

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

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TABLE OF CONTENTS

Authorization to copy	ii
Certification	iii
Acknowledgments	iv
Table of contents	v
List of Appendices	x
List of Abbreviations	xiii
List of Figures	xv
List of Schemes	xxi
List of Tables	xxii
Abstract	xxiii

CHAPTER ONE

1.0.	INTRODUCTION	1
1.1	Life Cycle of the Malaria Parasite and Progress of Disease in Man	3
1.2.	Biochemistry of Parasite	7
1.2.1.	Digestion of Hemoglobin	7
1.2.2.	Detoxification of Free Heme	14
1.2.3.	Degradation of Carbohydrates and Energy Production	15
1.2.4.	Role of Folate in Nucleotide Synthesis	17
1.2.5.	Redox Mechanism	17
1.3.	Mechanism of Drug Action and Resistance	18



1.3.1.	Inhibition of HemozoinBiocrystallization	19
1.3.2.	Antifolates	22
1.3.3.	Drugs that affect the Parasite's Redox Mechanism	26
1.4.	Drugs Currently in use for Malaria Chemotherapy	28
1.4.1.	Quinine Analogs	31
1.4.2.	4-Aminoquinoline Analogs	32
1.4.3.	8-Aminoquinolines	33
1.4.4.	9-Aminoacridines	36
1.4.5.	Guanidine Analogs (Biguanidines)	36
1.4.6.	Pyrimidine Analogs	40
1.4.7.	Sulphones	42
1.4.8.	Artemisinin	45
1.4.9.	Mefloquine	48
1.4.10.	Halofanthrine	48
1.4.11.	Lumefanthrine	51
1.5.	Computer Aided Drug Design (CADD)	51
1.5.1.	Ligand-Based Drug Design	55
1.5.2.	Structure-Based Drug Design	55
1.5.3.	Molecular Docking	56
1.5.3.1.	Pose Functions	63
1.5.3.2.	Scoring Functions	65
1.5.4.	History of Medicinal Chemistry and Advances in Molecular Modelling	69



CHAPTER TWO

2.0.	LITERATURE REVIEW	76
2.1.	Curcumin	76
2.2.	Curcumin as an Antimalarial Agent	79
2.3.	Search for Super Curcumin	82
2.4.	Curcumin Derivatives	87
2.5.	Curcumin Analogs	87
2.6.	Metal Complexes	88
2.7.	Aims and Objectives	96

CHAPTER THREE

3.0.	MATERIALS AND INSTRUMENTS	97
3.1.	General Details	97
3.2.	List of Reagents and Suppliers	98
3.3.	Software and X-Ray Crystallographic Data used for the Molecular Docking Study	98
3.4.	Experimental Animals for <i>In-Vivo</i> Assay	99
3.5.	Methodology	99
3.5.1.	Synthesis	99
3.5.1.1	(1 <i>E</i> ,4 <i>E</i>)-1,5-diphenylpenta-1,4-dien-3-one, 1a	99
3.5.1.2.	(1 <i>E</i> ,4 <i>E</i>)-1,5-bis(4-hydroxy-3-methoxyphenyl) penta-1,4-dien-3-one, 2a	100
3.5.1.3.	(1 <i>E</i> ,4 <i>E</i>)-1,5-bis(4-methoxyphenyl) penta-1,4-dien-3-one, 3a	100



3.5.1.4.	(1 <i>E</i> ,4 <i>E</i>)-1,5-bis(2-hydroxyphenyl) penta-1,4-dien-3-one, 4a	100
3.5.1.5.	(1 <i>E</i> ,4 <i>E</i>)-1-(naphthalene-1-yl)-5-(naphthalene-4-yl) penta-1,4-dien-3-one, 5a	101
3.5.1.6.	(1 <i>E</i> ,4 <i>E</i>)-1,5-bis(2-chloroquinolin-3-yl) penta-1,4-dien-3-one, 6a	101
3.5.1.7.	(1 <i>E</i> ,4 <i>E</i>)-1,5-bis(2-nitrophenyl) penta-1,4-dien-3-one, 7a	102
3.5.2.1	2,4-dinitrophenyl hydrazone of compound 1a	102
3.5.2.2.	2,4-dinitrophenyl hydrazone of compound 2a	103
3.5.2.3.	2,4-dinitrophenyl hydrazone of compound 3a	103
3.5.2.4.	2,4-dinitrophenyl hydrazone of compound 4a	104
3.5.2.5.	2,4-dinitrophenyl hydrazone of compound 5a	104
3.5.2.6.	2,4-dinitrophenyl hydrazone of compound 6a	105
3.5.2.7.	2,4-dinitrophenyl hydrazone of compound 7a	105
3.5.3.	2,4-dinitrophenyl hydrazone of curcumin, 11e	106
3.5.4.	Reaction Schemes	107
3.6.	Molecular Modelling (Docking) Studies	109
3.7.	Pharmacology	109
3.7.1.	Acute Toxicity Test	109
3.7.2.	<i>In-Vivo</i> : Chemosuppressive Test	110
CHAPTER FOUR		
4.0.	RESULTS AND DISCUSSION	111

4.1.	Chemistry	111
4.2.	Molecular Docking of Synthesized Compounds with PlasmeprinII	117
4.3.	Pharmacology	133
4.4.	DISCUSSION	142
	4.4.1. Infrared Spectroscopy	142
	4.4.2. Ultraviolet-Visible Spectroscopy	144
	4.4.3. Characterization of Pure Curcumin and its DNPDerivative.	144
	4.4.4. Characterization of Compound 1a .	147
	4.4.5. Characterization of Compound 2a and derivative	147
	4.4.6. Characterization of Compound 3a . and its derivatives.	149
	4.4.7. Molecular Modelling (Docking)	152
4.5.	Pharmacology	154
4.6.	Application of Lipid Based Drug Delivery System (LBDDS) in the Administration of Curcumin and Test Compounds	163
4.7.	Survival Index	170
4.8.	Short-Term Action and Parasite Recrudescence	171
4.9.	Compounds with Good Chemo Suppression and Survival Index Profiles	171
CHAPTER FIVE		
5.1.	CONCLUSION	177
5.2.	RECOMMENDATION	178

References	180
Appendix	204

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LIST OF APPENDICES

Appendix	Caption	Page
1	¹ H NMR Spectrum for Compound 1a	205
2a	¹ H NMR Spectrum for Compound 7a	206
2b	¹ H NMR Spectrum for Compound 7a with Aromatic Region Expanded	207
2c	¹³ C NMR Spectrum for Compound 7a	208
2d	¹³ C NMR Spectrum (Attached Proton Test, APT) for Compound 7a	209
3a	¹ H NMR Spectrum for Compound 7b	210
3b	¹ H NMR Spectrum for Compound 7b	211
3c	¹³ C NMR Spectrum for Compound 7b	212
3d	¹³ C NMR (Attached Proton Test, APT) Spectrum for Compound 7b	213
4a	¹ H NMR Spectrum for Compound 3a	214
4b	¹ H NMR Spectrum for Compound 3a	215
5	¹ H NMR Spectrum for Compound 3b	216
6a	¹ H NMR Spectrum for Compound 3c	217
6b	¹³ C NMR Spectrum for Compound 3c	218
6c	¹³ C NMR (Attached Proton Test, APT) Spectrum for Compound 3c	219
6d	Heteronuclear Correlation NMR Experiment (HETCOR) for Compound 3c	220
7	¹ H NMR Spectrum for Compound 2a	221
8	¹ H NMR Spectrum for Compound 2b	222
9	¹ H NMR Spectrum for Compound 11d	223



10	¹ H NMR Spectrum for Compound 11e	224
11	Infrared Spectrum for Compound 1a	225
12	Infrared Spectrum for Compound 1b	226
13	Infrared Spectrum for Compound 2a	227
14	Infrared Spectrum for Compound 2b	228
15	Infrared Spectrum for Compound 3a	229
16	Infrared Spectrum for Compound 3b	230
17	Infrared Spectrum for Compound 3c	231
18	Infrared Spectrum for Compound 4a	232
19	Infrared Spectrum for Compound 4b	233
20	Infrared Spectrum for Compound 5a	234
21	Infrared Spectrum for Compound 5b	235
22	Infrared Spectrum for Compound 6a	236
23	Infrared Spectrum for Compound 6b	237
24	Infrared Spectrum for Compound 7a	238
25	Infrared Spectrum for Compound 7b	239
26	Infrared Spectrum for Compound 11d	240
27	Infrared Spectrum for Compound 11c	241
28	Ultraviolet-Visible Spectrum for Compound 1a	242
29	Ultraviolet-Visible Spectrum for Compound 1b	243
30	Ultraviolet-Visible Spectrum for Compound 2a	244
31	Ultraviolet-Visible Spectrum for Compound 2b	245
32	Ultraviolet-Visible Spectrum for Compound 3a	246



33	Ultraviolet-Visible Spectrum for Compound 3b	247
34	Ultraviolet-Visible Spectrum for Compound 3c	248
35	Ultraviolet-Visible Spectrum for Compound 4a	249
36	Ultraviolet-Visible Spectrum for Compound 4b	250
37	Ultraviolet-Visible Spectrum for Compound 5a	251
38	Ultraviolet-Visible Spectrum for Compound 5b	252
39	Ultraviolet-Visible Spectrum for Compound 6a	253
40	Ultraviolet-Visible Spectrum for Compound 6b	254
41	Ultraviolet-Visible Spectrum for Compound 7a	255
42	Ultraviolet-Visible Spectrum for Compound 7b	256
43	Ultraviolet-Visible Spectrum for Compound 11d	257
44	Ultraviolet-Visible Spectrum for Compound 11e	258
45-55	3D and 2D Poses from FlexX for Compound 1a-8b	259

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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LIST OF FIGURES

Figure	Caption	Page
1.1	Extent of Malaria Transmission in Different Regions of the World as at 2010.	2
1.2	Data on Reported Cases of <i>P. falciparum</i> Resistance to Clinical Antimalarial Drugs	4
1.3	Life Cycle of the Malaria Parasite.	8
1.4	(Heterotetramer, $(\alpha\beta)_2$) Structure of Human Hemoglobin.	11
1.5	The Hemoglobin Digestion Pathway (Wiser, 2008);	13
1.6	Pathway Showing the Degradation of Glucose by the Plasmodium to Yield Energy (ATP).	16
1.7	The Accumulation of Chloroquine (CQ) in the Food Vacuole of the Parasite.	21
1.8	Simplified Scheme of Folate Metabolism.	24
1.9	Pathway Showing Actions of Nitroimidazoles.	29
1.10	The Quinine Alkaloids Extracted from the Bark of <i>Cinchona officinalis</i> Linne (<i>C. ledgeriana</i> Moens) are Potent Antimalarial Agents	31
1.11	Tautomerism of Amodiaquine Base into the Iminoquinone Form.	34
1.12	Hydroxychloroquine though an Antimalarial Drug is Also Effective in the Suppressive Treatment of Autoimmune Inflammatory Diseases such as Rheumatoid Arthritis and Systemic Lupus Erythromatosus.	35

1.13	8-Aminoquinoline Analogs Being Marketed as Antimalarial Drugs.	37
1.14	MepacrineMesylate and Mepacrine Hydrochloride Are Acridine Analogs	38
1.15	Aminoacrichinalong with its Structural Analogs Were Introduced as a Blend of 4-Aminoquinolone and 8-Aminoquinoline Features but they were not so Successful as a Result of their High Toxicity.	39
1.16	Structure of Proguanil and Mechanism of Cyclization to Form Cycloguanil the Active Metabolite.	41
1.17	Pyrimethamineand Trimethoprim which were Marketed with the Trademark Names by Burroughs Wellcome. Trimethoprim was also Marketed with the Brand Name Trimpexby Roche.	43
1.18	Sulfadoxine and Sulfametopyrazineare Sulfanilamide Based Drugs.	44
1.19	Dapsoneis 4,4-Diaminodiphenyl Sulfone and was Marketed as Avlosulfon [®] By Ayerst.	46
1.20	Artemisinin Derivatives Designed to be Soluble in Different Media, either Water Soluble or Lipid Soluble.	47
1.21	Mefloquinwas Developed by the United States Military Research Department.	49
1.22	Halofanthrine, a Phenantrene Methanol,is a Potent Inhibitor of the Formation of β -Hematin by the Malaria Parasite.	50
1.23	Lumefanthrine, a Fluorene Methanol, also a Potent Inhibitor of the Formation of β -Hematin and is Used in Conjunction with Artemisinin Derivative, Especially Artemether, in ACTs.	52
1.24	A Drug Discovery Cycle Highlighting both Ligand-Based	



(Indirect) and Structure-Based (Direct) Drug Design Strategies.	54
1.25 3D Representation of a Small Molecule Docked into a Binding Pocket of a Protein Molecule.	57
1.26 Some Docking Programs in a Pie Chart Showing the Extent to which they are Used Globally as at 2006.	62
1.27 Diagram Showing a Deviation of Only 20° in the Dihedral Φ of the Selective Estrogen Receptor Modulator Raloxifen which would Result in a Change of 2.5 Å in the Tertiary Amine Position, thereby Missing the Favorable Hydrogen-Bond Interaction with Asp351.	64
1.28 Scheme for Incremental Construction (<i>top line</i>) and Place-and-Join (<i>bottom line</i>) as Examples of the Mode of Operation of Systematic Search Algorithms.	66
2.1 (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione (diferuloyl methane)	77
2.2 Plant Derived Derivatives of Curcumin of which Curcumin is the Most Studied.	83
2.3 Some other Plant Derived Compounds that have Structural Moieties that Resemble Curcumin.	84
2.4 Synthetic Derivatives of Curcumin – The Aryloxy (Phenolic) Group has been Modified in the Two Cases.	85

2.5	Curcumin with Possible Positions for Structural Modification Highlighted.	86
2.6	Synthetic Analogs of Curcumin.	89
2.7	MonocarbonylCurcumin Analogs have the CH ₂ CO Group Removed.	90
2.8	MonocarbonylCurcumin Analogs Synthesized by Liang <i>et al.</i> , 2009b	91
2.9	The Monocarbonyl Curcumins Can be Synthesized by the Claisen Schmidt Condensation of Substituted Aromatic Aldehydes (2 Mols Equivalent) with One Mole Equivalent of Either Acetone, Cyclohexanone or Cyclopentanone.	92
2.10	Various Curcumin Derivatives – Review by Anand P. <i>et al.</i> , 2008.	93
2.11	A Synthesized Tetraoxane Ring System as Compared with the Trioxane Ring System in Artemisinin.	94
2.12	Novel FluoroKnoevenagel Condensates and their Schiff Bases which were used to form Copper Complexes (Milacicet <i>et al.</i> , 2008).	95
4.1	Structure of Plasmepsin II Deduced from X-Ray Crystallographic Data.	119
4.2	Chain A of Plasmepsin II with the Co-Crystallized Compound Embedded.	120
4.3	Binding Site in Chain A of Plasmepsin II with the Co-Crystallized Compound Embedded.	121
4.4	Binding Site in Chain A of Plasmepsin II with the Co-Crystallized Compound Embedded. The Binding Site has Been Converted into a Surface.	122
4.5	Binding Site in Chain A of Plasmepsin II without the Co-Crystallized Compound.	123
4.6	(a) 3D Pose-View of Chloroquine Docked with Plasmepsin II	



	(b) 2D Pose-View of Chloroquine Docked with Plasmepsin II.	124
4.7	(a) 3D Pose-View of Compound 5a Docked with Plasmepsin II	
	(b) 2D Pose-View of Compound 5a Docked with Plasmepsin II	125
4.8	(a) 2D Pose-View of Compound 5b Docked with Plasmepsin II	
	(b) 3D Pose-View of Compound 5b Docked with Plasmepsin II	126
4.9	(a) 2D Pose-View of Compound 6a Docked with Plasmepsin II	
	(b) 3D pose-view of compound 6a docked with plasmepsin II	127
4.10	(a) 3D Pose-View of Compound 6b Docked with Plasmepsin II	
	(b) 2D Pose-View of Compound 6b Docked with Plasmepsin II	128
4.11	(a) 3D Pose-View of Compound 7a Docked with Plasmepsin II	
	(b) 2D Pose-View of Compound 7a Docked with Plasmepsin II	129
4.12	(a) 3D Pose-View of Compound 7b Docked with Plasmepsin II	
	(b) 2D Pose-View of Compound 7b Docked with Plasmepsin II	
4.13	(a)3D Pose-View of Compound 10a Docked with Plasmepsin II	
	(b)2D Pose-View of Compound 10a Docked with Plasmepsin II	131
4.14	(a) 3D Pose-View of Compound 10b Docked with Plasmepsin II	
	(b) 2D Pose-View of Compound 10b Docked with Plasmepsin II	132
4.15	Chart Showing % Chemosuppression and Survival Indices of Compound 1a and 1b at Three Different Doses (50, 100 and 200 mg/kg) in Comparison with Chloroquine at 10 mg/kg.	135
4.16	Chart Showing % Chemosuppression and Survival Indices of Compound 2a and 2b at Three Different Doses (50, 100 and 200 mg/kg)	



	in Comparison with Chloroquine at 10 mg/kg.	136
4.17	Chart Showing % Chemosuppression and Survival Indices of compound 3a and 3b at Three Different Doses (50, 100 and 200 mg/kg) in Comparison with Chloroquine at 10 mg/kg.	137
4.18	Chart Showing % Chemosuppression and Survival Indices of Compound 4a and 4b at Three Different Doses (50, 100 and 200 mg/kg) in Comparison with Chloroquine at 10 mg/kg.	138
4.19	Chart Showing % Chemosuppression and Survival Indices of Compound 5a and 5b at Three Different Doses (50, 100 and 200 mg/kg) in Comparison with Chloroquine at 10 mg/kg.	139
4.20	Chart Showing % Chemosuppression and Survival Indices of Compound 6a and 6b at Three Different Doses (50, 100 and 200 mg/kg) in Comparison with Chloroquine at 10 mg/kg.	140
4.21	Chart Showing % Chemosuppression and Survival Indices of Compound 11d and 11e at Three Different Doses (50, 100 and 200 mg/kg) in Comparison with Chloroquine at 10 mg/kg.	141
4.22	Keto-Enol Tautomerism Showing the Extension in Conjugation that the Extra CH ₂ CO Affords and the Hydrogen Bonding within a Pseudo Six-Membered Ring System.	143
4.23	The Suggested Cyclized Product from the Reaction of Curcumin with 2,4-Dinitrophenyl Hydrazine. The Product is Thought to be a Pyrazole which is Typical for Reactions of Curcumin with Hydrazino Derivatives from Literature.	146



4.24	(a) Comparison of Compound 2a with Curcumin. Compound 2a is the Monocarbonyl Curcumin Analog that Resembles Curcumin the most.	
	(b) Analysis of the ^1H NMR Spectrum of Compound 2b	148
4.25	(a) Analysis of the ^1H NMR Spectrum of Compound 2e Showing the Symmetry in the Aromatic Rings and the Entire Molecule.	
	(b) Analysis of the ^1H NMR Spectrum of compound 4e Showing the Loss of Symmetry in the Entire Molecule and the Splitting of the CH Protons in the Pyrazoline Moiety of the Molecule	151
4.26	Schematic Diagram Showing the Route of the Drug After Oral Ingestion and Through the Gastro Intestinal Tract, Depicting the Extent to Which the Drug is Absorbed. (Goodman and Gilman, 2006).	156
4.27	Pathway Showing the Degradation of Curcumin when Administered Orally and when Administered Intraperitoneal or Intravenous.	159
4.28	The Portion (CH_2CO) of the Parent Curcumin that is Excluded in the Monocarbonyl Curcumin Analog	161
4.29	The Biopharmaceutics Classification System (BCS) as Defined by the FDA	166
4.30	Structures of Some of the Compounds Synthesized in this Work that Have a Good Chemo Suppression and Survival Index Profile. Some of These Drugs May Be Considered for Further Tests.	176

LIST OF SCHEMES

Scheme	Caption	Page
3.1	Scheme Showing the Reaction of Substituted Benzaldehydes with Acetone to form the Monocarbonyl Curcumin Analogs.	107
3.2	Scheme showing the Reaction of the Synthesized Monocarbonyl Curcumins with 2,4-dinitrophenyl Hydrazine (DNP) to form the Corresponding DNP Hydrazones (1-7) b , Reaction of Compound 3a with 4-nitro Phenyl Hydrazine to form a Pyrazoline Derivative (3c) and Reaction of Pure Curcumin (11d) with DNP to form a DNP Pyrazolone Derivative (11e).	108



LIST OF TABLES

Table	Caption	Page
1.1	Food vacuole proteases in <i>Plasmodium falciparum</i>	10
1.2	Antifolate combinations used in malaria chemotherapy	26
1.3	Possible redox agents	28
1.4	Table of docking softwares with country of origin and year of publication	59
4.1	Table of physicochemical properties	112
4.2	Nuclear magnetic resonance spectroscopic data of some of the synthesized compounds	113
4.3	Nuclear magnetic resonance spectroscopic data for pure curcumin and the DNP pyrazole	115
4.4	Prominent Infrared absorption bands from the spectra of the synthesized compounds	116
4.5	Binding energy of synthesized compounds with plasmepsin II	118

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, ^1H 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. knowlesi* and *P. ovale*. (Chinet *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

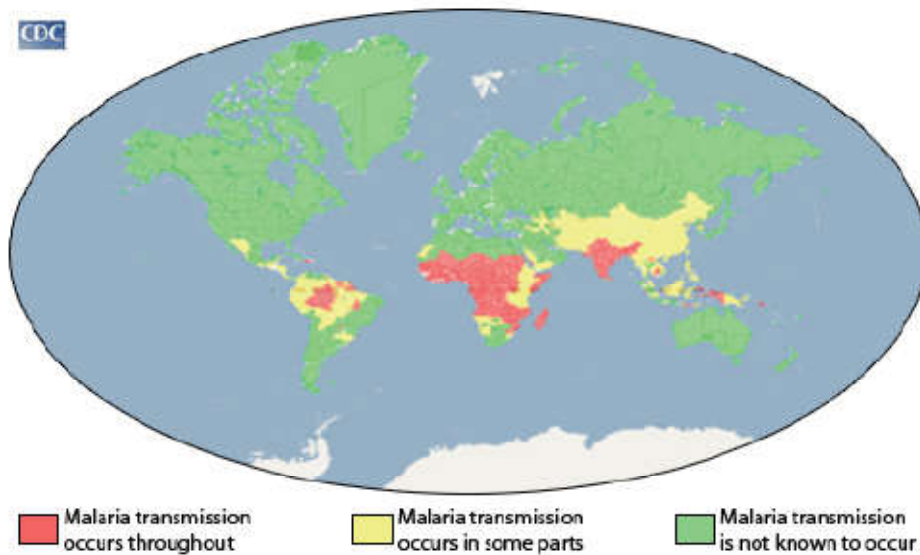


Figure 1.1: Extent of Malaria Transmission in Different Regions of the World as at 2010.

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 artemisinin. 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

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