GENETIC AND *IN SILICO* STUDIES OF 6--PYRUVOYLTETRAHYDROPTERIN SYNTHASE IN *Plasmodium falciparum* ISOLATES

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE, (M.Sc) IN BIOCHEMISTRY IN THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY, OTA,OGUNSTATE, NIGERIA

AUGUST 2023

ACCEPTANCE

This is to attest that this dissertation is accepted in partial fulfilment of the requirements for the award of the degree Master of Science (M.Sc.) in Biochemistry in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Nigeria.

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DECLARATION

I, **BELLA-OMUNAGBE, MERCY** (**21PCP02249)**, declare that I carried out this research under the supervision of Prof. Shalom N. Chinedu of the Department of Biochemistry, College of Science and Technology, Covenant University, Ota Nigeria. I attest that the dissertation has not been presented wholly or partially for the award of any degree elsewhere. All the sources of materials and scholarly publications used in the dissertation have been duly acknowledged.

BELLA-OMUNAGBE, MERCY

Signature and Date

CERTIFICATION

We certify that this dissertation titled "**GENETIC AND *IN SILICO* STUDIES OF 6--PYRUVOYLTETRAHYDROPTERIN SYNTHASE IN *Plasmodium falciparum* Isolates**" is an original work carried out by **BELLA-OMUNAGBE, MERCY (21PCP02249)** in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria, under the supervision of Prof. Shalom N. Chinedu. We have examined and found the work acceptable as part of the requirements for the award of a degree of Master of Science (M.Sc.) in Biochemistry.

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DEDICATION

I would like to dedicate this work to Ipu'Ojaken. Thank you for your love.

ACKNOWLEDGMENT

I want to begin by thanking the Lord God Almighty for His mercies, grace, and strength throughout this work.

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LIST OF ABBREVIATIONS

6-HMDP - 6-Hydroxymethyl-7-8-Dihydropterin

ACT – Artemisinin Combination Therapy

ADME – Absorption, Distribution, Metabolism and Excretion

ADMET – Absorption, Distribution, Metabolism, Excretion and Toxicity

AL- Artemether-Lumefantrine

ART - Artemisinin

AS-AQ – Artesunate-Amodiaquine

AS-PY – Artesunate-Pyronaridine

CASTp – Computed Atlas of Surface Topography of Proteins

CG - Cycloguanil

CHREC - Covenant Health Research Ethics Committee

CQ - Chloroquine

CYP – Cytochrome P450

CYP1A2 – Cytochrome P450 1A2

CYP2D6 - Cytochrome P450 2D6

DHA - Dihydroartemisinin

DHA-PPQ - Dihydroartemisinin-Piperaquine

DHFR – Dihydrofolate Reductase

DHNTP - Dihydroneopterin Triphosphate

DHPS – Dihydropterate Synthase

DNA – Deoxyribonucleic Acid

GTPC – Guanosine Triphosphate Cyclohydrolase

P13P - Phosphatidylinositol 3-Phosphate

PCR – Polymerase Chain Reaction

*Pf*CRT – *Plasmodium falciparum* Chloroquine Resistance Transporter

*Pf*P13K - *Plasmodium falciparum* Phosphatidylinositol-3-Kinase

PG - Proguanil

PTPS – 6-Pyruvoyltetrahydropterin Synthase

PYR - Pyrimethamine

T-Fold – Tunneling Fold

WHO – World Health Organization

ABSTRACT

The lyase enzyme, 6-pyruvoyltetrahydropterin Synthase (6-PTPS) is involved in the biosynthesis of tetrahydrobiopterin. In *Plasmodium* species, where Dihydroneopterin Aldolase (DHNA) is absent, it acts in the synthesis of folate which is crucial for the optimum growth, development and survival of the parasite. This has been noted as a potential target for the development of antimalarial drugs. This study validated the presence of *Pf*PTPS in *P. falciparum*-infected isolates, identified a relationship between parasite density and Cycle threshold and predicted compounds that could inhibit the enzyme and thereby serve as potential drugs against *P. falciparum* infection. Quantitative PCR was used to amplify the *Pf*PTPS gene in isolates, and the resulting Cycle Threshold was compared with the parasite density. Molecular docking was done with PyRx to identify potential inhibitors of *Pf*PTPS. The results show an inverse relationship between the parasite density and Cycle threshold. The first seven best hits ranked by the docking scores with better binding affinity than the control ligand, Biopterin were selected for visualisation, ADMET and toxicity studies. They include three conformers of the compound (I.D.140296439), and four other compounds (I.D. 140296495, 144380406, 135573878 and 136075207). In silico ADMET studies predicted good pharmacokinetic properties of the compounds and reported a high risk of irritant toxicity in 140296439 and 144380406. The study highlighted 140296439, 140296495, 144380406, 135573878 and 136075207 as potential inhibitors of *Pf*PTPS and possible compounds for antimalarial drug development.

Keywords: 6-Pyruvoyltetrahydropterin synthase, molecular docking, drug targets, antimalarial drug development, qPCR