OBAFEMI AWOLOWO UNIVERSITY

SYNTHESIS, CHARACTERIZATION AND ANTIMALARIA STUDIES OF SOME MONO CARBONYL CURCUMIN ANALOGS AND THEIR ARYL HYDRAZONE DERIVATIVES

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CERTIFICATION

We certify that this project work was carried out by Mr. FADARE OlatomideAyodeji and Supervised by Prof. C.A. Obafemi (Chemistry Department, Obafemi Awolowo University, Ile-Ife) and Co-supervised by Prof. E.O. Iwalewa (Pharmacology Department, University of Ibadan, Ibadan) in partial fulfillment of the requirements for the award of Ph.D. degree in Chemistry of the Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria.

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LIST OF ABBREVIATIONS

ACT: Artemisinin combination therapies

ADME: Absorption, Distribution, Metabolism and Elimination

ATP: Adenosine triphosphate

BCS: Biopharmaceutical classification system

Caco-2 cells: Heterogeneous human epithelial colorectal adenocarcinoma cells

CDC: Centre for disease transmission and control

CQ: Chloroquine

CRT: Chloroquine resistant transporter

DDT: Dichlorodiphenyltrichloroethane

DHC: Dihydrocurcumin

DHFR: Dihydrofolate reductase

DHPS: Dihydropteroate synthase

DNA: Deoxyribonucleic acid

DNP: Dinitrophenyl hydrazine/Dinitrophenyl hydrazone

DPAP: Dipeptidyl aminopeptidase

dTMP: Thymidine monophosphate

dUMP: Uridine monophosphate

FaSSIF: A patented complex of taurocholate and lecithin

GIT: Gastro intestinal tract

GTP: Guanosine-5'-triphosphate

Hb: Hemoglobin

HHC: Hexahydrocurcumin

Hz: Hemozoin

IgG:Immunoglobulin G

IL-6: Interleukin 6

LBDDS: Lipid based drug delivery system

LDH: Lactate dehydrogenase

LPS: lipopolysaccharide

MDR: Multi drug resistance

MDR1:Multi drug resistance transporter

NAD: Nicotinamide adenine dinucleotide

NADH: Reduced form of NAD

NADPH: Nicotinamide adenine dinucleotide phosphate

OHC: Octahydrocurcumin

pABA: para-Aminobenzoic acid

PFOR: Pyruvate-ferredoxin oxidoreductase

PL: Phospholipid

ROI: Reactive oxygen intermediate

RT-PCR: Reverse transcription polymerase chain reaction

SI: Survival index

SMEDDS: Self-micro emulsifying drug delivery system

SNEDDS: Self-nano emulsifying drug delivery system

SOD: Superoxide dismutase

TCA: Tricarboxylic acid

TG: Triglyceride

THC: Tetrahydrocurcumin

TNF: Tumor necrosis factor

WHO: World health organization

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ABSTRACT

The study synthesized mono carbonyl analogs of curcumin, the aryl hydrazone derivatives of the mono carbonyl curcumin analogs and a 2,4-dinitrophenyl pyrazolone derivative of curcumin itself. The synthesized compounds were then characterized, docked with plasmepsin II, and the acute toxicity of the synthesized compounds as well as the percentage chemosuppression of the rodent strain of malaria parasite (*Plasmodium berghei*) NK65(CS) were determined. This was with a view to establishing the compounds suitable for a curative assay and discovering new potent and relatively non-toxic synthetic analogs of curcumin that could be used to combat *Plasmodium falciparum* which is the malaria causal organism in man.

The monocarbonyl curcumins were synthesized by a simple Claisen-Schmidt condensation reaction by reacting two molar equivalents of a substituted benzaldehyde with a molar equivalent of acetone in acidic/basic conditions to yield compounds **1a-7a.** The monocarbonyl curcumins were then reacted with a molar equivalent of 2,4-dinitrophenyl hydrazine with stirring in ethanol (at room temperature) under acid catalysis for 18 hrs to yield the corresponding DNP hydrazone**1b-7b**. Compound **3a** was reacted with 4-nitro phenylhydrazine under the same conditions as DNP which resulted into a pyrazoline, **3c**. Curcumin was also reacted with DNP under the same condition to yield a pyrazolone, **11e**. The synthesized compounds were then characterized using spectroscopic techniques such as UV-Visible, IR, ^{1}H and ^{13}C NMR spectroscopy. The synthesized compounds were docked with plasmepsin II, one of the enzymes used by the parasite to digest haemoglobin, using flexX, a part of the LeadIT tools to estimate the binding affinity of the compounds for the protein as a function of antimalarial activity. All

the synthesized compounds were tested *in-vivo* using a four day chemosuppressive assay for their antimalarial activity. The test animals were monitored afterwards for 24 days to assess the long term effect of the drug on the test models and to estimate the survival index (SI) of the test models with respect to the test compounds. The compounds were administered using a lipid based drug delivery system (cotton seed oil was used as the vehicle for the compounds administered orally).

The binding energies computed for the compounds ranged from -19.29 to -35.96 kJ/mol. Chloroquine was used as a control molecule and all the compounds had binding affinity greater than that of chloroquine (-17.02 kJ/mol). Some of the compounds docked had high affinity for the plasmepsin II. Compounds **1b**, **4b**, **5b**, **6a**, **6b**, **7b** and **10b** had binding energies ranging from -25 to -36 kJ/mol. Among the compounds listed, only compound **6a** was a monocarbonyl curcumin analog of curcumin. Compounds **1a** (83.72 % chemosuppression at 200 mg/kg and SI of 68.75 %), **2b** (81.93% chemosuppression at 200 mg/kg and SI of 50 %), **3a** (58.62 % chemosuppression at 200 mg/kg and SI of 100 %), **5a** (66.59 % chemosuppression at 100 mg/kg and SI of 68.75 %), **6a** (71.2 % chemosuppression at 50 mg/kg and SI of 46.15 %) and **11e** (74.09 % chemosuppression at 50 mg/kg and SI of 54.55 %)were the compounds that had the best combination of survival index and chemosuppression profiles.

This study concluded that the compounds **1a**, **2b**, **3a**, **5a**, **6a** and **11e** had high chemosuppression compared to curcumin which was comparable to chloroquine and could therefore be selected for a curative *in-vivo* assay.

CHAPTER ONE

1.0. INTRODUCTION

Malaria is a deadly infectious disease caused by a blood-borne protozoan of the genus *Plasmodium* (*P*) and is transmitted by the female *Anopheles* mosquito. There are more than 120 species of the protozoan from the genus *Plasmodium* of which five are currently known to infect humans: *P. falciparum, P. vivax, P. malariae, P.knowelsi and P. ovale*. (Chin*et al*., 1965; Jongwutiwes *et al*., 2004; Cox-Singh *et al*., 2008). Malaria is the second leading cause of death from infectious disease in Africa, where 89 % of worldwide malaria deaths occur (Figure 1.1) (About malaria – CDC, 2014). According to the world health organization's statistics on malaria, over 200 million people (including children) are infected yearly. In 2012 malaria caused 207 million clinical episodes, and 627, 000 people died from malaria and the number has increased steadily since then.

Malaria is characterized by periodic bouts of severe chills and high fever. Serious cases of malaria can result in death if left untreated. Among the five species of the plasmodium known to afflict humans, *P. falciparum* causes the most severe form of human malaria and results in a majority of the reported fatalities worldwide.

After repeated infections, people who live in regions where malaria is prevalent develop a limited immunity to the disease. This partial protection does not prevent them from developing malaria again, but does protect them against the most serious effects of the infection. They generally develop a mild form of the disease that does not last long and is unlikely to be fatal. Infants and children are especially vulnerable to malaria because they have not yet built up immunity to the parasite. Some people have genetic traits that help them resist malaria. Sickle-

cell anemia and thalassemia, for example, are inherited blood disorders linked to malaria resistance. Over the years, the malaria parasite has developed resistance to existing drugs used

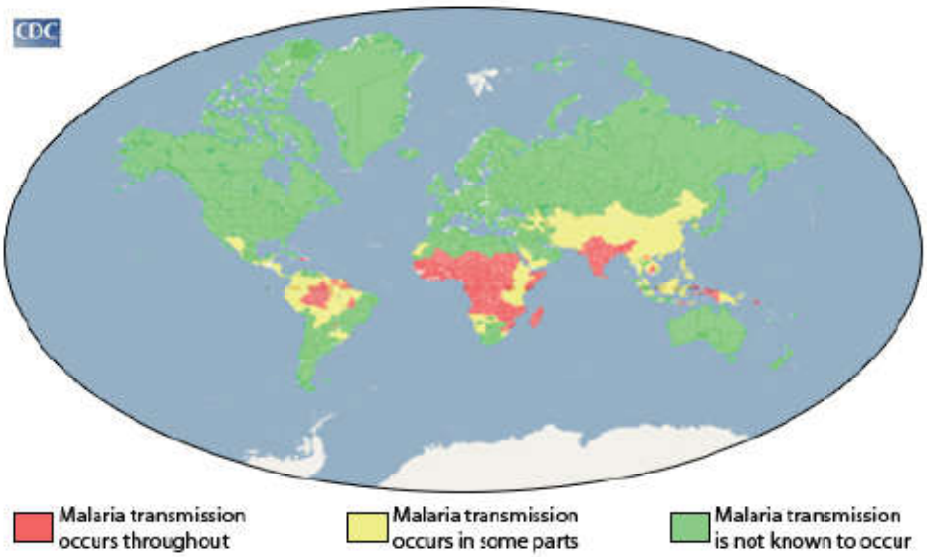

Figure 1.1: Extent of Malaria Transmission in Different Regions of the World as at 2010.

Source is Centre for Disease Transmission and Control, United States of America, www.cdc.gov ("Guidelines for the Treatment of Malaria" World Health Organization.<http://helid.digicollection.org/en/d/Js13418e/14.6.html>).

to treat the disease. Recent data reveals documented cases of drug resistance by Plasmodium falciparum – the strain that causes the most severe clinical manifestation of the disease in man (Figure 1.2).

Artemisinin in combination with other antimalarial drugs is the preferred mode of treatment nowadays due to low resistance of the parasite to artemisisnin. Recently, however, cases of resistance to artemisinin have been reported in Cambodia and Thailand (Dondorp *et al.*, 2009). The development of resistance to existing drugs by the parasites makes it expedient that new antimalaria drugs are developed for the treatment of the disease.

The disease vector (female anopheles mosquito) also constitute a problem in the eradication of the disease. Attempts to eradicate the disease in the absence of a vaccine has proved abortive over the years because of the continued existence of the disease vector in areas where the disease is endemic. When individuals are treated effectively with existing drugs, it cannot be guaranteed that they will not come down with the disease again since they are still exposed to the disease vector. The areas where the disease is endemic are areas where the vector thrives and is difficult to eradicate. The vector thrives in warm regions of the earth. The females which transmit the disease lay their eggs in water where their larvae develop and mature. Due to the prevalence of the mosquito in malaria endemic regions, basic antimosquito measures have been employed such as draining sites where mosquitoes lay their eggs, covering water channels, use of insecticide-

treated bed nets, spraying of insecticides and introducing into ponds fish that feed on mosquito larvae. The United States virtually eradicated malaria in the late 1940s and early 1950s through the use of the insecticide DDT. However, DDT was later banned in the United States and many other countries because of its