IN VITRO AND *EX VIVO* ACTIVITIES OF NOVEL HYDROXAMIC ACID DERIVATIVE AGAINST *Plasmodium falciparum*

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ACCEPTANCE

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

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DECLARATION

I, **OFFOR Gloria Nwabugwu** (14PCP00958), declare that this M.Sc. dissertation titled: "*In vitro* and *Ex vivo* Activities of Novel Hydroxamic Acid Derivative Against *Plasmodium falciparum*" was undertaken by me under the supervision of Dr. A.H. Adebayo. The work presented in this dissertation has not been presented, either wholly or partly for the award of any degree elsewhere. All sources of scholarly information used in this dissertation were duly acknowledged.

Offor, Gloria Nwabugwu **……**

(Student) Signature and Date

CERTIFICATION

We certify that the dissertation titled: "*In vitro* and *Ex vivo* Activities of Novel Hydroxamic Acid Derivative Against *Plasmodium falciparum*" is an original work carried out by OFFOR, Gloria Nwabugwu with Matriculation Number: 14PCP00958, of Biochemistry Programme in the Department of Biological Sciences, College of Science and Technology, Covenant University Ota, Ogun State, Nigeria. We have examined the work and found it acceptable for the award of Master of Science (M.Sc.) degree in Biochemistry.

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DEDICATION

To an ever faithful God whose unquantifiable mercy, favour and grace saw me to the end of this programme

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

DMSO	dimethyl sulfoxide
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
RBC	red blood cell
RPMI	Roswell Park Memorial Institute
RSA	ring-stage survival assay
WBC	white blood cell
BSA	Bovine Serum Albumin
MSP1	Merozoite Surface Protein 1
MSP2	Merozoite Surface Protein 2
Pfcrt	*P. falciparum C*hloroquine resistance transporter gene
PCR	Polymerase chain reaction
*pfmdr*1	*P. falciparum* multidrug resistance gene 1
Pfnhe1	*plasmodium falciparum* sodium hydrogen exchanger
Pfmrp	*P. falciparum* multidrug resistance-associated protein
cytb	*Cytochrome b*
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthase
DNA	Deoxyribonucleic acid
HRP II	Histidine-rich protein II
IC_{50}	50% inhibitory concentration
WHO	World Health Organization
CQ	chloroquine
ART	artemisinin
OA4	hydroxamic acid derivative
L	liter
mL	milliliter
μL	microliter
mM	millimolar
nM	nanomolar
MW	molecular weight
RT	room temperature

ABSTRACT

Emergence of drug-resistant strains of *Plasmodium* has recently led to increased efforts to discover and develop new antimalarial drugs both for the prophylaxis and treatment of malaria infection. The present study was conducted to evaluate the antiplasmodial activity of novel hydroxamic acid derivative against *Plasmodium falciparum* 3D7 strain and the wild type isolate from malaria infected patients. Chloroquine-sensitive *P. falciparum* 3D7 was grown *in vitro* in O^+ human red blood cells in RPMI 1640 medium supplemented with 10% heat inactivated AB human serum, 25 mM HEPES buffer, 50 µg/ml penicillin and 50 µg/ml streptomycin under an atmosphere of 90% N_2, 5% O_2 and 5% CO_2. Serially diluted drugs were placed in the wells of 96 well micro titre plates and incubated with aliquots of parasite culture medium containing asynchronized rings at a parasitemia of 0.2% and a haematocrit of 4%. For the *ex vivo* assay, aliquots of the washed infected red blood cells in complete medium were incubated with varying concentrations of the drugs. The parasitemia for both assays were determined microscopically using Giemsa-stained smears. Chloroquine and artemisinin used as the positive controls as well as the drug-free negative control were all assayed in duplicates simultaneously with the test compound (OA4). Results from the half maximal inhibitory concentrations (IC_{50}) analyses showed the *in vitro* and *ex vivo* IC_{50} values of OA4 to be 28200 nM and 154.2 nM respectively. OA4 lowered the parasitemia across all the drug-treated wells when compared to the drug-free negative control wells. The result from the erythrocyte stabilization assay revealed that OA4 did not induce any alteration on the morphology of red blood cells. In conclusion, these results demonstrated that the hydroxamic acid derivative OA4 could be a promising antimalarial drug candidate and is therefore a useful hit compound for further medicinal chemistry optimization.

Keywords: Malaria, *P. falciparum*, hydroxamates, drug sensitivity, cell morphology