalone tool. The tool used in the study Auto dock 4 was developed by Morris GM et al. after its first release in 1990.¹¹ This tool enables us to perform the docking of a macromolecule with the ligand which performs efficiently by foretelling binding conformations and binding energies between macromolecule and ligand under consideration. This program performs by initially looking for hefty conformational space for the ligand in the region of protein which is carried out by Lamarckian genetic

algorithm.⁹ The auto dock uses a grid-based method for the calculation of binding energies of the conformations. In this approach, the target macromolecule is entrenched in a grid after which the investigative atom is placed chronologically in each grid point and their respective binding energy is noted in each grid. This binding energy is used later during the docking imitation. This tool also allows the user to carry out a simulated annealing search and traditional genetic algorithm search.¹⁰ In the present study, effort has been made to study the efficacy of the natural herbal molecule in comparison to commercially available anti-malarial drugs.

Materials and Methods

Prediction of Anti-malarial Activity

Bioinformatics tool <http://way2drug.com/> is available to predict the biological activity of the compound. It provides access to the PASS Online server to obtain the predicted biological activity, mechanism of action of the selected compound.

Installation of the Tools

The latest available version of the AutoDock was downloaded from <http://autodock.scripps.edu/downloads/autodock-registration/autodock-4-2-download-page/> for the windows. The program was installed in the default directory C:\Program Files (x86)\.

Retrieval of the Macromolecule and Ligand

The macromolecule used in the study was ub-c reductase. This macromolecule was docked with the ligand carvacrol with the help of autodock4. As the tools read pdb file format of the macromolecule and ligand thus the 3D structure of ub-c reductase was retrieved from <https://www.rcsb.org/> in pdb format. The structure of the ub-c reductase is stored under the PDB ID: 5XTE. Similarly, carvacrol structure was retrieved from <https://pubchem.ncbi.nlm.nih.gov> in the sdf format which was then converted to pdbqt format by using Open Babel¹². Finally, both the pdb and pdbqt files of the macromolecule and ligand were saved under C:\workspace.

Setting up the Macromolecule

While performing the analysis under mention protocol was followed:

Initially open the ub_c_reductase.pdb file in the text editor. Furthermore, A and B chain atom line was selected and stored as a newly edited file as "ub_c_reductase_a_b_chain" followed by its docking.

The autoDock tool was opened by clicking on its desktop shortcut. Under the main menu the path used was: click File> Read Molecule> browse the workspace Folder > select the macromolecule file named as ub_c_reductase_a_b_chain.pdb > click open. Removed water by Edit> click on Delete Water option. Hydrogen atoms were added to the

macromolecule by Edit > Hydrogens > add > All Hydrogens > ok option. Non-Polar hydrogen were merged by Edit > Hydrogen > Merge Non-Polar, then Click Edit > Charges > Compute Gasteiger > Total Gasteiger charge added = -14.9962 > Click ok and saved the file using pathway File > Save > Write pdb > Ok which writes the newly modified pdb file to the workspace folder. Then after, clicked on Grid > Macromolecule > choose > select macromolecule > ok > saved as "protein.pdbqt" in the same folder containing the pdb files.

Table 1. Predicted biological activity of (A) carvacrol and (B) atovaquone, selected Pa>Pi where Pa is the probability of the compound being active for a particular activity and Pi is the probability of inactive

Pa	Pi	Activity
0.931	0.004	Ubiquinol-cytochrome-c reductase inhibitor
0.908	0.001	SULT1A3 substrate
0.898	0.003	Antiseptic
0.888	0.003	Alkane 1-monooxygenase inhibitor
0.884	0.002	Carminative

Pa	Pi	Activity
0.775	0.041	Ubiquinol-cytochrome-c reductase inhibitor
0.730	0.010	27-Hydroxycholesterol 7alpha-monooxygenase inhibitor
0.734	0.014	Aldehyde oxidase inhibitor
0.759	0.046	CYP2C12 substrate
0.717	0.011	CYP2C9 substrate

Table 2. Binding energy and RMSD value of 10 conformations obtained after AutoDock

Bound conformation Number	Binding energy (Kcal/mol)	Distance from finest bound conformation
		RMSD Lower Bound
1	-5.4	0.000
2	-5.3	0.479
3	-5.3	22.504
4	-5.1	22.059
5	-5.1	36.325
6	-5.0	30.695
7	-4.9	20.552
8	-4.6	21.551
9	-4.6	8.153

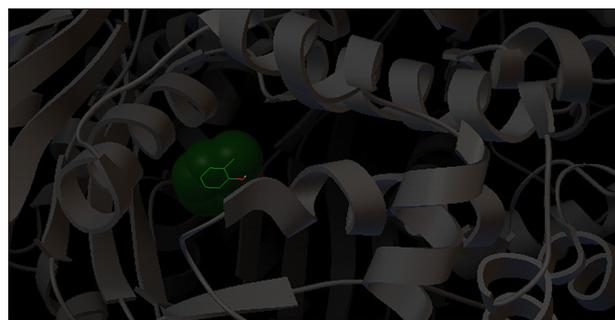


Figure 1. Carvacrol and ubiquinol-c-reductase docked conformation with the lowest binding energy

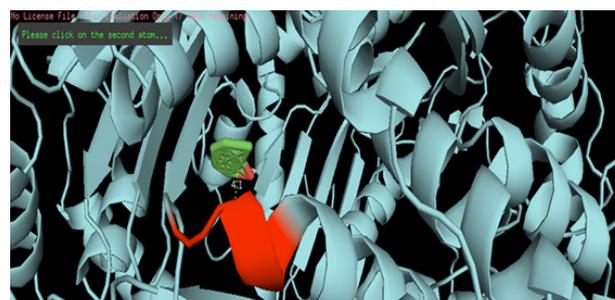


Figure 2. Conformation depicting distance between the carvacrol and binding site of ubiquinol-c-reductase

Setting up the Ligand

In AutoDock clicked on Ligand > input > open > select ligand file "carvacrol.pdbqt" > open AutoDock automatically modified the ligand in terms of addition of charges and also detects the frequency of rotatable bonds in the ligand. In this case, 6 aromatic carbons were found in the ligand and one rotatable bond was detected. TORSDOF was set to 1 automatically with respect to detected rotatable bonds. Further, clicked on Ligand > Torsion Tree > Detect root option wherein root was indicated by the green circle in the Autodock visualization window.

Setting Grid Space

At this point boundary of the grid box for macromolecule was set under which the ligand was studied for its potential binding. To study the grid space setting, clicked on Grid > Macromolecule > Choose > Select the file ub_c_reductase_a_b_chain.pdb > saved the modified macromolecule in pdbqt extension in the same workspace destination. Further, for ligand, clicked on Grid > Set map types > choose ligand > select carvacrol > click ligand options and clicked on Grid > Grid Box finally.¹² The following values were entered to set the grid box as shown in Figure 1.

X center=51.019

Y center=92.826

Z center=94.059

Number of points in x dimension=92

Number of points in y dimension=74

Number of points in z dimension=94

Spacing=1.000 angstrom

Clicked on File > Close saving current option. Clicked on Grid > output > save gpf > give file name "grid.gpf" > save to the workspace folder.

Performing Autogrid

To perform auto grid the option selected was Run > Run Autogrid > Autogrid which was further followed by the selection of path under the provided options in the Autogrid window.

Program Pathname=C:/Program Files (x86)/MGLTools-1.5.6/4.2.6/autogrid4.exe

Parameter Filename=C:/workspace/grid.gpf

Log File Name=C:/workspace/grid.glg (automatically write while the parameter filename path was added) shown in figure 1. To launch the autogrid clicked Launch option and AutoDock process manager for the window to appear. On the successful completion, the process manager window disappeared.

Performing Autodock

Docking is performed absolutely by means of several search strategies. The best approach is the Lamarck genetic set of rules (LGA), however, it's far traditional genetic algorithms and simulated annealing are also available. For traditional structures, AutoDock starts over and over to give extra anchored conformations and examine the anticipated power and the consistency of the results. Autodock analysis was done after complete run.

Result

Prediction of Anti-Malarial Activity

Comparative evaluation of biological efficiency of Atovaquone and Carvacrol was conducted using Pass Online software and the results obtained were shown in table 1.

Atovaquone showed Pa-value 0.775 (where Pa value is the probability of the compound being active for specific activity) as Ubiquinol-c-reductase inhibitor which is less than the Pa value of Carvacrol (Pa-value=0.931). The obtained data revealed that the Carvacrol inhibits the activity of Ubiquinol-cytochrome-c reductase more effectively as compared to Atovaquone with Pa value of 0.931 which is also the target receptor of commercialized drug Malarone.

Molecular Docking

After predicting the receptor of Carvacrol, docking of the ubiquinone-c-reductase with ligand Carvacrol was performed by using AutoDock, in return on accessing the option in AutoDock "Play ranked by energy" it was

observed that the entire conformation of Carvacrol docked with Ubiquinone-c-reductase in accordance with their free binding energies. A total of 9 docked conformations were returned by the AutoDock after its successful completion. The binding energy and RMSD (root-mean-square deviation) value for all the conformations obtained is as under in Table 2.

The conformation with the lowest energy returned by AutoDock is shown below as Figure 1, (Delano) wherein Ubiquinone-c-reductase has been shown in ribbon view and Carvacrol with lines and atomic spheres respectively³.

Carvacrol binds to ubiquinone-c-reductase at position 264-267 amino acids namely histidine, proline, aspartic acid, and asparagine respectively. The distance measured between the Carvacrol and ubiquinone-c-reductase binding cavity was 4.1Å, which implies the strong interaction showed in Figure 2.

Discussion

In the present study, the biological activity of Carvacrol was predicted and its potential as an anti-malarial drug was compared with the components of the commercially available drug namely, Malarone. Malarone comprises Atovaquone/ Proguanil combination which retards the growth of parasite by inhibiting the activity of Ubiquinol-c-reductase in host red blood cells. Ubiquinol-cytochrome-c reductase is one of the three major enzymes involved in ETC Pathway, playing a crucial role in synthesizing ATP from ADP with the help of ATP synthase. Ubiquinol-cytochrome-c reductase catalyzes Fe-S protein reduction reaction and thus transfers an electron to cytochrome c.¹²

Atovaquone showed Pa value 0.775 (where Pa value is the probability of the compound being active for a specific activity) as Ubiquinol-c-reductase inhibitor which is less than the Pa value of Carvacrol (Pa value=0.931). The obtained data revealed that the Carvacrol inhibits the activity of Ubiquinol-cytochrome-c reductase more effectively as compared to Atovaquone with Pa value of 0.931 which is also the target receptor of commercialized drug Malarone.

It is a well-known fact that the lower binding energy leads to better binding and if binding energy value is negative that means binding is spontaneous and does not require any energy for the binding to take place, therefore, the conformation with most negative binding energy/ affinity will be the most preferable conformation when we opt to choose the best conformation on the basis of binding energy/ affinity. Results indicate that the conformation with the minimum binding energy (-5.4 Kcal/ mol) is the most preferable out of all conformations of Ubiquinone-c-reductase docked with Carvacrol. We also predicted that Carvacrol acts as a non-competitive inhibitor of cytochrome-c-reductase which binds at the A chain of the enzyme

without interrupting the binding of ubiquinone. Therefore, this study shows that Carvacrol can be used as an anti-malarial drug as a substitute to Atovaquone in combination with Proguanil as antimalarial drug.

The above result of biological prediction and docking reveals that the Carvacrol binds effectively with Ubiquinol-c-reductase and thereby can work as an Ubiquinol-c-reductase inhibitor of parasite *P. falciparum* and ultimately retards the growth of the parasite.

Conclusion

Antimalarial drug Malarone, a combination of Atovaquone/Proguanil interferes with the growth of the parasite in red blood cells of the host organism by inhibiting the activity of Ubiquinol-cytochrome-c-reductase. The biological activity of Atovaquone and Carvacrol was studied using the way2drug tool. The result showed that Carvacrol acts as an effective inhibitor of Ubiquinol cytochrome c reductase as compared to Atovaquone. To confirm the above findings, we have performed docking studies by using Autodock 4. The result suggests consistency with our findings.

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Conflict of Interest: None

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