Identification of some novel natural inhibitors of proteins in the life cycle of the malarial parasite

Dissertation/thesis submitted to the Central University of Punjab

For the award of

M.Sc. life sciences (Biochemistry)

In

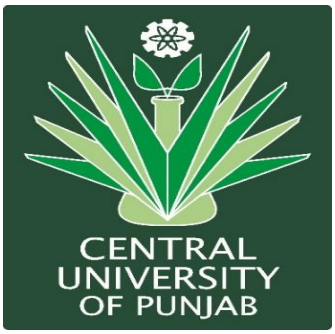
Biochemistry and microbial sciences

By

PRIYANKA KUMARI

Supervisor

Dr. Shashank Kumar



Centre for Biochemistry and Microbial Sciences

School of Basic and Applied Sciences

Central University of Punjab, Bathinda

, 2018

CERTIFICATE

I declare that the project entitled ***"Identification of some novel natural inhibitors of proteins in the life cycle of the malarial parasite"*** has been prepared by me under the guidance of Dr Shashank Kumar, Assistant Professor, Department of Biochemistry and Microbial Sciences, School of Basic and Applied Sciences, Central University of Punjab. No part of project has formed the basis for the award of any degree or fellowship previously.

Priyanka kumari

M.Sc. Life Sciences with specialization in Biochemistry,

Department of Biochemistry and Microbial Sciences,

School of Basic and Applied Sciences,

Central University of Punjab,

Bathinda-151001

Date-

CERTIFICATE

I certify that Priyanka kumari Malik has prepared her Project entitled "**Identification of some novel natural inhibitors of proteins in the life cycle of the malarial parasite"** for the award of M.Sc. Life Sciences with specialization in Biochemistry degree of Central University of Punjab, under my guidance. She carried out this work at the Department of Biochemistry and Microbial Sciences, School of Basic and Applied Sciences, Central University of Punjab.

(SHASHANK KUMAR)
M.Sc. Life Sciences with specialization in Biochemistry,
Department of Biochemistry and Microbial Sciences,
School of Basic and Applied Sciences,
Central University of Punjab,
Bathinda-151001
Date-

ABSTRACT

Identification of some novel natural inhibitors of proteins in the life cycle of the malarial parasite

Name of the student	: Priyanka kumari
Registration Number	: 16mslsbc05
Degree for which submitted	: Master of Science
Supervisor	: Dr.Shashank K.
Department	: Biochemistry and Microbial Sciences
School	: Basic and Applied Sciences

Keywords: Antimalarial potential, In silico, Target proteins, Lead compounds.

Antimalarial phytoconstituents were identified and analysed.We studied receptor based molecular docking of protein drug targets(Plasmepsin 2 (1lf2),HSP90 (3k60), PfATPase (1u5n), Human orotidine 5 – decarboxylase domain (2v30), and Plasmodium orotidine 5- decarboxylase domain (3n3m) against the natural phytochemical .The dock score were in range of -6.1 to -10.7 for antimalarial targets respectively. Some ligands showed better dock score than standard inhibitors of the respected proteins from the range (-6.9 to -10.7).

Priyanka kumari Dr.Shashank Kumar

ACKNOWLEDGEMENT

First and foremost I extend gratitude to my revered seminar advisor Dr. Shashank K., Assistant Professor, Department of Biochemistry and Microbial Sciences, for instilling in me the confidence and drive for doing an arduous task and having faith in my abilities to accomplish any assignment which I set out to do. I thank him also for being a guardian, a friend as well as an erudite scientist to troubleshoot the problems encountered during the scientific report presentation preparation.

I extend my heartfelt thanks to Prof. Ramakrishna Wusirika (HOD) and other faculty members of the Department for their mature and scholarly guidance in making this study fruitful.

I wish to thank Mr Prem Prakash Kushwaha (Ph.D. scholar) and my classmates for their kind cooperation and support.

At last, I am thankful to all people who have helped and inspired me during my seminar preparation and wishing for my bright future.

Priyanka kumari

Date

LIST OF ABBREVIATIONS

Sr. No.	Full Form	Abbreviation
1	Absorption, Distribution, Metabolism, Excretion and Toxicity	ADME/T
2	Auto Dock Tool	ADT
3	Ames Test	AT
4	Blood Brain Barrier	BBB
5	Computer-Aided Drug Design	CADD
6	Carcino Rat	CR
7	Cytochrome P450	CYP
8	Colorectal adenocarcinoma cells	16
9	Chloroquinone	CQ
10	1, 1Diphenyl-2-Picryl Hydrazyl	DPPH
11	Deoxyribonucleic acid	DNA
12	Enzyme-Linked Immunosorbent Assay	ELISA
13	High Throughput Screening	HTS
14	Human Intestinal Absorption	HIA
15	The Human Ether-a-go-go-Related Gene	HERG
17	Inhibitor	I
18	[3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]	MTT
19	Madin Darby Canine Kidney Cells	MDCK
20	Non	N
21	Shattered Kingdom log	SK Log
22	Traditional Chinese Medicine	TCM
23	World Health Organization	WHO
24	Zone of inhibition	ZOI

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 • INTRODUCTON

Introduction

Malaria is one of the most infectious diseases across the globe, especially in Africa and South Asia. *Plasmodium falciparum* is a microscopic parasite which is responsible for malaria. The disease transmits through the Anopheles genus a mosquito species. The Severity of the diseased condition leads to death with multiple symptoms of cold fever. Prevention measurements need to be taken to kill the malaria-carrying mosquitos . The house walls should be sprayed with insecticides and some anti-malarial drugs with different combinations and concentrations. For large malarial disease controlling international boards are established across the globe.

The major issues related to malaria lead to arising in the development of anti-malarial drugs especially natural products called phytochemicals, which are easily available and cost-effective drugs can be produced on the large scale. There are reports suggesting the prominent roles of phytochemicals in treating deadliest diseases. In the field of pharmacology and medicine (Lahlou,2013). the natural products are giving better outcome which is approved internationally and they become revolutionary in drug discovery systems. There are multiples of natural products which are approved by FDA in clinical trials. The new era of computer-Aided Drug Discovery (CADD) become so prominent in identifying hits for developing leads compounds. For optimization of lead compounds and their validation, it requires the studies of phytochemicals physicochemical and pharmaceuticals screening need to be done with multiple parameters including Absorption, metabolism, excretion, toxicity, and distribution (ADME/T) properties(Kumar, 2016).

The life cycle of *Plasmodium falciparum* as parasite vertebrates and invertebrate spreads the population and disease. By achieving the prevention and treatment of both the cycles that prevent the chances of the malarial condition in humans. For this, the deep study of these two cycles in the mosquito to humans can find and

elucidate the key roles of Plasmodium and their functions give the better treatment approaches. Initially, the primary host anopheles mosquito which carrying parasite bites a person introduces the parasite into the bloodstream as a secondary host. Then immediately parasite turns to haploid sporozoite that turns into schizont. There are more than a thousand haploids contains in schizont are called merozoites. Within a week after an infectious bite schizonts rupture and kill hepatocytes and releases millions of merozoites into the bloodstream, This is called as asexual exo-erythrocytic schizogony. Later in the liver cycle, the dormant parasite called as hypnozoites. And it will establish cause relapses in *P.vivax* and *P.ovale* malaria in humans. The characteristics of relapses such as asymptomatic latency remain up to months or years. The malarial parasite remains in the body can be treated but that doesn't clear whole parasites in the bloodstream. Even after the treatment sometimes the chances of getting the disease may happen. During the cycle of erythrocytic schizogony, the merozoites lead to mitotic amplification. After the maturation of parasite into trophozoites that will survive by hemoglobin present in the erythrocytes as a feed. Early merozoites are produced by the maturation of trophozoites into schizonts. Schizont and erythrocytes will rupture after 2-3days releasing merozoites. That rigorously invades other uninfected erythrocytes. The major symptoms can be seen in the person who affected and going through these cycles will have paroxysm such as cold fever. The infected erythrocytes containing merozoites releasing toxins into the bloodstream. Some fractions of erythrocytes are turns to gametocytes. The whole scenario of vertebrate cycles leads to malaria and the anopheles feed on the person's blood in gametocytes. The gametocytes turn into male and female haploid gametes present mosquito gut within minutes. The male mosquito fertilizes the female gametes for producing a diploid zygote. Ookinete is developed by the meiotic division of zygote. The ookinete which is highly motile migrates and invades semipermeable membranes of the mosquito gut wall this process is called the peritrophic matrix. The ookinete turns into oocyte when it residing in between the gut wall and basal lamina. In the hemocoel cavity the oocyte breaks and releases sporozoites. These sporozoites migrate and invade the system of the mosquito and established in the salivary glands of mosquito and that

sporozoites are passed into the bloodstream of a host when it bites for a meal. This whole process of transforming into malaria and their population development process will lead to the spread of disease. To eradicate these malarial conditions we develop different strategies for inhibiting a function of a specific cycle in both primary and secondary host systems (Kaur et al, 2009).

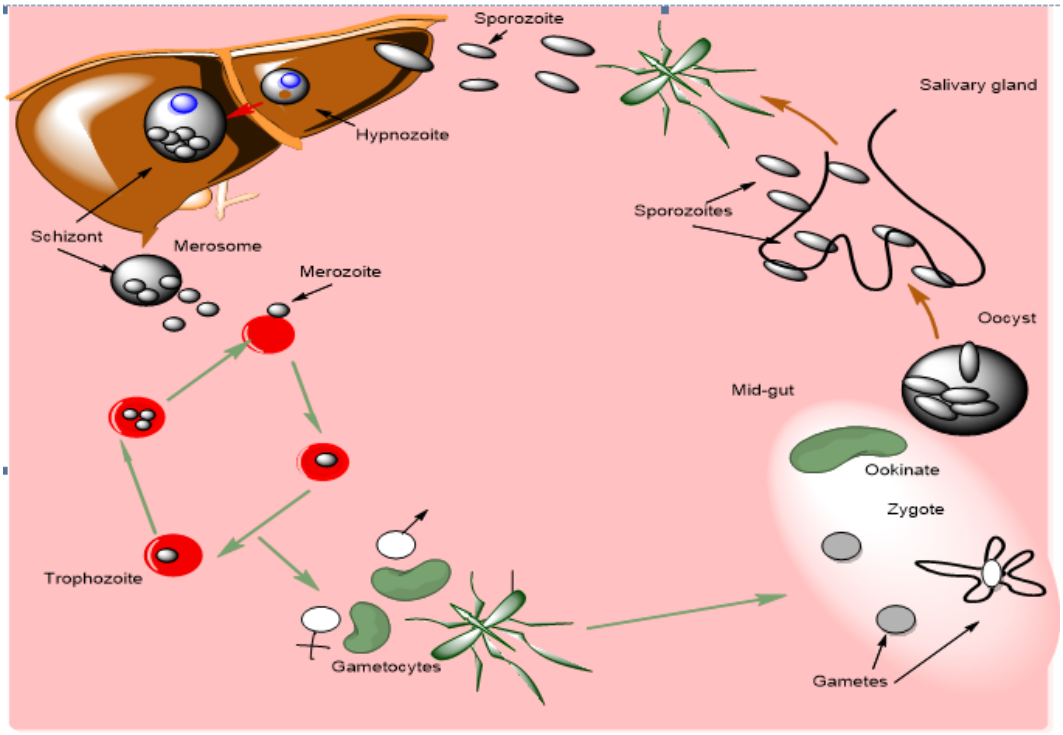


Fig 1.1: Life cycle of *Plasmodium falciparum*

1.1 Hypothesis

The present study has been designed to identify the natural phytochemicals against malaria using *in silico* approach.

Objective

In silico antimalarial potential screening of phytochemicals.

2 • REVIEW OF LITERATURE

Review of Literature

2.1 Natural antimalarial products

Natural compounds are the best-known anti-malarial till now. Current surveys have shown that numerous extracts of many organisms (mostly plants) as possessing anti-plasmodial activity. Because of their antimalarial properties, Cinchona species are widely known and the component alkaloid quinine is still known as an effective drug. The Chinese conventional treatment for malaria comprises the utilization of *Artemisia annua* (Compositae) specifically its active compound, artemisinin, which is recently under substantial interest (Phillipson and O'Neill, 1987).

Possibly the less well-known stereoisomer quinidine is at least as potent as, and probably more effective than quinine (White, 1985). Artemisinin has a greater chemotherapeutic index in comparison to chloroquine and it affects the chloroquine-resistant strains of human malaria (Warhurst, 1985). Another species utilized as an antimalarial drug in Chinese conventional medicine is *Dichroic febrifuga* (Saxifragaceae) (Anonymous, 1975). The active principle, febrifugine has been utilized clinically on *P. vivax* and *P. ovale* but the liver toxicity of this compound marks it improper as a suitable antimalarial drug (Steck, 1972).

2.2 History of natural antimalarial products

Plants have a history of being used as a source of medicine and continue to function as the foundation for many pharmaceuticals used nowadays. The medicinal uses of plants are labeled already on Assyrian clay tablets dated about 2000 B.C. and acknowledged in the Egyptian culture, in Indian Ayurveda (Patwardhan, 2005), in conventional Chinese Medicine (TCM) (Kong et al., 2009) and in numerous European documents. Conventional medicines not only offer valued clues to find

new drugs, but also might assist to move the drug discovery paradigm from 'finding new-entity drugs' to 'combining existing agents', and may even lead the combinations among such agents (Wagner and Ulrich-Merzenich, 2009). A current structural correlation among ~10,000 traditional TCM components and ~8,000 new drugs or candidates, recognized 908 agent pairs which are structurally alike (with similarity 0.85) and 327 agent pairs which are identical in structure. Plants remain to function as the foundation for numerous pharmaceuticals used nowadays (Newman and Cragg, 2007). Even though the modern pharmaceutical industry was born from botanical medicine, synthetic methods to drug discovery have become standard, more recently. Though a broad review of human drugs presented between 1981 and 2006 specifies that around 62% of new small-molecule drugs were natural products, derived from natural products (usually semi-synthetically) and natural product–inspired pharmacophores (which can be considered as natural-product analogs), the synthetic combinatorial chemistry and high throughput screening (HTS) of promising drug targets disengaged the historic link among plants and medicines. Nowadays, though, the minor output of modern antimalarial pharmaceutical research and development has inspired new interests in the potential of natural compounds. In the pharmaceutical industry, the high-throughput screening of chemical libraries against potential targets was emphasized although, in parallel, screening of natural product diminished in the 1990s.

2.3 Importance of natural antimalarial products

Search for new antimalarial drugs and therapies is still a priority of malarial therapy because of the origination of resistance to chemotherapeutic drugs. Natural products signify a source of promising new pharmacophores, the weapons that are required to kill the parasites. Herbal medicinal products are frequently supposed as being safe by the patient community and in disease-endemic countries, many have been used by communities for generations. Furthermore, natural products possess structural and chemical diversity and they are a good source of active therapeutic agents.

.2.4 Currently used natural antimalarial products

Quinine, a 4-methanolquinoline alkaloid isolated by Pelletier and Caventou, from the bark of *Cinchona species* in 1820, is among the oldest and most significant antimalarial drugs in use till date.(Sebisubi and Tan, 2006). This alkaloid constitutes the sole active principle effective against *P. falciparum.* For the development of the synthetic 4- and 8-aminoquinolineclasses of antimalarial drug it is supposed to be the prototype, as exemplified by chloroquine (CQ) and primaquine respectively. The only effective and affordable drug applied for the treatment of malaria was CQ. Quinine, a stage-specific blood schizonticide, since it acts mainly on the mature trophozoite stage of parasite's life cycle. The Chinese conventional treatment of malaria comprises the utilization of *Artemisia annua* and its active compound, artemisinin that is recently under significant interest (Philipson and O'Neill, 1987). Artemisinin has a greater chemotherapeutic index in comparison to chloroquine and is effective against chloroquine-resistant strains of human malaria (Warhurst, 1985). Another species utilized as an antimalarial drug in Chinese conventional medicine is *Dichroic febrifuga* (Anonymous, 1975). The active principle, febrifugine has been utilized clinically on *P. vivax* and *P. ovale* but its liver toxicity marks it improper as a suitable antimalarial drug (Steck, 1972).

2.5 Computer-based drug designing

Most of the antimalarial drugs are small molecules that are designed to bind, interact and modulate the biological activity of the receptors. Computer-aided drug designing (CADD) is being applied to recognize hits, pick leads and optimize drug leads by studying their physicochemical, pharmaceutical and ADME\T (absorption, distribution, metabolism, excretion, and toxicity) properties (Kumar, 2016). The main goal of CADD is to amplify the set of molecules with practically active, drug-like properties and eliminate compounds with unsuitable properties such as inactive, reactive, toxic, poor ADME/T. The ADEM/T property of compounds provides few important *in silico* predictions of the lead compounds which comprises bioavailability, blood-brain barrier crossing and inhibition of K ion channels etc. *In silico* screening method is the principal technique for initial identification of natural products as inhibitors of targets protein and predicting their biological binding mode. In other

words, *in silico* modeling is utilized significantly to reduce time and resource supplies of chemical synthesis and biological *in vitro* and *in vivo* testing (Kumar, 2016)

3 • MATERIALS & METHODS

Material and Methods

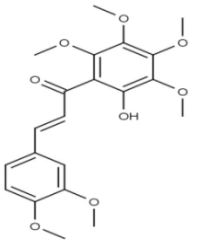
3.1 *In silico*, potential screening

3.1.1 Protein preparation

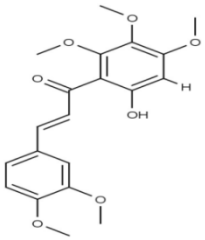
The crystal structure HSP90 (3K60), PFATP6 (1U5N), PForotidine 5'-monophosphate decarboxylase (3N3M), Human orotidine 5' decarboxylase domain uridine monophosphate (2V30), will be retrieved from protein data bank. All the heteroatoms will be removed leaving only the residues of the receptor. Preparation of the target protein with ADT involved the addition of polar hydrogens to the macromolecule, an essential step to correct the calculation of partial charge. Finally, Gasteiger charges will be calculated for each atom of the macromolecule.

3.1.2 Ligand preparation

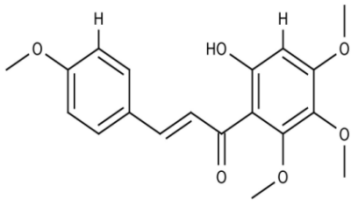
Different literature database has been searched for methylated flavonoids, phytochemicals present in bulbine phytochemicals, and sesquiterpene lactone. At the end of a literature survey, we found the presence of 14 flavonoids, 21 bulbine phytochemicals, and 1557 sesquiterpene lactone. The structure of the phytochemicals is shown in figure (3.1), (3.2) and (3.4).



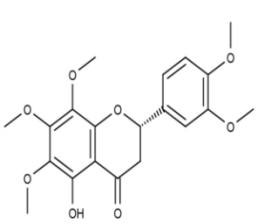
1



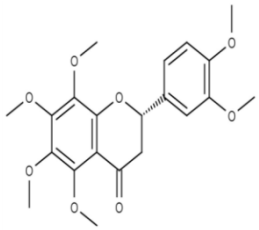
2



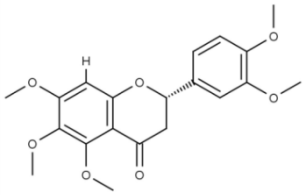
3



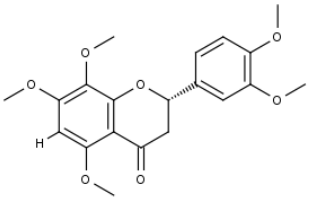
4



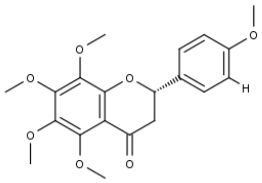
5



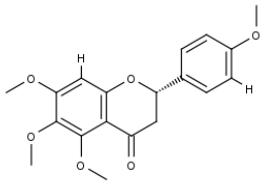
6

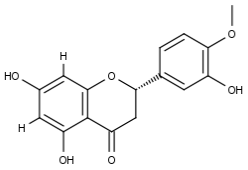


8

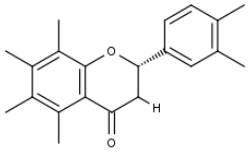


9

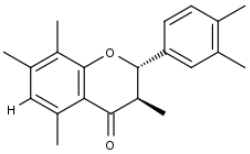




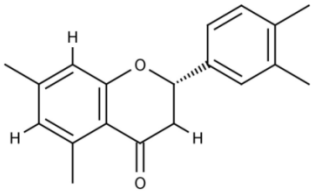
10



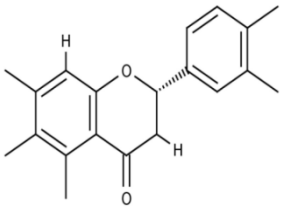
11



12



13

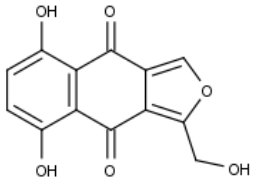


14

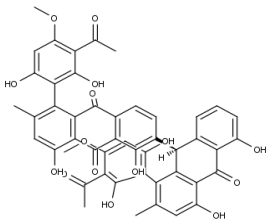
7

Figure 3.1: Structure of methylated flavanoids; (1); (2E)-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-3,4,5,6-tetramethoxyphenyl)prop-2-en-1-one**, (2);** (2E)-3-(3,4-dimethoxyphenyl)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)prop-2-en-1-one, **(3);** (2E)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, **(4);** (2S)-2-(3,4-dimethoxyphenyl)-5-hydroxy-6,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(5);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxy-3,4-dihydro-2H-1-benzopyran-one, **(6);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(7);** (2S)-2-(3,4-dimethoxyphenyl)-5,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(8);** (2S)-5,6,7,8 tetramethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(9);** (2S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(10);** (2S)-

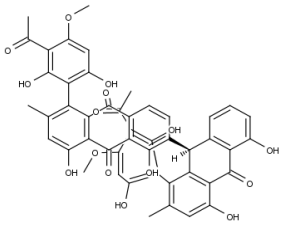
5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,4- dihydro-2H-1-benzopyran-4-one**, (11) ;** (2S)-2-(3,4-dimethylphenyl)-5,6,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(12);** 2-(3,4-dimethylphenyl)-3,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one , **(13);** (2S)-2-(3,4-dimethylphenyl)-5,7-dimethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(14);** (2S)-2-(3,4-dimethylphenyl)-5,6,7-trimethyl-3,4-dihydro-2H-1-benzopyran-4-one.



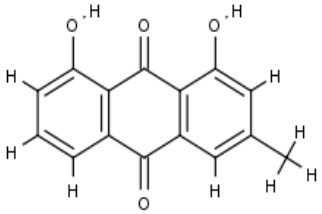
1



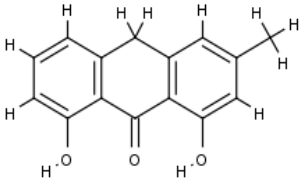
2



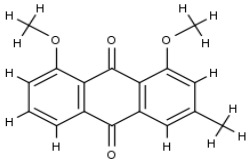
3



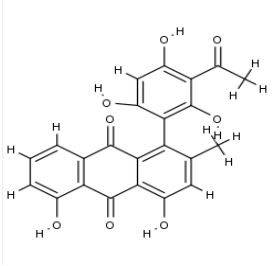
4



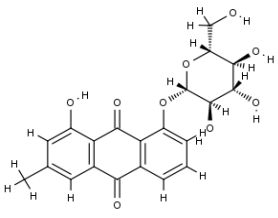
5



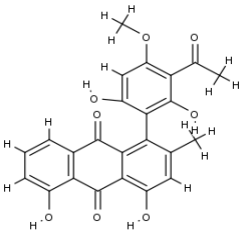
6



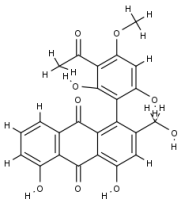
7



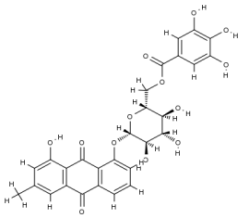
8



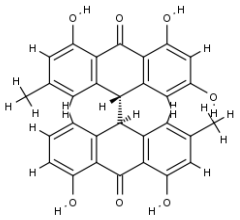
9



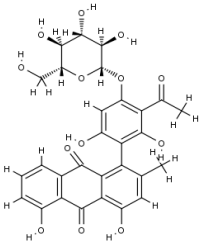
10



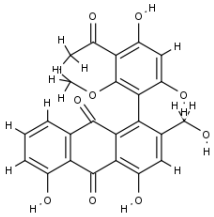
11



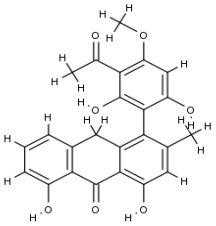
12



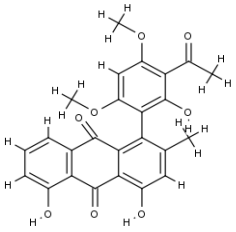
13



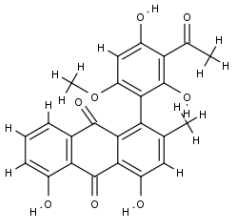
14



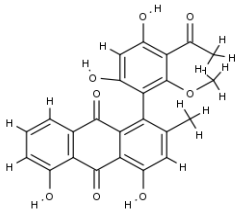
15



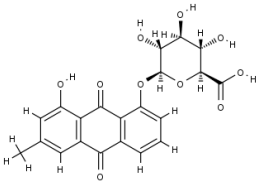
16



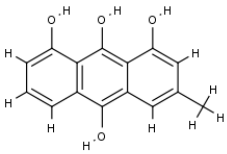
17



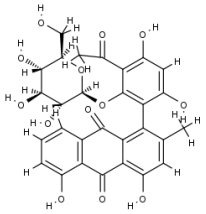
18



19



20

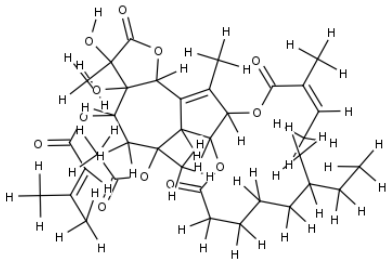


21

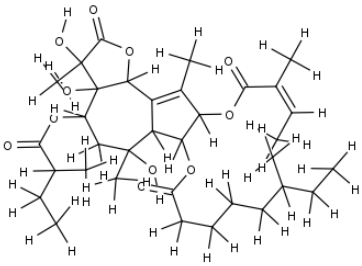
Figure 3.2: Structure of bulbine phytochemicals; (1); 5,8-dihydroxy-1-(hydroxymethyl)-4H,9H-naphtho[2,3-c]furan-4,9-dione, **(2);** 1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-6-[(9S)-1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methyl-10-oxo-9,10-dihydroanthracen-9-yl]-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene 9,10-dione, **(3);** 1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-6-[(9S)-1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methyl-10-oxo-9,10dihydroanthracen-9-yl]-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione, **(4);** 1,8-dihydroxy-3-methyl-9,10-dihydroanthracene-9,10-dione, **(5);**

1,8-dihydroxy-3-methyl-9,10-dihydroanthracen-9-one, **(6);** 1,8-dimethoxy-3-methyl-9,10-dihydroanthracene-9,10-dion, **(7);** 1-(3-acetyl-2,4,6trihydroxyphenyl)-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione, **(8);** 1-hydroxy-3-methyl-8-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-9,10-dihydroanthracene-9,10-dione, **(9);** 1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione, **(10);** 1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-(hydroxymethyl)-9,10-dihydroanthracene-9,10-dione, **(11);** [(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[(8-hydroxy-6-methyl-9,10-dioxo-9,10-dihydroanthracen-1-yl)oxy]oxan-2-yl] methyl 3,4,5-trihydroxybenzoate, **(12);** (10R)-10-[(9R)-4,5-dihydroxy-2-methyl-10-oxo-9,10-dihydroanthracen-9-yl]-1,3,8-trihydroxy-6-methyl-9,10-dihydroanthracen-9-one, **(13);** 1-(3-acetyl-2,6-dihydroxy-4-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}phenyl)-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione, **(14);** 1-(3-acetyl-4,6-dihydroxy-2-methoxyphenyl)-4,5-dihydroxy-2-(hydroxymethyl)-9,10-dihydroanthracene-9,10-dione, **(15);** 4-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-1,8-dihydroxy-3-methyl-9,10-dihydroanthracen-9-one, **(16);** 1-(3-acetyl-2-hydroxy-4,6-dimethoxyphenyl)-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione, **(17);** 1-(3-acetyl-2,4-dihydroxy-6-methoxyphenyl)-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione, **(18);** 1-(3-acetyl-4,6-dihydroxy-2-methoxyphenyl)-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione, **(19);** (2S,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[(8-hydroxy-6-methyl-9,10-dioxo-9,10-dihydroanthracen-1-yl)oxy]oxane-2-carboxylic acid, **(20);** 3-methylanthracene-1,8,9,10-tetrol, **(21);** 1-(3-acetyl-4,6-dihydroxy-2-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}phenyl)-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione.

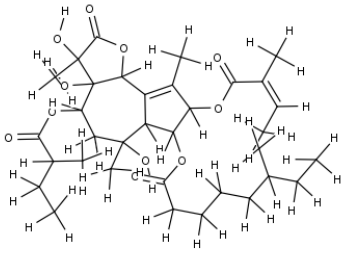
(Structure of only active ligands are shown)



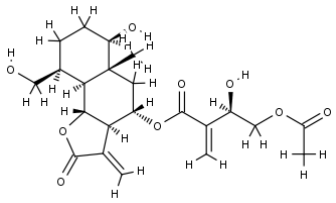
1



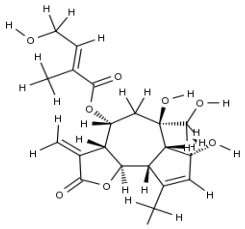
2



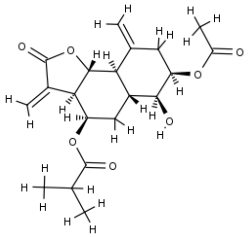
3



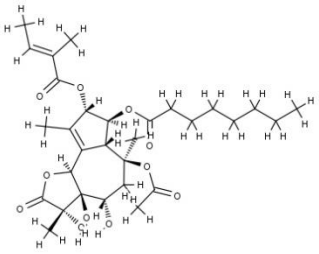
4



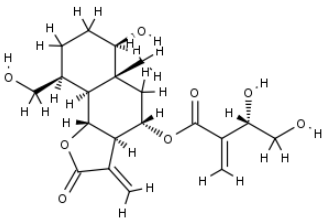
5



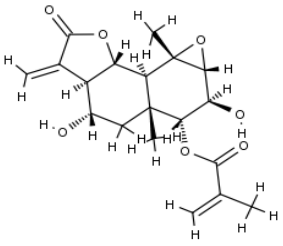
6



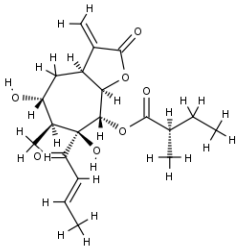
7



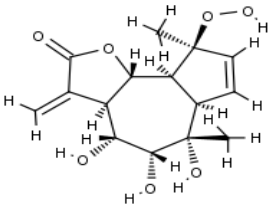
8



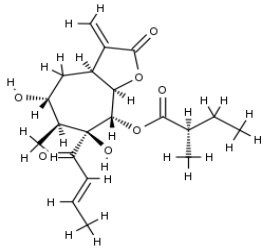
9



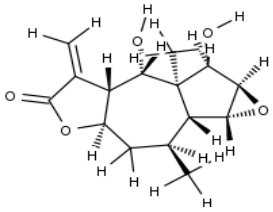
10



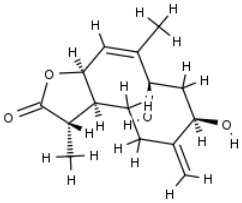
11



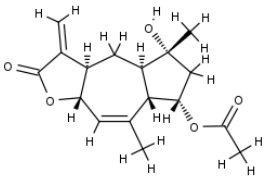
12



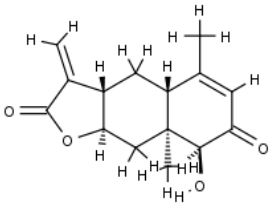
13



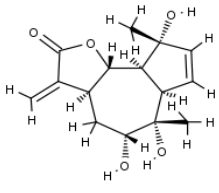
14



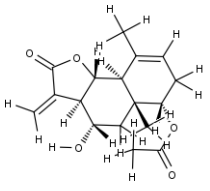
15



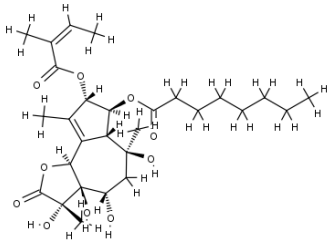
16



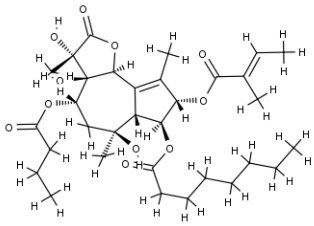
17



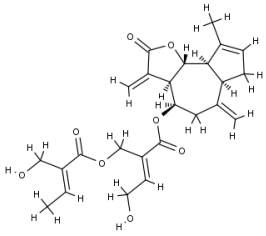
18



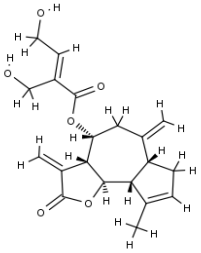
19



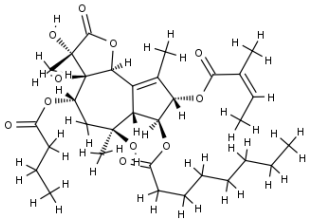
20



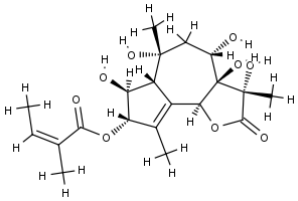
21



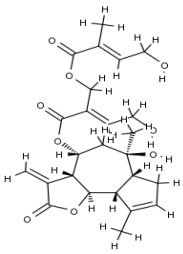
22



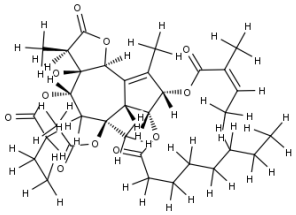
23



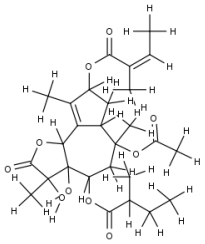
24



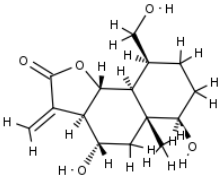
25



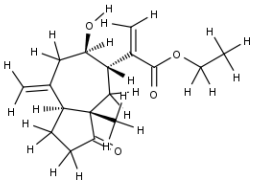
26



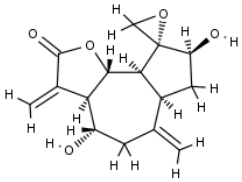
27



28



29



30

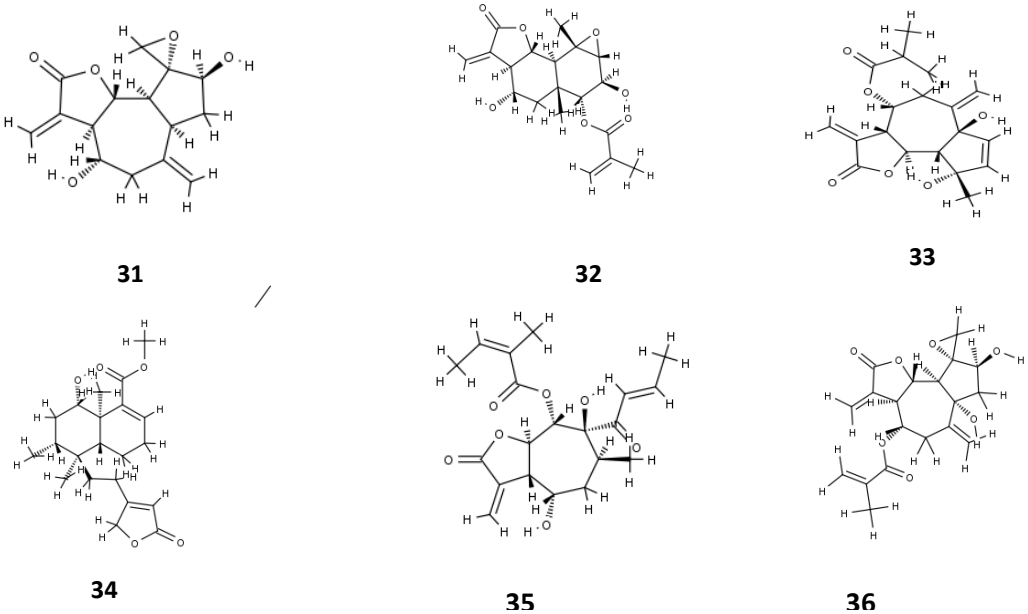


Figure 3.3: Structure of sesquiterpene lactone; (1); 6-(acetyloxy)-3,3a-dihydroxy-3,6,9-trimethyl-4-[(3-methylbut-2-enoyl)oxy]-8-{[(2Z)-2-methylbut-2-enoyl]oxy}-2-oxo-2H,3H,3aH,4H,5H,6H,6aH,7H,8H,9bH-azuleno[4,5-b]furan-7-yl6-methyloctanoate, **(2);** 3,3a,6-trihydroxy-3,6 **35** -{[(2Z)-2-methylbu **36** }-4-[(2-methylbutanoyl)oxy]-oxo2H,3H,3aH,4H,5H,6H,6aH,7H,8H,9bH-azuleno[4,5-b]furan-7-yl 6-methyloctanoate, **(3);** (3S,3aS,4S,6S,6aR,7S,8S,9bS)-6-(acetyloxy)-4-(butanoyloxy)-3,3a-dihydroxy-3,6,9-trimethyl-8-[(2-methylprop-2-enoyl)oxy]-2-oxo-2H,3H,3aH,4H,5H,6H,6aH,7H,8H,9bH-azuleno[4,5-b]furan-7-yl octanoate, **(4);** (3aR,4S,5aR,6R,9S,9aS,9bR)-6-hydroxy-9-(hydroxymethyl)-5a-methyl-3-methylidene-2-oxo-dodecahydronaphtho[1,2-b]furan-4-yl (3R)-4-(acetyloxy)-3-hydroxy-2-methylidenebutanoate, **(5);** (3aR,4R,6R,6aS,7R,9aR,9bR)-6,7-dihydroxy-6-(hydroxymethyl)-9-methyl-3-methylidene-2-oxo-2H,3H,3aH,4H,5H,6H,6aH,7H,9aH,9bH-azuleno[4,5-b]furan-4-yl (2E)-4-hydroxy-2-methylbut-2-enoate, **(6);** (3aR,4R,5aR,6S,7R,9aR,9bR)-7-(acetyloxy)-6-hydroxy-3,9-dimethylidene-2-oxo-dodecahydronaphtho[1,2-b]furan-4-yl2-methylpropanoate, **(7);** (3S,3aR,4S,6S,6aR,7S,8S,9bS)-6-(acetyloxy)-3,3a,4-trihydroxy-3,6,9-trimethyl-8-{[(2E)-2-methylbut-2-enoyl]oxy}-2-oxo-2H,3H,3aH,4H,5H,6H,6aH,7H,8H,9bH-azuleno[4,5-b]furan-7-yl octanoate, **(8);** (3aR,4S,5aR,6R,9S,9aS,9bR)-6-hydroxy-9-(hydroxymethyl)-5a-methyl-3-methylidene-2-oxo-dodecahydronaphtho[1,2-b]furan-4-yl (3S)-3,4-dihydroxy-2 methylidenebutanoate, **(9);** (1S,2S,6R,7S,9R,10R,11S,12R,14S)-7,11-dihydroxy- 9,14-dimethyl-5-methylidene-4-oxo-3,13-dioxatetracyclo[7.5.0.0^{2,6}.0^{12,14}]tetradecan- 10-yl 2-methylprop-2-enoate, **(10);**

(3aS,5R,6S,7S,8S,8aR)-7-[(2E)-but-2-enoyl]-5,7-dihydroxy-6-methyl-3-methylidene-2-oxo-octahydro-2H-cyclohepta[b]furan-8-yl (2R)-2-methylbutanoate, **(11);** (3aR,4R,5R,6S,6aR,9S,9aS,9bS)-9-hydroperoxy-4,5,6-trihydroxy-6,9-dimethyl-3-methylidene-2H,3H,3aH,4H,5H,6H,6aH,9H,9aH,9bH-azuleno[4,5-b]furan-2-one, **(12);** (3aS,5R,6S,7S,8S,8aR)-7-[(2E)-but-2-enoyl]-5,7-dihydroxy-6-methyl-3-methylidene-2-oxo-octahydro-2H-cyclohepta[b]furan-8-yl (2R)-2-methylbutanoate, **(13);** (1S,2S,3R,7R,9S,10R,11R,13R,14R)-2,14-dihydroxy- 1,9-dimethyl-4-methylidene-6,12-dioxatetracyclo[8.4.0.0^{3,7}.0^{11,13}]tetradecan-5- one, **(14);** (3S,3aS,7R,9S,11aR)-7,9-dihydroxy-3,10-dimethyl-6-methylidene-2H,3H,3aH,4H,5H,6H,7H,8H,9H,11aH cyclodeca[b]furan-2-one, **(15);** (3aR,4aR,5R,7S,7aS,9aS)-5-hydroxy-5,8-dimethyl-3-methylidene-2-oxo-2H,3H,3aH,4H,4aH,5H,6H,7H,7aH,9aH-azuleno[6,5-b]furan-7-yl acetate, **(16);** (3aS,4aR,8S,8aS,9aR)-8-hydroxy-5,8a-dimethyl-3-methylidene-2H,3H,3aH,4H,4aH,7H,8H,8aH,9H,9aH-naphtho[2,3-b]furan-2,7-dione, **(17);** (3aS,5R,6S,6aR,9R,9aS,9bS)-5,6,9-trihydroxy-6,9-dimethyl-3-methylidene-2H,3H,3aH,4H,5H,6H,6aH,9H,9aH,9bH-azuleno[4,5-b]furan-2-one, **(18);** (3aR,4S,5aR,6S,9aS,9bR)-4-hydroxy-5a,9-dimethyl-3-methylidene-2-oxo2H,3H,3aH,4H,5H,5aH,6H,7H,9aH,9bH-naphtho[1,2-b]furan-6-yl acetate, **(19);** (3S,3aR,4S,6S,6aR,7S,8S,9bS)-3,3a,4,6-tetrahydroxy-3,6,9-trimethyl-8-{[(2Z)-2-methylbut-2-enoyl]oxy}-2-oxo-2H,3H,3aH,4H,5H,6H,6aH,7H,8H,9bH-azuleno[4,5-b]furan-7-yl octanoate, **(20);** (3S,3aR,4S,6S,6aR,7S,8S,9bS)-4-(butanoyloxy)-3,3a,6-trihydroxy-3,6,9-trimethyl-8-{[(2E)-2-methylbut-2-enoyl]oxy}-2-oxo-2H,3H,3aH,4H,5H,6H,6aH,7H,8H,9bH-azuleno[4,5-b]furan-7-yl octanoate, **(21);** (3aR,4R,6aR,9aR,9bR)-9-methyl-3,6-dimethylidene-2-oxo-2H,3H,3aH,4H,5H,6H,6aH,7H,9aH,9bH-azuleno[4,5-b]furan-4-yl (2E)-4-hydroxy-2-({[(2E)-2-(hydroxymethyl)but-2-enoyl]oxy}methyl)but-2-enoate, **(22);** (3aR,4R,6aR,9aR,9bR)-9-methyl-3,6-dimethylidene 2oxo2H,3H,3aH,4H,5H,6H,6aH,7H,9aH,9bH-azuleno[4,5-b]furan-4-yl(2E)-4-hydroxy-2-(hydroxymethyl)but-2-enoate, **(23);** (3S,3aR,4S,6S,6aR,7S,8S,9bS)-4-(butanoyloxy)-3,3a,6-trihydroxy-3,6,9-trimethyl-8-{[(2Z)-2-methylbut-2-enoyl]oxy}-2-oxo 2H,3H,3aH,4H,5H,6H,6aH,7H,8H,9bH-azuleno[4,5-b]furan-7-yloctanoate, **(24);** (3S,3aR,4S,6R,6aR,7S,8S,9bS)-3,3a,4,6,7-pentahydroxy-3,6,9-trimethyl-2-oxo 2H,3H,3aH,4H,5H,6H,6aH,7H,8H,9bH-azuleno[4,5-b]furan-8-yl(2Z)-2-methylbut-2-enoate, **(25);** (2E)-3-{[(3aR,4R,6R,6aR,9aR,9bR)-6-hydroxy-6,9-dimethyl-3-methylidene-2-oxo-2H,3H,3aH,4H,5H,6H,6aH,7H,9aH,9bH-azuleno[4,5-b]furan-4-yl]oxy}-2-(2-hydroxyethylidene)-3-oxopropyl (2E)-4-hydroxy-2-methylbut-2-enoate, **(26);** (3R,3aR,4S,6S,6aR,7R,8S,9bS)-6-(acetyloxy)-4-(butanoyloxy)-3a-hydroxy-3,6,9-trimethyl-8-{[(2Z)-2-methylbut-2-enoyl]oxy}-2-oxo-2H,3H,3aH,4H,5H,6H,6aH,7H,8H,9bH-azuleno[4,5-b]furan-7-yl octanoate, **(27);** 6-(acetyloxy)-3,3a-dihydroxy-3,6,9-trimethyl-4-[(2-methylbutanoyl)oxy]-2-oxo-2H,3H,3aH,4H,5H,6H,6aH,7H,8H,9bH-azuleno[4,5-b]furan-8-yl (2Z)-2-methylbut-2-enoate, **(28);** (3aR,4S,5aR,6R,9S,9aS,9bR)-4,6-dihydroxy-9-(hydroxymethyl)-5a-methyl-3-methylidene-dodecahydronaphtho[1,2-b]furan-2-one, **(29);** ethyl 2-[(3aS,5S,6R,8aS)-6-hydroxy-3a-methyl-8-methylidene-3-oxo-decahydroazulen-5-yl]prop-2-

enoate, **(30);** (3aR,4S,6aR,8S,9R,9aS,9bS)-4,8-dihydroxy-3,6-dimethylidene-decahydro-2H-spiro[azuleno[4,5-b]furan-9,2'-oxirane]-2-one, **(31);** (3aR,4S,6aR,8S,9R,9aS,9bS)-4,8-dihydroxy-3,6-dimethylidene-decahydro-2H-spiro[azuleno[4,5-b]furan-9,2'-oxirane]-2-one, **(32);** (1S,2S,6R,7S,9R,10R,11S,12R,14S)-7,11-dihydroxy- 9,14-dimethyl-5-methylidene-4-oxo-3,13-dioxatetracyclo[7.5.0.0^{2,6}.0^{12,14}]tetradecan- 10-yl 2-methylprop-2-enoate, **(33);** (3aR,4R,6aS,9S,9aS,9bS)-6a,9-dihydroxy-9-methyl-3,6-dimethylidene-2-oxo-2H,3H,3aH,4H,5H,6H,6aH,9H,9aH,9bH-azuleno[4,5-b]furan-4-yl 2 methylpropanoate, **(34);** methyl (4aR,5S,6R,8S,8aR)-8-hydroxy-5,6,8a-trimethyl-5-[2-(5-oxo-2,5-dihydrofuran-3-yl)ethyl]-3,4,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylate, **(35);** (3aS,4S,6S,7S,8S,8aR)-7-[(2E)-but-2-enoyl]-4,7-dihydroxy-6-methyl-3-methylidene-2-oxo-octahydro-2H-cyclohepta[b]furan-8-yl (2Z)-2-methylbut-2-enoate, **(36);** (3aR,4S,6aS,8S,9R,9aR,9bS)-6a,8-dihydroxy-3,6-dimethylidene-2-oxo-decahydro-2H-spiro[azuleno[4,5-b]furan-9,2'-oxirane]-4-yl 2-methylprop-2-enoate

The literature based phytoconstituents will be used as ligand for molecular docking. The 3D or 2D structure of phytochemicals of targeted proteins like HSP90 (3K60), Plasmepsin 2(1lf2), *Plasmodium falciparum* ATPase (1u5n), Human orotidine 5' decarboxylase (2v30), Plasmodium orotidine 5'decarboxylase (3n3m) and respective reported inhibitors of particular protein will be retrieved from NCBI PubChem in sdf format. Open Babel molecule format converter will be used for conversion of 2D to 3D conformation, Marvin Sketch software will perform the conversion from sdf to pdb (for docking) and mol (for molecular properties prediction) file. Ligands energy was minimized by applying mmff94 force field and conjugate gradients optimization algorithm using PyRx-Python prescription 0.8 (Olson, 2015) for 200 steps.

3.1.3 Molecular docking

Crystal structure of target HSP90 (3K60), PFATP6 (1U5N), PForotidine 5'-monophosphate decarboxylase (3N3M), Human orotidine 5' decarboxylase domain uridine monophosphate (2V30), will be obtained from the RCSB protein data bank. The preparation of the target enzyme with the Auto Dock Tools involved in the addition of hydrogen atoms to the target enzyme, which is a necessary step for the computation of partial atomic charges. Gasteiger charges will be considered for each atom present in the target in Auto Dock 4.2.

3.2 *In vitro* anticancer activity by (MTT assay)

In vitro anticancer potential of 005 against various cancer cell lines was performed using MTT [3-(4,5- dimethylthiazol-2-yr)-2,5-diphenyltetrazolium bromide] (Tian et. al., 2003). Cell suspension (100 μL) will be incubated for 24 h followed by addition of 100 μL extracts (100 μg/well) and further was incubated for 72 h. MTT solution (10 μL) will be added to each of the 96 wells, and then plates was be wrapped with aluminum foil and incubated at 37˚C for 4 h leading to the formation of MTT-formazon crystals. Absorbance was measured by ELISA reader at 540 nm. Controls and samples were assayed in triplicate. The results was shown as mean ± SD (Kumar et al., 2013).

(%)I=[(Ac−As)/Ac] ×100

3.3 Antioxidant assay by DPPH assay

The free radical scavenging activity of the samples was measured *in vitro* by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Appropriate DPPH solution concentration will be prepared in methanol followed by addition of 1 mL of the test sample at different concentration. The content will be mixed and allowed to stand at measured by recording the absorbance at 517 nm. The percentage scavenging activities (%Inhibition) at different concentrations of the extracts will be calculated using the following formula:

(%)I=[(Ac−As)/Ac] ×100

where I am inhibition and AC and AS are the absorbance values of the control and the sample, respectively. Three replicates were made for each sample and results were expressed as mean ± SD (Kumar et. al., 2014).

3.4 Disc diffusion method

The antimicrobial activity of compound a against *E.coli* will be determined using Kirby-Bauer disc diffusion method (Bauer et. al, 1966). The inoculum suspension of bacterial strains will be swabbed on the entire surface of LB agar. Sterile 6 mm

diameter paper discs (Himedia) saturated with 20 µL of phytochemicals (drug) prepared in DMSO (containing 2 mg extract/disc) will be aseptically placed on the upper layer of the inoculated agar surfaces and plates will be incubated at 37°C for 24 hours. Antibacterial activity will be determined by measuring the diameter of the zone of inhibition (ZOI) surrounding discs. Standard antibiotic discs of penicillin (10 µg/disc) and norfloxacin (10 µg/disc) will be used as positive control. Discs containing 20 µL DMSO will be used as a negative control. The antimicrobial assay will be performed in triplicate and results will be reported as the average of three replicates (Bauer, 1966).

4 • RESULT & DISCUSSION

Results

4.1 Ligand-Protein Binding analysis

The ligands were docked against targeted proteins to predict the binding interaction. The result of molecular docking study is summarized in the table (4.1.1), table (4.1.2) and figure (4.1.1). The table contains dock score for phytochemicals and the standards. It includes lowest binding energy for the proteins against the ligands and also for the known standard inhibitors.The ligands such as (2S)-2-(3,4-dimethylphenyl)-5,6,7-trimethyl-3,4-dihydro-2H-1-benzopyran-4-one,(2S)-2-(3,4-dimethylphenyl)-5,6,7,8 tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one, 3,3a,6-trihydroxy-3,6,9-trimethyl-8-{[(2Z)-2-methylbut-2-enoyl]oxy}-4-[(2-methyl butanol)oxy]-2-oxo 2H,3H,3aH,4H,5H,6H,6aH,7H,8H,9bH-azuleno[4,5-b]furan-7-yl 6-methyl octanoate, (1S,2S,6R,7S,9R,10R,11S,12R,14S)-7,11-dihydroxy- 9,14-dimethyl-5-methylidene-4-oxo-3,13- dioxatetracyclo [7.5.0.0^{2,6}.0^{12,14}] tetradecane- 10-yr 2-methylprop-2-enoate, showed better binding energy between (-8.5 kcal/mol to -10.7 kcal/mol) against targeted proteins such as Plasmepsin 2(1lf2), HSP90(3k60), ATPase(1u5n), Plasmodium omp 5’ decarboxylase(3n3m) as compared to standard inhibitors.

4.1.1 Binding affinity of phytochemicals against different target proteins.

Different groups of phytochemicals such as methylated flavonoids, phytochemicals present in Bulbine frutescens and sesquiterpene lactones were used as ligands to dock against different target proteins. Binding potential of standard inhibitors and test phytochemicals (methylated flavonoids, Bulbine frutescens, and sesquiterpene lactones) are given in table (4.1.1), table (4.1.2) and figure (4.1.1) respectively.

Table 4.1.1: Comparative table of flavonoid showing binding affinity of the ligands with different targeted proteins

		Protein PDB ID				
S. No	**Standard compound**	**1lf2**	**1u5n**	**2v30**	**3k60**	**3n3m**
1	RS370	-9.4	-	-	-	-
2	Artemisinin	-	-7.3	-	-	-
3	{[(2R,3S,4R,5R)-5-(2,4-dioxo-1,2,3,4tetrahydropyrimidin-1-yl)-3,4-dihydroxyoxolan-2-yl]methoxy}phosphonic acid	-	-	-5.8	-	-
4	Tanespimycin	-	-	-	-6.9	-
5	{[(2R,3S,4R,5R)-5-(2,6-dioxo-2,3,6,9-tetrahydro-1H-purin-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxy}phosphonic	-	-	-	-	-6.9
S. No	**Methylated flavonoids**	**1lf2**	**1u5n**	**2v30**	**3k60**	**3n3m**
1	(2E)-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy3,4,5,6Tetramethoxyphenyl)prop-2-en-1-one	-7.7	-5.9	-5.5	-7.2	-6.4
2	(2E)-3-(3,4-dimethoxyphenyl)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)prop-2-en-1-one	-6.2	11.9	-6.4	-7	-6.6
3	(2E)-1-(6-hydroxy-2,3,trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one	-5.9	-6.5	-5.9	-7	-6.4
4	(2S)-2-(3,4-dimethoxyphenyl)-5-hydroxy 6,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one	-6.6	-7.7	-6.6	-7.1	-6.6
5	(2S)-2-(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxy-3,4-dihydro-2H-1-benzopyran-4-one	-7.1	-6.3	-5.7	-6.7	-6.6
6	(2S)-2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one	-6.7	-6.2	-6.2	-4.9	-6.3
7	(2S)-2-(3,4-dimethoxyphenyl)-5,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one	-6.5	-7.8	-6.1	-7.5	-6.8
8	(2S)-5,6,7,8-tetramethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one	-6.5	-6.4	-6	-7	-6.1
9	(2S)-5,6,7-trimethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one	-6.5	-6.7	-6.2	-7.3	-8.2

10	(2S)-5,7-dihydroxy-2-(3-hydroxy-4methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one	-7.1	-6.7	-8.8	-7.4	-8.8
11	(2S)-2-(3,4-dimethylphenyl)-5,6,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one	-8.5	-8.8	-7.4	-8.5	-7.5
12	2-(3,4-dimethylphenyl)-3,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one	-8.4	-9.1	-6.3	-8.4	-8.3
13	(2S)-2-(3,4-dimethylphenyl)-5,7-dimethyl-3,4-dihydro-2H-1-benzopyran-4-one	-8.4	-8.7	-8.7	-8.3	-8.8
14	(2S)-2-(3,4-dimethylphenyl)-5,6,7-trimethyl-3,4-dihydro-2H-1-benzopyran-4-one	-8.5	-8.8	-7.4	-8.3	-8.8

Table 4.1.2: Comparative table of Bulbine frutescens showing binding affinity of the ligands with different targeted proteins.

		Protein PDB ID				
S. No	**Standard compounds**	**1lf2**	**1u5n**	**2v30**	**3k60**	**3n3m**
1	RS370	-9.4	-	-	-	-
2	Artemisinin	-	-7.3	-	-	-
3	{[(2R,3S,4R,5R)-5-(2,4-dioxo-1,2,3,4tetrahydropyrimidin-1-yl)-3,4-dihydroxyoxolan-2-yl]methoxy}phosphonic acid	-	-	-5.8	-	-
4	Tanespimycin	-	-	-	-6.9	-
5	{[(2R,3S,4R,5R)-5-(2,6-dioxo-2,3,6,9-tetrahydro-1H-purin-9-yl)-3,4-dihydroxyoxolan-2yl]methoxy}phosphonic	-	-	-	-	-6.9
S. No	**Bulbine frutescens**	**1lf2**	**1u5n**	**2v30**	**3k60**	**3n3m**
1	5,8-dihydroxy-1-(hydroxymethyl)-4H,9H-naphtho[2,3-c]furan-4,9-dione	-5.3	-7.3	-5.8	-6.3	-73

2	1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-6-[(9S)-1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methyl-10-oxo-9,10dihydroanthracen-9-yl]-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione	-6.3	-8	-6.6	-6.1	-8.2
3	1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-6-[(9S)-1-3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methyl-10-oxo-9,10dihydroanthracen-9-yl]-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione	-6.5	-8.5	-7.3	-6.3	-7.8
4	1,8-dihydroxy-3-methyl-9,10-dihydroanthracene-9,10-Dione	-5.9	-8	-6.5	-7.1	-7.8
5	1,8-dihydroxy-3-methyl-9,10-dihydroanthracen-9-one	-5.9	-7.8	-6.7	-7.3	-7.7
6	1,8-dimethoxy-3-methyl-9,10-dihydroanthracene-9,10-Dione	-5.8	-7.9	-6.4	-6	-7.3
7	1-(3-acetyl-2,4,6-trihydroxyphenyl)-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione	-6.3	-8	-7.2	-7.7	-8.3
8	1-hydroxy-3-methyl-8-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-9,10-dihydroanthracene-9,10-dione	-6.3	-8.2	-7.2	-8.1	-8.3
9	1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione	-6.3	-8.4	-6.9	-7.4	-7.9
10	1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-(hydroxymethyl)-9,10-dihydroanthracene-9,10-dione	-6.1	-7.5	-7.1	-7.1	-8.1
11	[(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[(8-hydroxy-6-methyl-9,10-dioxo-9,10-dihydroanthracen-1-yl)oxy]oxan-2-yl]methyl 3,4,5-trihydroxybenzoate	-6.6	-8.9	-8.1	-6.5	-9.2
12	(10R)-10-[(9R)-4,5-dihydroxy-2-methyl-10-oxo-9,10-dihydroanthracen-9-yl]-1,3,8-trihydroxy-6-methyl-	-7.9	-8	-8.7	-8.2	-9.8

	9,10-dihydroanthracen-9-one					
13	1-(3-acetyl-2,6-dihydroxy-4-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}phenyl)-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione	-6.3	-7.5	-7.5	-8.2	-8.5
14	1-(3-acetyl-4,6-dihydroxy-2-methoxyphenyl)-4,5-dihydroxy-2-(hydroxymethyl)-9,10-dihydroanthracene-9,10-dione	-5.7	-7.5	-6.7	-7	-7.7
15	4-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-1,8-dihydroxy-3-methyl-9,10-dihydroanthracen-9-one	-6.3	-7.5	-6.9	-7.6	-7.7
16	1-(3-acetyl-2-hydroxy-4,6-dimethoxyphenyl)-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione	-7.1	-7.5	-6.8	-7.3	-7.9
17	1-(3-acetyl-2,4-dihydroxy-6-methoxyphenyl)-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione	-7.3	-8.1	-6.9	-7.5	-8.3
18	1-(3-acetyl-4,6-dihydroxy-2-methoxyphenyl)-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione	-7	-8.1	-7.5	-7.2	-6.6
19	(2S,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[(8-hydroxy-6-methyl-9,10-dioxo-9,10-dihydroanthracen-1-yl)oxy]oxane-2-carboxylic acid	-6.3	-8.6	-7.6	-8.4	-7.9
20	3-methylanthracene-1,8,9,10-tetrol	-6	-7.2	-6.7	-7.2	-6.6
21	1-(3-acetyl-4,6-dihydroxy-2-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2, yl]oxy}phenyl)-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione	-6.4	-6.4	-6.7	-7.7	-7.5

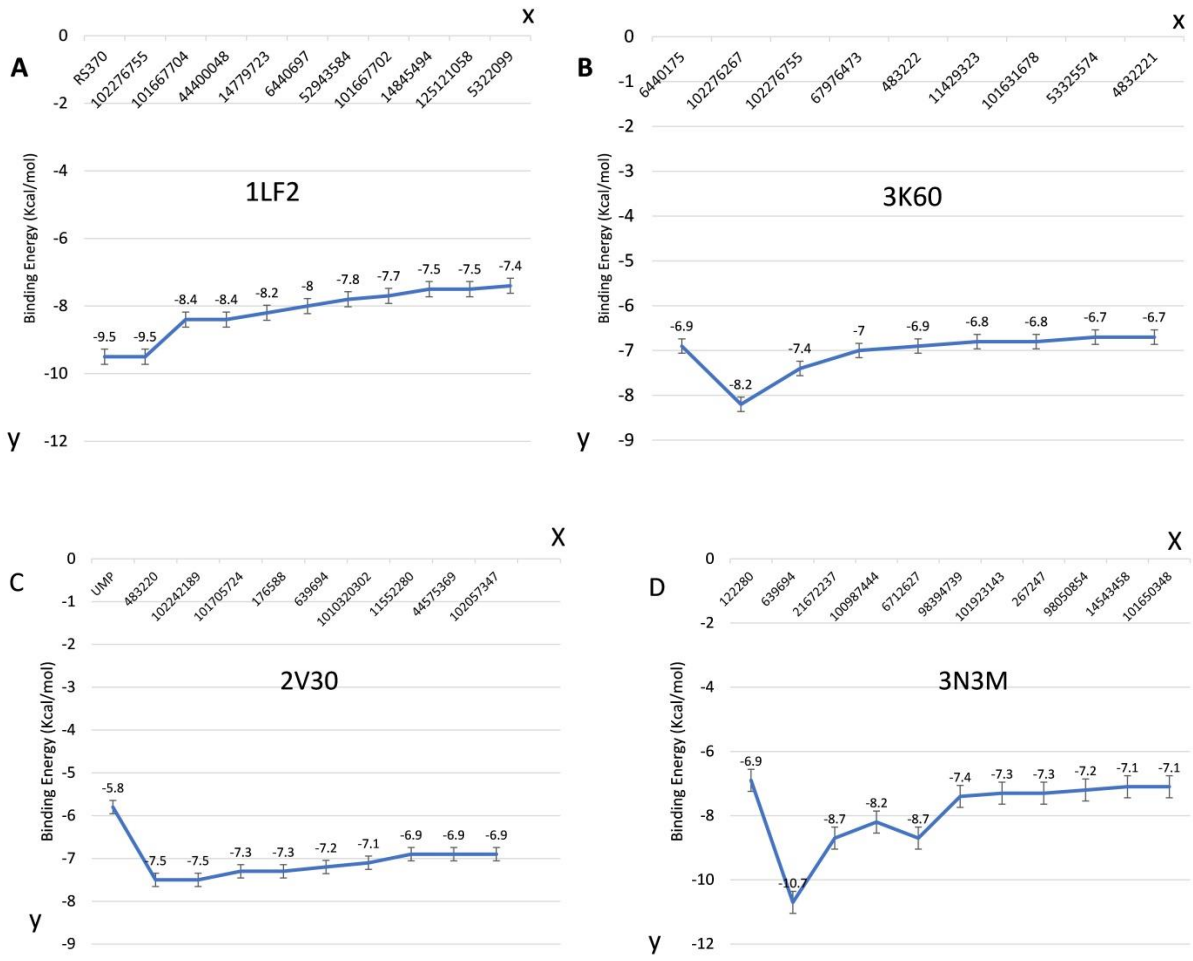
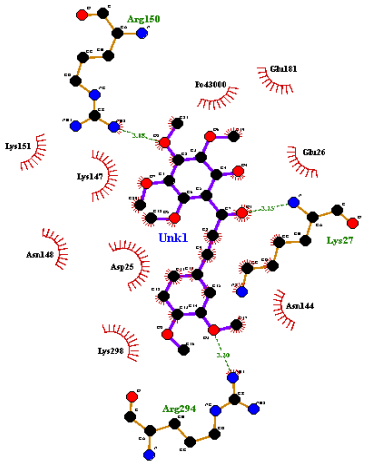


Figure 4.1: Comparative table of binding affinity of the ligands with respectively targeted proteins. **(A);**1LF2- Plasmepsin 2 **(B);**3K60 – Heat Shock Protein 90, **(C);**2V30- Human Orotidine 5' decarboxylase, **(D);** 3N3M- Plasmodium Orotidine Decarboxylase .PubChem CID showed in figure **(a), (b), (c), and (d)** are given in figure **(3.3).**

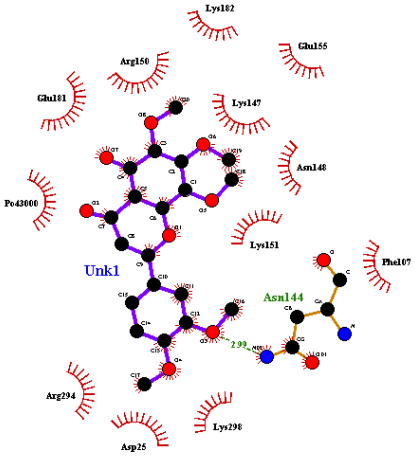
4.2 Protein-Ligand Interaction and Lig Plot

The protein-ligand complexes in PDB format are displayed, edited and run via the software Lig Plot+ (Version v.1.4.5) for generation of Lig Plot schematic diagrams. The protein-ligand interaction along with hydrogen bonding and hydrophobic

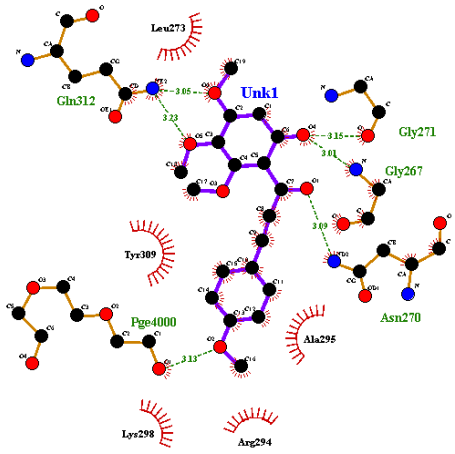
interaction with the complex binding residues are given for the lead phytochemicals. The hydrogen bonding and hydrophobic interaction of methylated flavonoids, Bulbine frutescens and sesquiterpene lactones with the target proteins, plasmepsin2, HSP90, ATPase, Plasmodium orotidine 5' decarboxylase, Human orotidine 5' decarboxylase are shown in figure (4.2.1), (4.2.2), and (4.2.3) respectively.



1

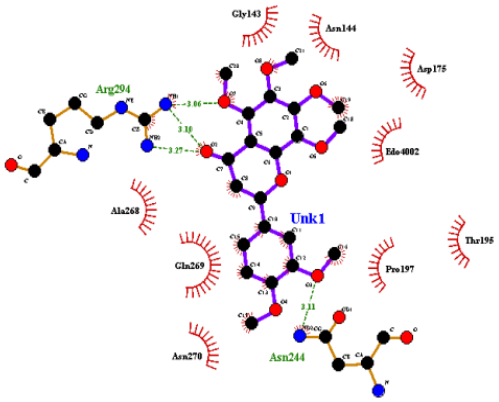


2

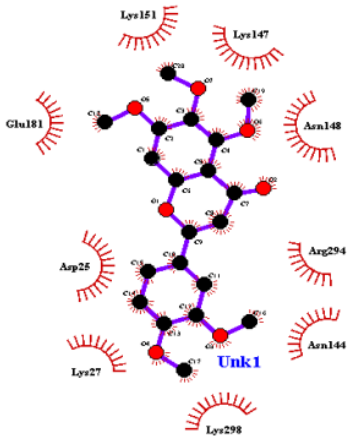


3

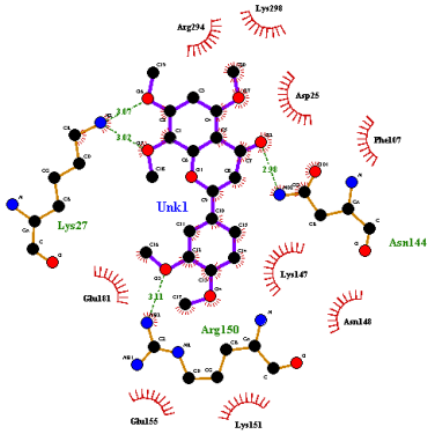
4


Gly143
Asn144
Asp175
Arg294
Edo4002
Ala268
Unk1
Thr196
Gln269
Pro197
Asn270
Asn244

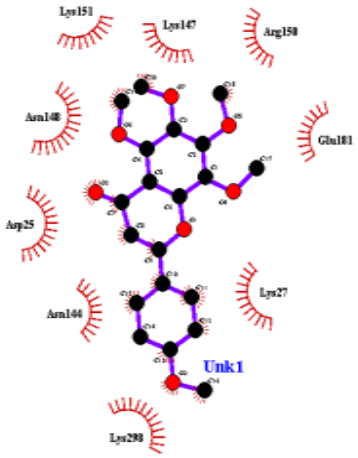
5


Lys151
Lys147
Glu181
Asn148
Arg294
Asp25
Asn144
Lys27
Unk1
Lys298

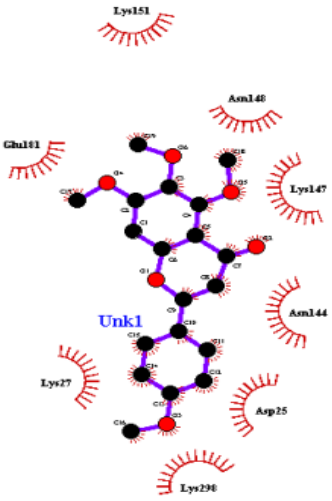
6


Lys290
Arg294
Asp25
Phe107
Unk1
Lys27
Asn144
Lys147
Glu181
Asn148
Arg150
Glu155
Lys151

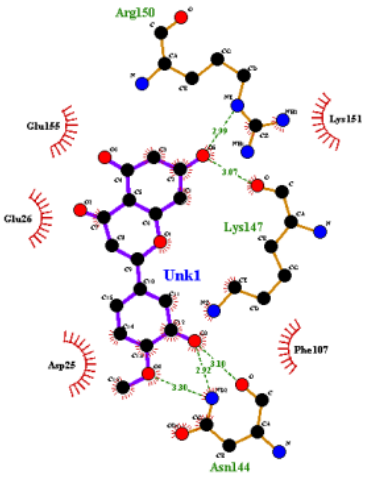
7


Lys151
Lys147
Arg150
Asn148
Glu181
Asp25
Lys27
Asn144
Unk1
Lys298

8


Lys151
Asn148
Glu181
Lys147
Asn144
Unk1
Lys27
Asp25
Lys290

9


Arg150
Glu155
Lys151
Glu26
Lys147
Unk1
Asp25
Phe107
Asn144

10

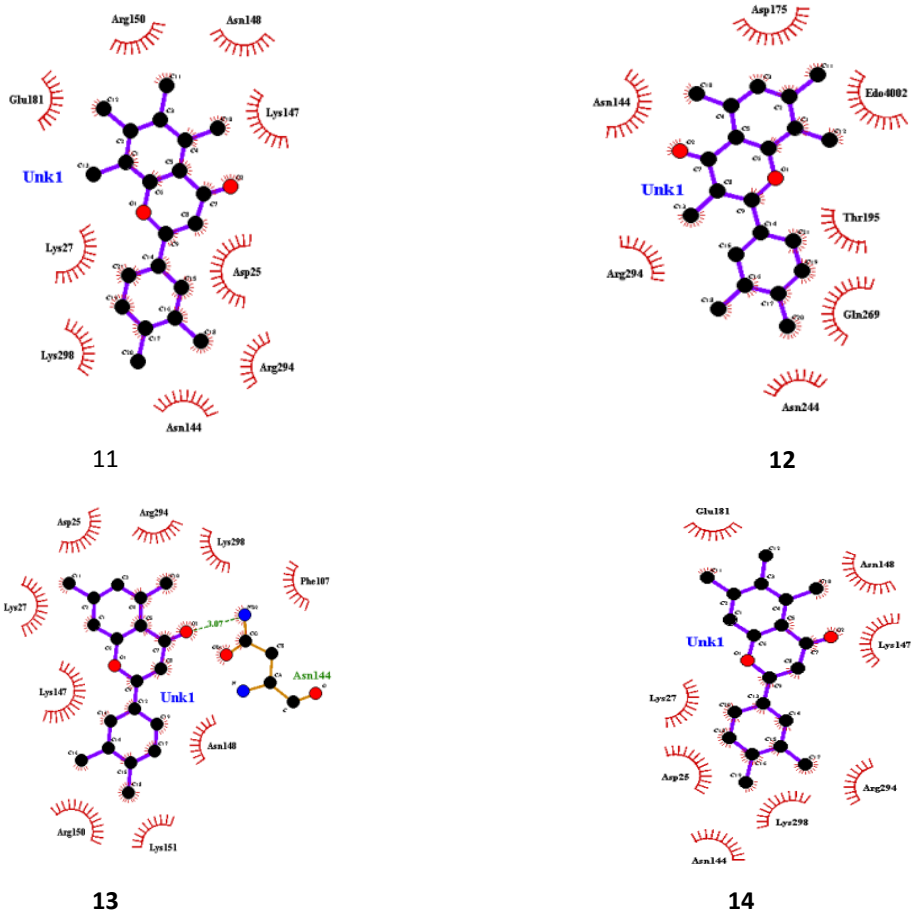
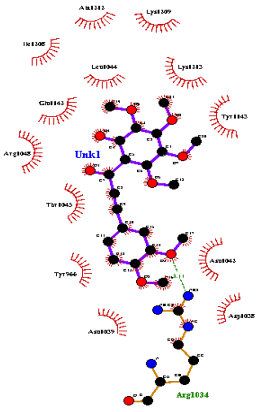
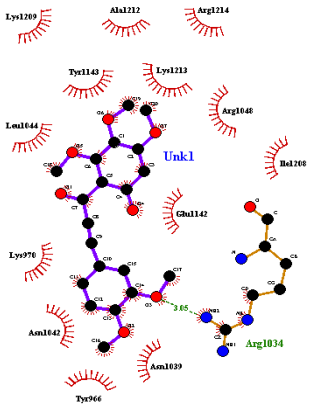


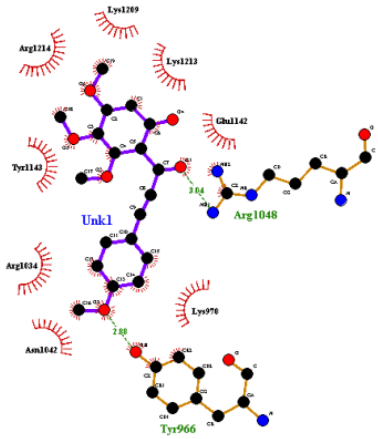
Fig 4.2.1: Lig plot showing interaction between plasmodium omp 5' decarboxylase and methylated flavonoids. **(1);** (2E)-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-3,4,5,6-tetramethoxyphenyl)prop-2-en-1-one, **(2);** (2E)-3-(3,4-dimethoxyphenyl)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)prop-2-en-1-one, **(3);** (2E)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, **(4);** (2S)-2-(3,4-dimethoxyphenyl)-5-hydroxy-6,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(5);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxy-3,4-dihydro-2H-1-benzopyran-one, **(6);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(7);** (2S)-2-(3,4-dimethoxyphenyl)-5,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(8);** (2S)-5,6,7,8 tetramethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one **,(9);** (2S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(10);** (2S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(11);** (2S)-2-(3,4-dimethylphenyl)-5,6,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(12);** 2-(3,4-dimethylphenyl)-3,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(13);** (2S)-2-(3,4-dimethylphenyl)-5,7-dimethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(14);** (2S)-2-(3,4-dimethylphenyl)-5,6,7-trimethyl-3,4-dihydro-2H-1-benzopyran-4-one.


Unk1
Arg1034

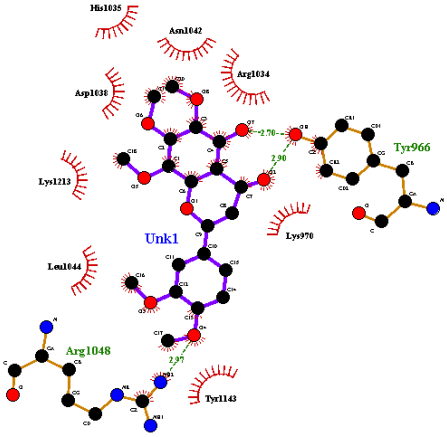
1


Lys1209
Ala1212
Arg1214
Tyr1143
Lys1213
Arg1048
Leu1044
Unk1
Ile1208
Glu1142
Lys976
2.65
Asn1042
Arg1034
Asn1039
Tyr966

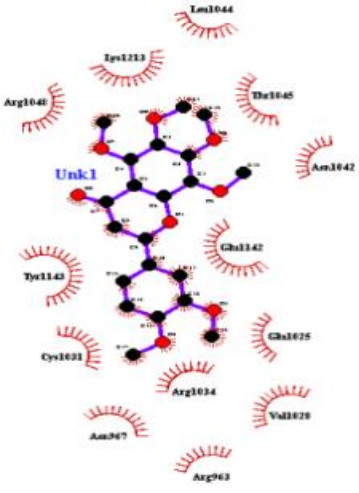
2


Lys1209
Arg1214
Lys1213
Glu1142
Tyr1143
3.04
Arg1048
Unk1
Arg1034
Lys970
2.88
Asn1042
Tyr966

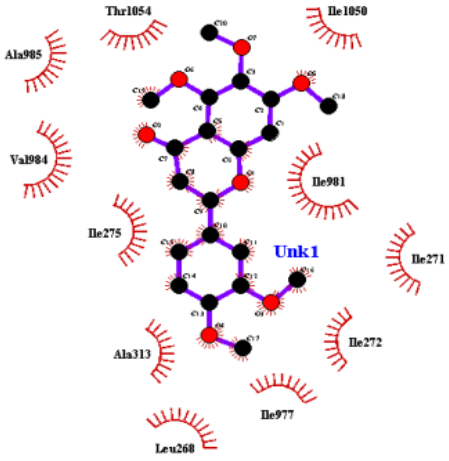
3


His1035
Asn1042
Arg1034
Asp1038
2.70
2.90
Tyr966
Lys1213
Lys970
Unk1
Leu1044
Arg1048
2.97
Tyr1143

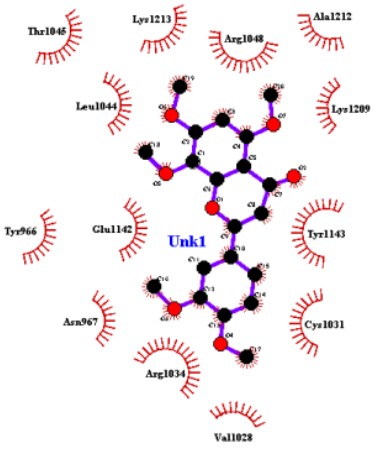
4


Leu1044
Lys1213
Arg1048
Thr1045
Asn1042
Unk1
Glu1142
Tyr1143
Glu1025
Cys1031
Arg1034
Val1029
Asn967
Arg963

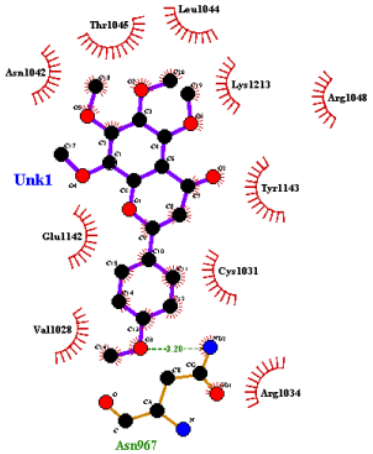
5


Thr1054
Ile1050
Ala985
Val984
Ile981
Ile275
Unk1
Ile271
Ile272
Ala313
Ile977
Leu268

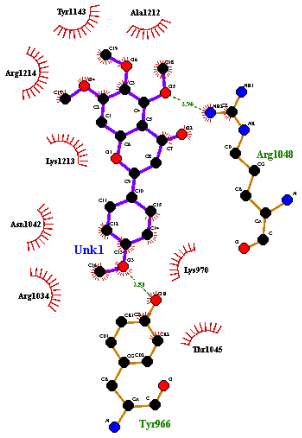
6


Thr1045
Lys1213
Arg1048
Ala1212
Leu1044
Lys1209
Tyr966
Glu1142
Unk1
Tyr1143
Asn967
Cys1031
Arg1034
Val1028

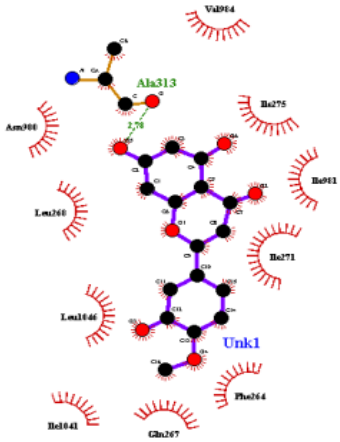
7


Leu1044
Thr1045
Asn1042
Lys1213
Arg1048
Unk1
Tyr1143
Glu1142
Cys1031
Val1028
Arg1034
Asn967

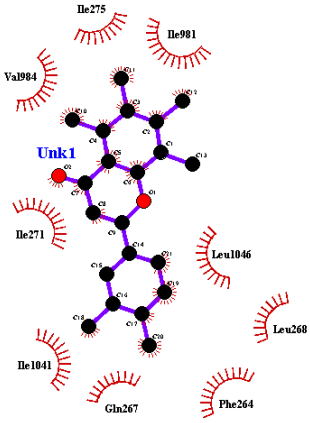
8


Tyr1143
Ala1212
Arg1214
Arg1048
Lys1213
Asn1042
Unk1
Lys970
Arg1034
Thr1045
Tyr966

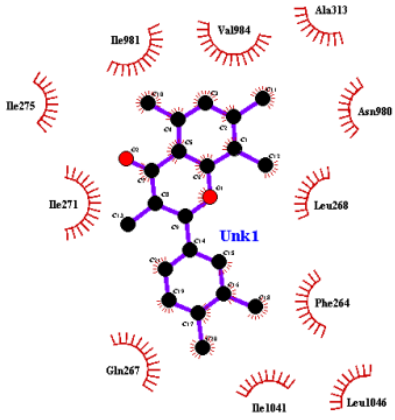
9


Val984
Ala313
Asn980
Ile275
Ile981
Leu260
Ile271
Leu1046
Unk1
Phe264
Ile1041
Gln267

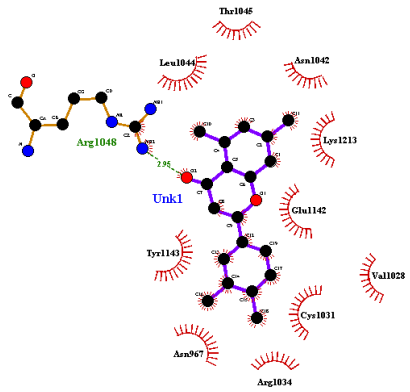
10


Ile275
Ile981
Val984
Unk1
Ile271
Leu1046
Leu268
Ile1041
Gln267
Phe264

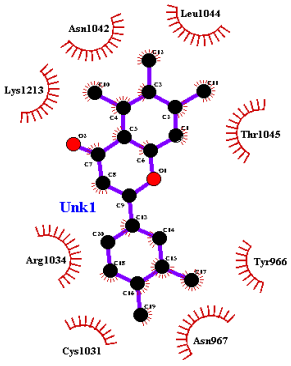
11


Ala313
Val984
Ile981
Asn980
Ile275
Ile271
Leu268
Unk1
Phe264
Gln267
Ile1041
Leu1046

12

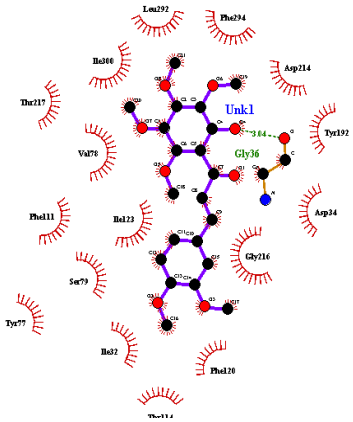


13

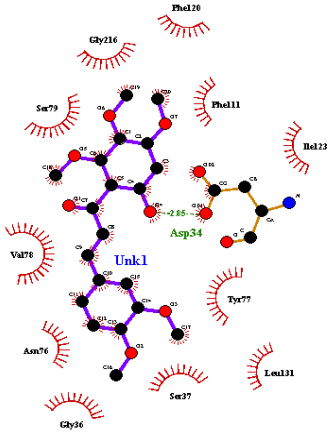


14

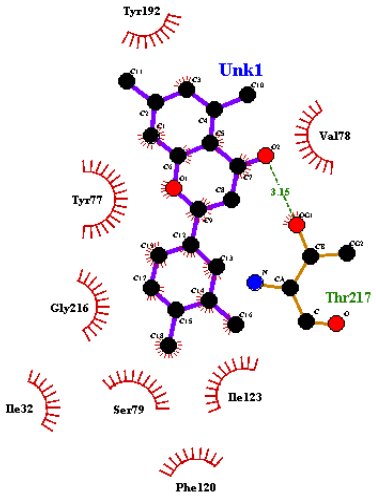
Figure 4.2.2: Lig plot showing interaction between ATPase and methylated flavonoids. (1); (2E)-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-3,4,5,6-tetramethoxyphenyl)prop-2-en-1-one, **(2);** (2E)-3-(3,4-dimethoxyphenyl)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)prop-2-en-1-one, **(3);** (2E)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, **(4) ;** (2S)-2-(3,4-dimethoxyphenyl)-5-hydroxy-6,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(5);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxy-3,4-dihydro-2H-1-benzopyran-one, **(6);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(7);** (2S)-2-(3,4-dimethoxyphenyl)-5,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(8);** (2S)-5,6,7,8 tetramethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(9);** (2S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(10);** (2S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(11);** (2S)-2-(3,4-dimethylphenyl)-5,6,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(12);** 2-(3,4-dimethylphenyl)-3,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(13);** (2S)-2-(3,4-dimethylphenyl)-5,7-dimethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(14);** (2S)-2-(3,4-dimethylphenyl)-5,6,7-trimethyl-3,4-dihydro-2H-1-benzopyran-4-one.


Leu292
Phe294
Ile300
Asp214
Thr217
Unk1
Tyr192
Val78
Gly36
Asp34
Phe111
Ile123
Gly216
Ser79
Tyr77
Ile32
Phe120

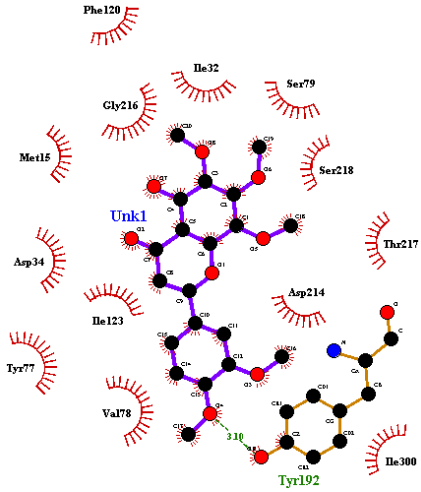
1


Phe120
Gly216
Ser79
Phe111
Ile123
Asp34
Unk1
Val78
Tyr77
Asn76
Leu131
Ser37
Gly36

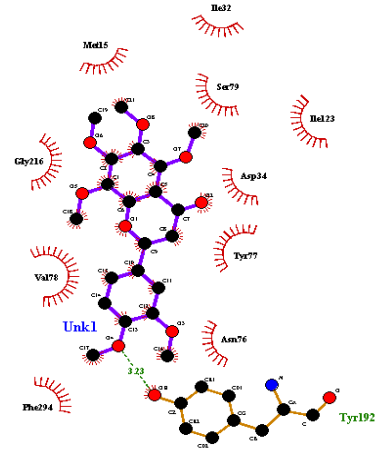
2


Tyr192
Unk1
Val78
Tyr77
Thr217
Gly216
Ile32
Ser79
Ile123
Phe120

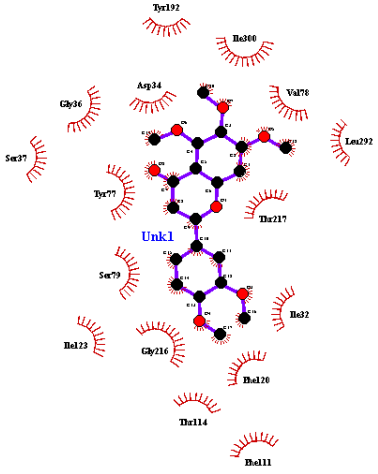
3


Phe120
Ile32
Ser79
Gly216
Met15
Ser218
Unk1
Thr217
Asp34
Asp214
Ile123
Tyr77
Val78
Ile300
Tyr192

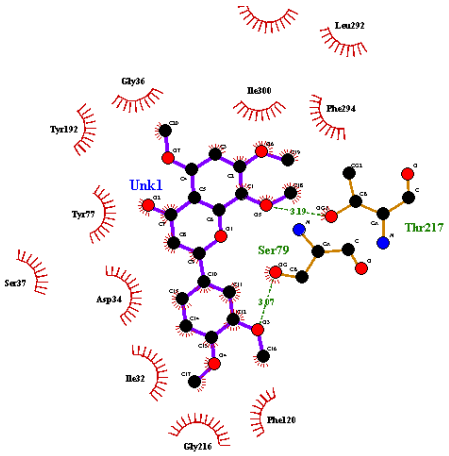
4


Ile32
Met15
Ser79
Ile123
Gly216
Asp34
Tyr77
Val78
Unk1
Asn76
Phe294
Tyr192

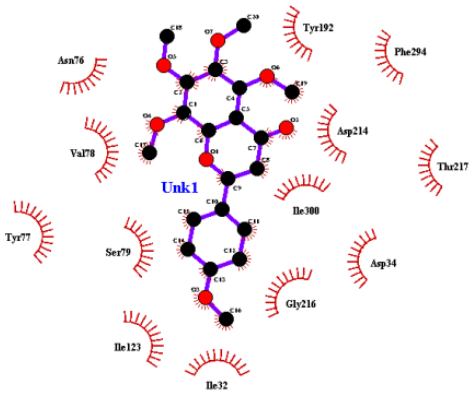
5


Tyr192
Ile300
Asp34
Val78
Gly36
Leu292
Ser37
Tyr77
Thr217
Unk1
Ser79
Ile32
Ile123
Gly216
Ile120
Thr114
Ile111

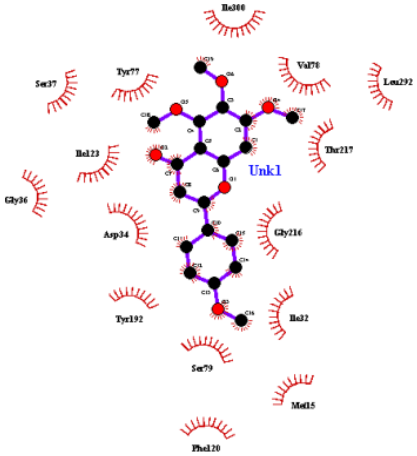
6


Leu292
Gly36
Ile300
Phe294
Tyr192
Unk1
Tyr77
Thr217
Ser79
Ser37
Asp34
Ile32
Phe120
Gly216

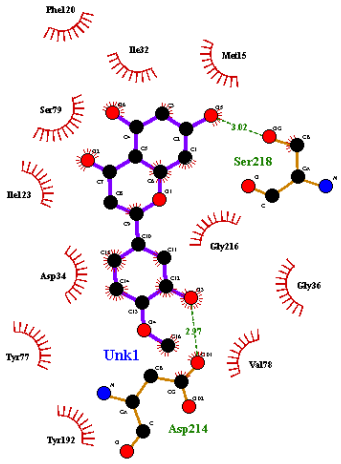
7


Tyr192
Phe294
Asn76
Asp214
Val78
Thr217
Unk1
Ile300
Tyr77
Ser79
Asp34
Gly216
Ile123
Ile32

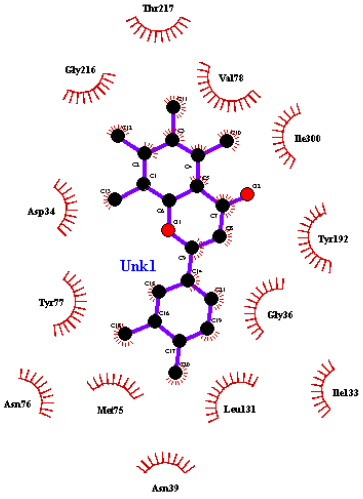
8


Ile300
Ser37
Tyr77
Val78
Leu292
Ile123
Thr217
Unk1
Gly36
Asp34
Gly216
Tyr192
Ile32
Ser79
Met15
Phe120

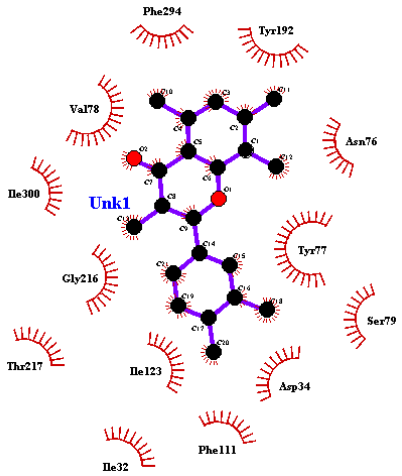
9


Phe120
Ile32
Met15
Ser79
Ser218
Ile123
Gly216
Asp34
Gly36
Tyr77
Unk1
Val78
Tyr192
Asp214

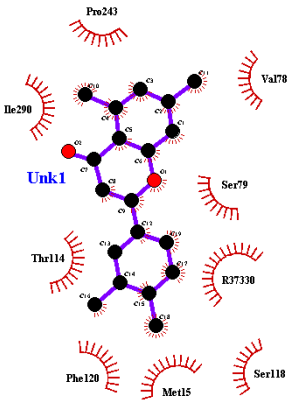
10


Thr217
Gly216
Val78
Ile300
Asp34
Tyr192
Unk1
Tyr77
Gly36
Asn76
Met75
Leu131
Ile133
Asn39

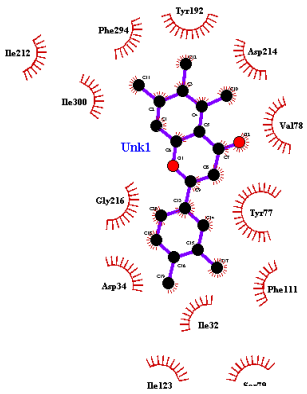
11


Phe294
Tyr192
Val78
Asn76
Ile300
Unk1
Tyr77
Gly216
Ser79
Thr217
Ile123
Asp34
Phe111
Ile32

12

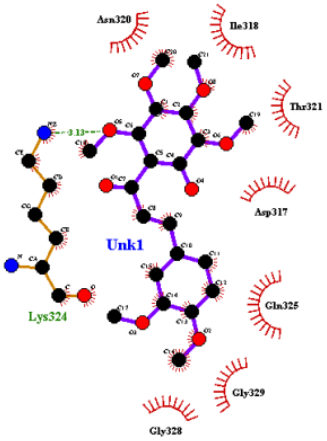


13

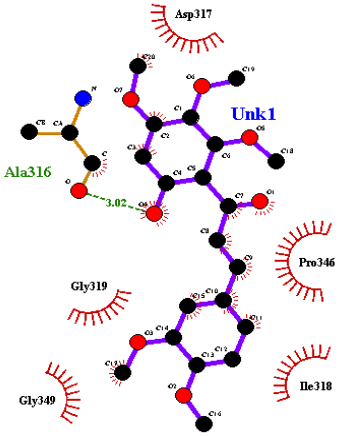


14

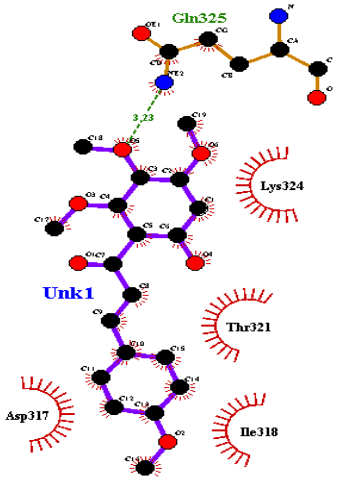
Fig 4.2.3: Lig plot showing interaction between Plasmepsin2 and methylated flavonoids. **(1);** (2E)-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-3,4,5,6-tetramethoxyphenyl)prop-2-en-1-one, **(2);** (2E)-3-(3,4-dimethoxyphenyl)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)prop-2-en-1-one, **(3);** (2E)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, **(4) ;** (2S)-2-(3,4-dimethoxyphenyl)-5-hydroxy-6,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(5);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxy-3,4-dihydro-2H-1-benzopyran-one, **(6);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(7);** (2S)-2-(3,4-dimethoxyphenyl)-5,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(8);** (2S)-5,6,7,8 tetramethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(9);** (2S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(10);** (2S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(11);** (2S)-2-(3,4-dimethylphenyl)-5,6,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(12);** 2-(3,4-dimethylphenyl)-3,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(13);** (2S)-2-(3,4-dimethylphenyl)-5,7-dimethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(14);** (2S)-2-(3,4-dimethylphenyl)-5,6,7-trimethyl-3,4-dihydro-2H-1-benzopyran-4-one.


Asn320
Ile318
Thr321
Asp317
Unk1
Lys324
Gln325
Gly329
Gly328

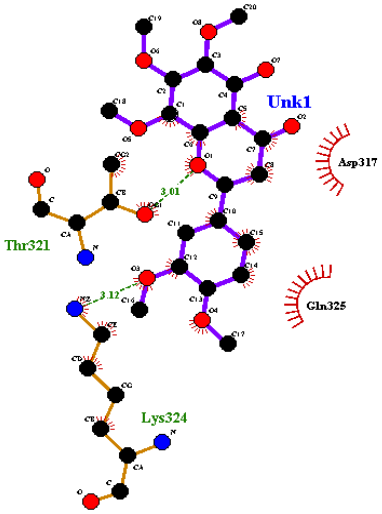
1


Asp317
Unk1
Ala316
3.02
Pro346
Gly319
Gly349
Ile318

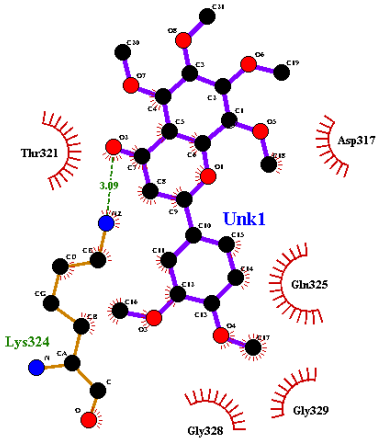
2


Gln325
3.23
Lys324
Unk1
Thr321
Asp317
Ile318

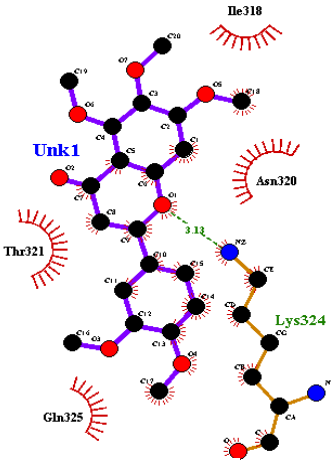
3


Unk1
Asp317
3.01
Thr321
3.12
Gln325
Lys324

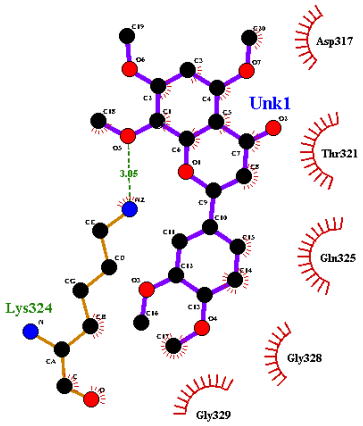
4


Thr321
Asp317
3.09
Unk1
Gln325
Lys324
Gly329
Gly328

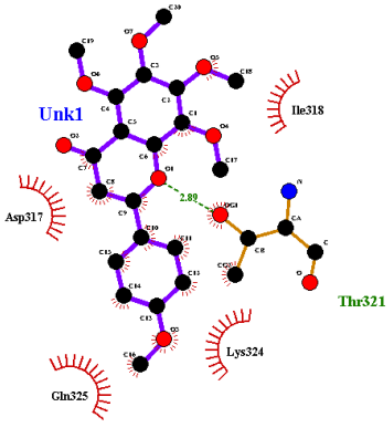
5


Ile318
Unk1
Asn320
Thr321
3.13
Lys324
Gln325

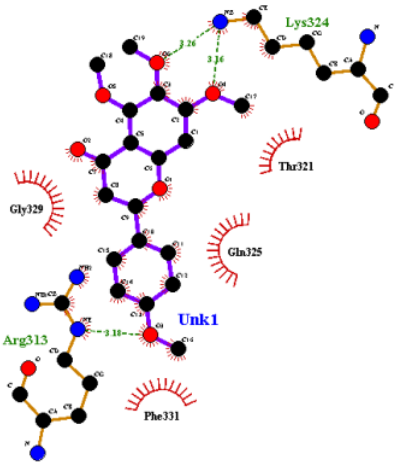
6


Asp317
Unk1
Thr321
Gln325
Lys324
Gly328
Gly329

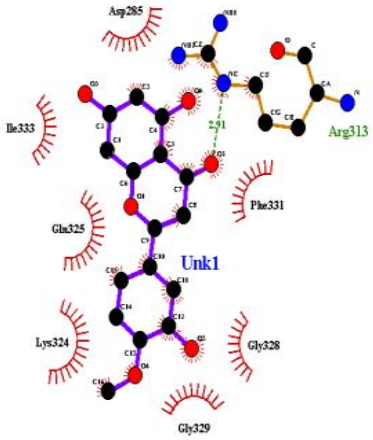
7


Unk1
Ile318
Asp317
Thr321
Lys324
Gln325

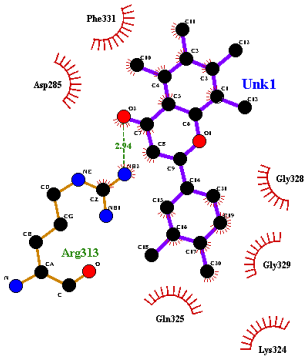
8


Lys324
Thr321
Gly329
Gln325
Unk1
Arg313
Phe331

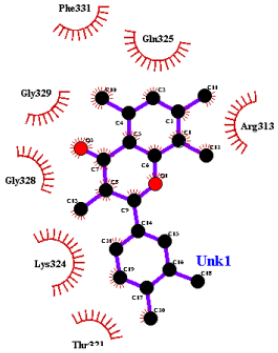
9


Asp285
Ile333
Arg313
Phe331
Gln325
Unk1
Lys324
Gly328
Gly329

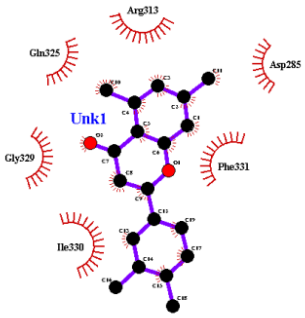
10


Phe331
Asp285
Unk1
Gly328
Arg313
Gly329
Gln325
Lys324

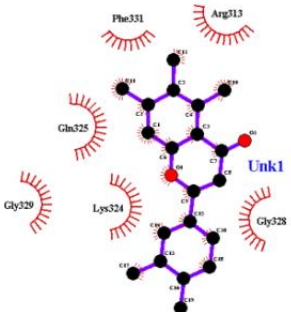
11


Phe331
Gln325
Gly329
Arg313
Gly328
Lys324
Unk1

12

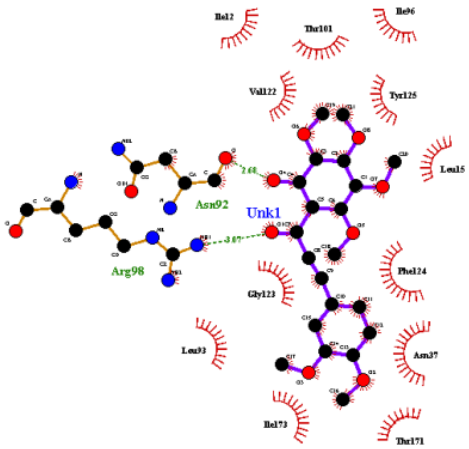


13

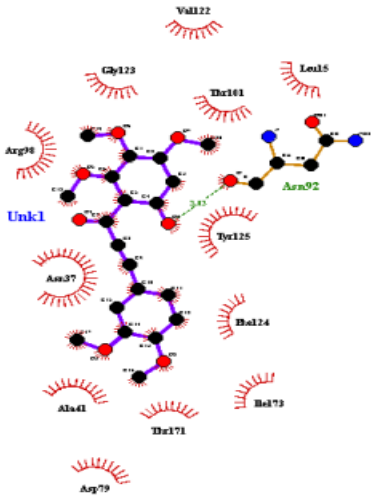


14

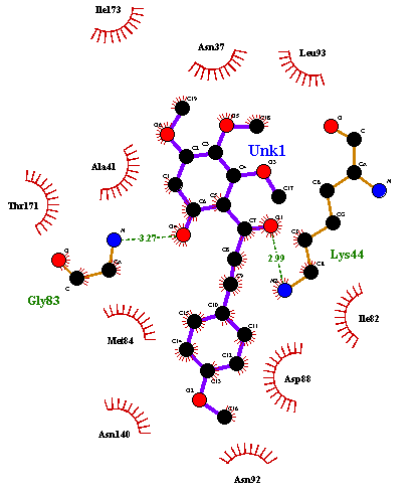
Figure 4.2.4: Lig plot showing interaction between Human orotidine 5' decarboxylase and methylated flavonoids. **(1);** (2E)-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-3,4,5,6-tetramethoxyphenyl)prop-2-en-1-one, **(2);** (2E)-3-(3,4-dimethoxyphenyl)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)prop-2-en-1-one, **(3);** (2E)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, **(4) ;** (2S)-2-(3,4-dimethoxyphenyl)-5-hydroxy-6,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(5);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxy-3,4-dihydro-2H-1-benzopyran-one, **(6);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(7);** (2S)-2-(3,4-dimethoxyphenyl)-5,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(8);** (2S)-5,6,7,8 tetramethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(9);** (2S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(10);** (2S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(11);** (2S)-2-(3,4-dimethylphenyl)-5,6,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(12);** 2-(3,4-dimethylphenyl)-3,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(13);** (2S)-2-(3,4-dimethylphenyl)-5,7-dimethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(14);** (2S)-2-(3,4-dimethylphenyl)-5,6,7-trimethyl-3,4-dihydro-2H-1-benzopyran-4-one.


Ile12
Thr101
Ile96
Val122
Tyr125
Leu15
Asn92
Unk1
Arg98
Phe124
Gly123
Leu93
Asn37
Ile73
Thr171

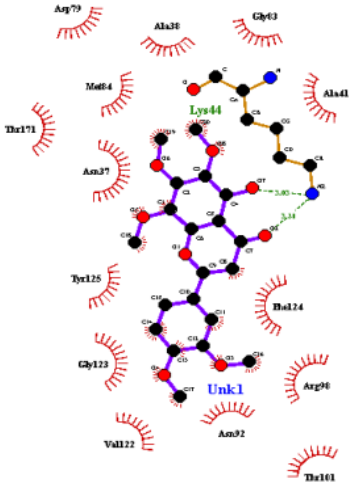
1


Val122
Gly123
Leu15
Thr101
Arg98
Asn92
Unk1
Tyr125
Asn37
Phe124
Ala41
Ile73
Thr171
Asp79

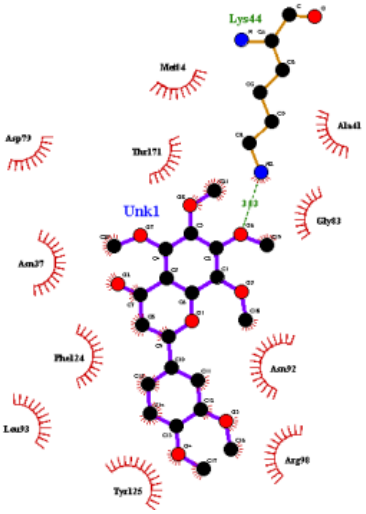
2


Ile73
Asn37
Leu93
Unk1
Ala41
Thr171
Gly83
Lys44
Met84
Ile82
Asp88
Asn140
Asn92

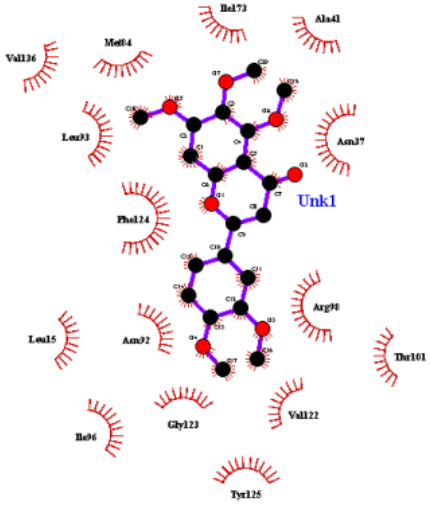
3


Asp79
Ala38
Gly83
Met84
Ala41
Lys44
Thr171
Asn37
Tyr125
Phe124
Gly123
Unk1
Arg98
Asn92
Val122
Thr101

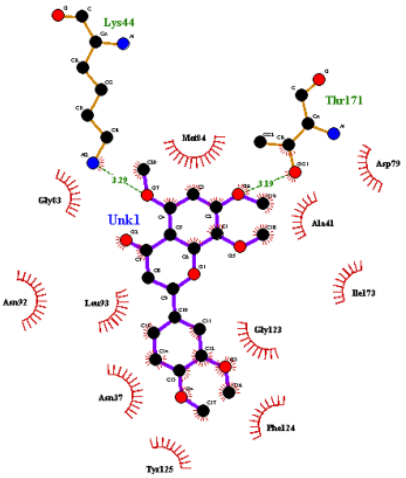
4


Lys44
Met84
Asp79
Ala41
Thr171
Unk1
Gly83
Asn37
Phe124
Asn92
Leu93
Arg98
Tyr125

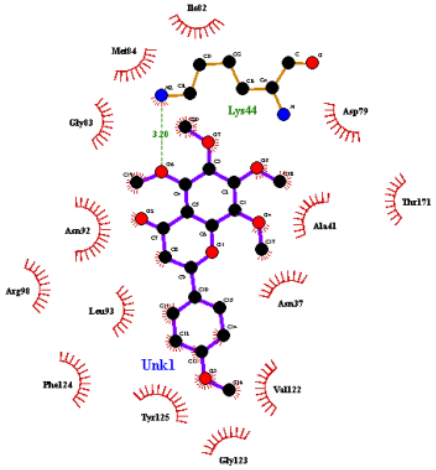
5


Ile73
Ala41
Met84
Val136
Leu93
Asn37
Unk1
Phe124
Arg98
Leu15
Asn92
Thr101
Gly123
Val122
Ile96
Tyr125

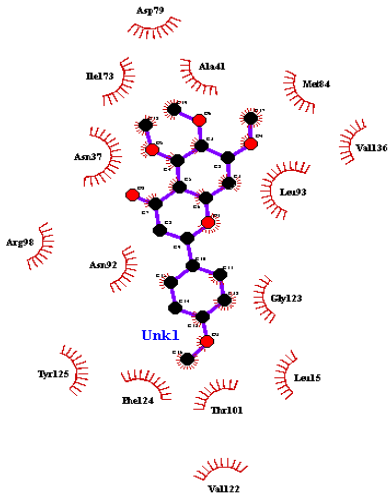
6


Lys44
Thr171
Met84
Asp79
Gly83
Unk1
Ala41
Asn92
Leu93
Ile173
Gly123
Asn37
Phe124
Tyr125

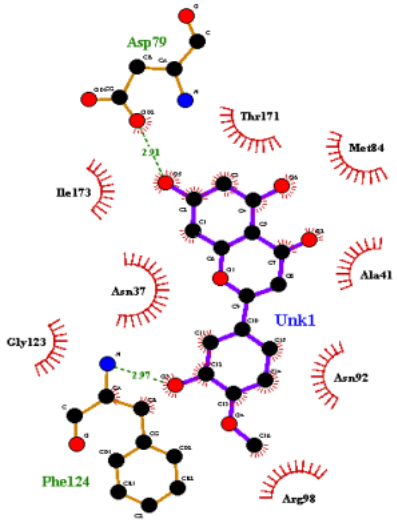
7


Ile82
Met84
Lys44
Asp79
Gly83
Thr171
Asn92
Ala41
Arg98
Leu93
Asn37
Unk1
Phe124
Val122
Tyr125
Gly123

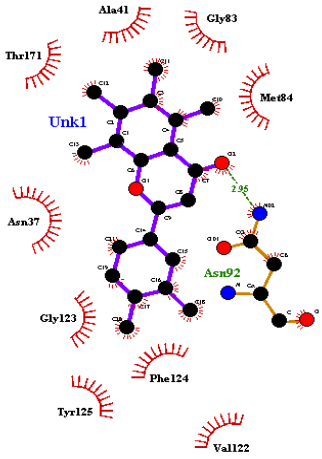
8


Asp79
Ala41
Ile173
Met84
Val136
Asn37
Leu93
Arg98
Asn92
Gly123
Unk1
Tyr125
Phe124
Leu15
Thr101
Val122

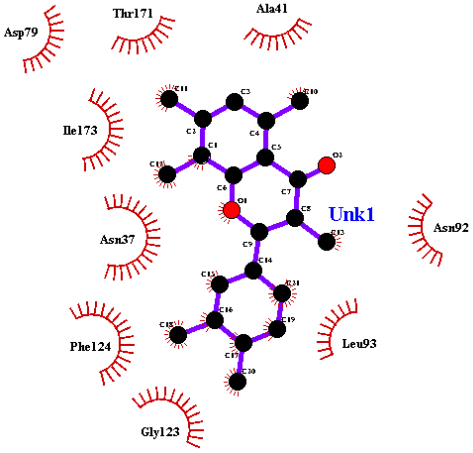
9


Asp79
Thr171
Met84
Ile173
Ala41
Asn37
Unk1
Gly123
Asn92
Phe124
Arg98

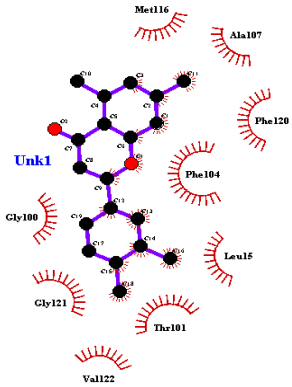
10


Ala41
Gly83
Thr171
Met84
Unk1
Asn37
Asn92
Gly123
Phe124
Tyr125
Val122

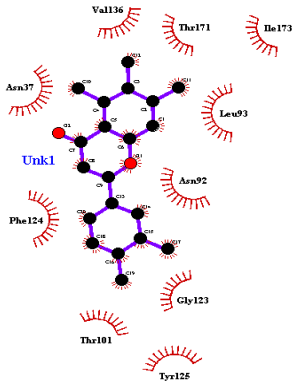
11


Asp79
Thr171
Ala41
Ile173
Unk1
Asn92
Asn37
Phe124
Leu93
Gly123

12

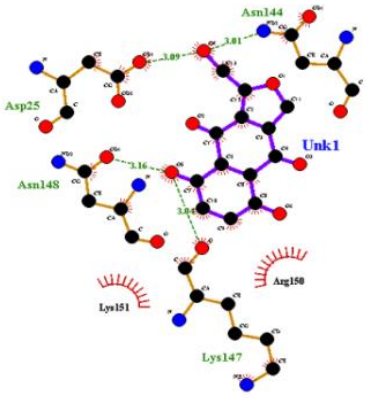


13

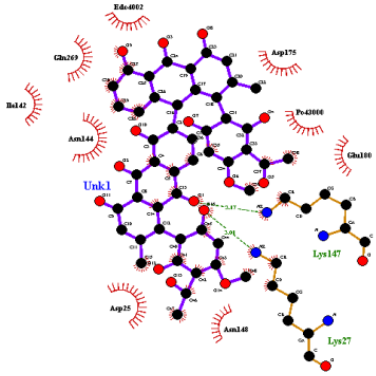


14

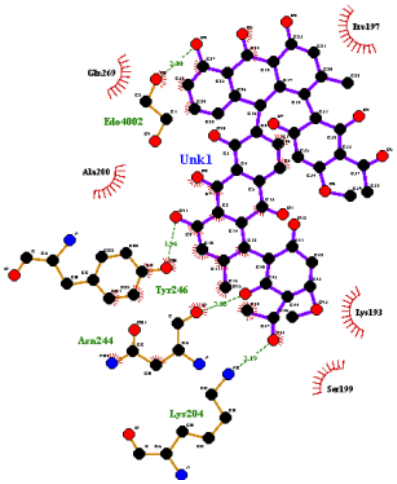
Figure 4.2.5: Lig plot showing interaction between HSP90 and methylated flavonoids. (1); (2E)-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-3,4,5,6-tetramethoxyphenyl)prop-2-en-1-one, **(2);** (2E)-3-(3,4-dimethoxyphenyl)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)prop-2-en-1-one, **(3);** (2E)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, **(4) ;** (2S)-2-(3,4-dimethoxyphenyl)-5-hydroxy-6,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(5);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxy-3,4-dihydro-2H-1-benzopyran-one, **(6);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(7);** (2S)-2-(3,4-dimethoxyphenyl)-5,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(8);** (2S)-5,6,7,8 tetramethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(9);** (2S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(10);** (2S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(11);** (2S)-2-(3,4-dimethylphenyl)-5,6,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(12);** 2-(3,4-dimethylphenyl)-3,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(13);** (2S)-2-(3,4-dimethylphenyl)-5,7-dimethyl-3,4-dihydro-2H-1-benzopyran-4-one, **(14);** (2S)-2-(3,4-dimethylphenyl)-5,6,7-trimethyl-3,4-dihydro-2H-1-benzopyran-4-one


Asn144
Asp25
Unk1
Asn148
Arg150
Lys151
Lys147

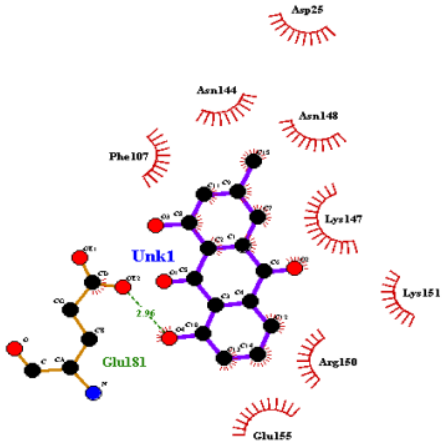
1


Edo4002
Gln269
Asp175
Asn144
Unk1
Glu180
Lys147
Asp25
Asn148
Lys27

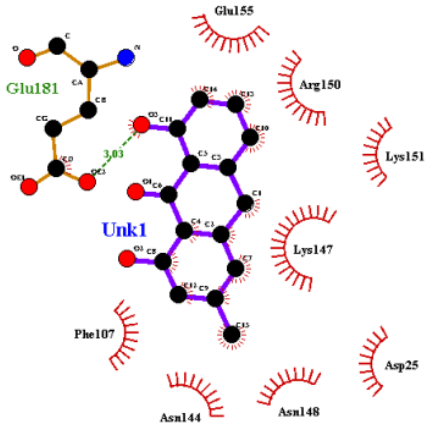
2


Ile197
Gln269
Edo4002
Unk1
Ala200
Tyr246
Lys193
Asn244
Ser199
Lys204

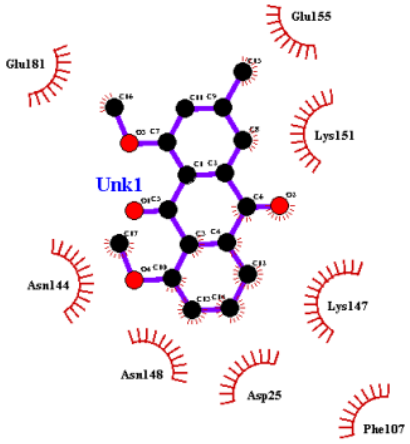
3


Asp25
Asn144
Asn148
Phe107
Lys147
Unk1
Lys151
Glu181
Arg150
Glu155

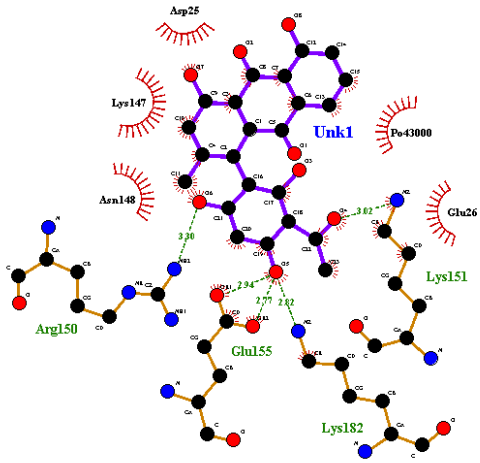
4


Glu155
Glu181
Arg150
Lys151
Unk1
Lys147
Phe107
Asp25
Asn144
Asn148

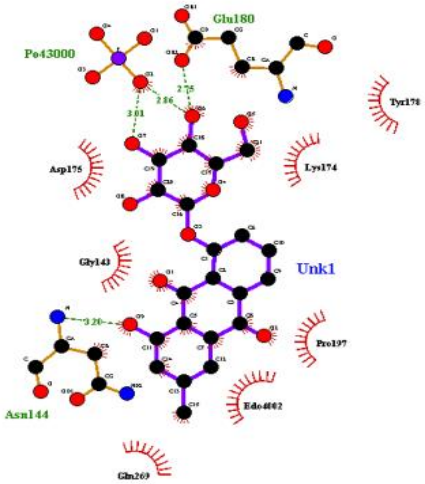
5


Glu155
Glu181
Lys151
Unk1
Asn144
Lys147
Asn148
Asp25
Phe107

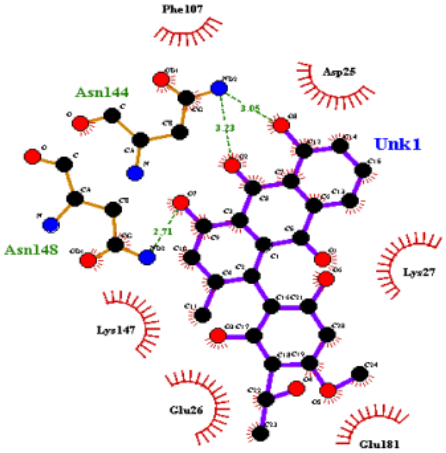
6


Asp25
Lys147
Unk1
Po43000
Asn148
Glu26
Lys151
Arg150
Glu155
Lys182

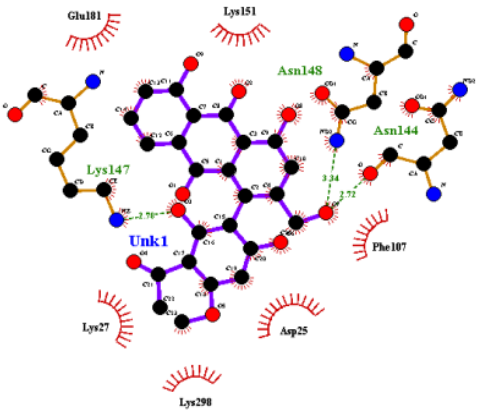
7


Glu180
Po43000
Tyr178
Asp175
Lys174
Gly143
Unk1
Pro197
Asn144
Edo402
Gln269

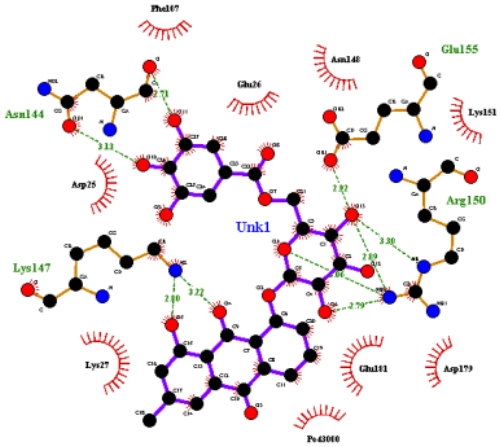
8

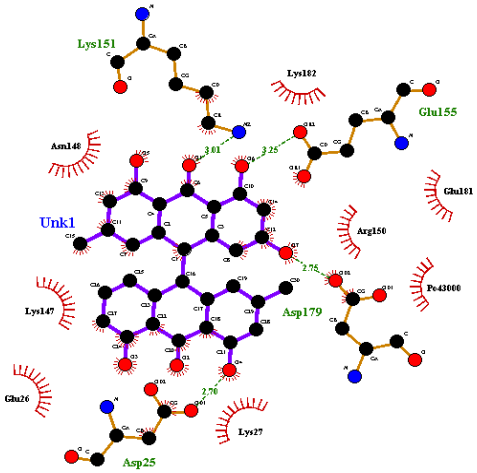

Phe107
Asp25
Asn144
Unk1
Asn148
Lys27
Lys147
Glu26
Glu181

9

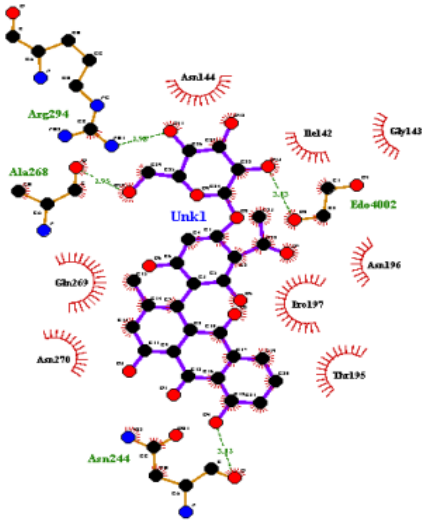

Glu181
Lys151
Asn148
Asn144
Lys147
Unk1
Phe107
Lys27
Asp25
Lys298

10

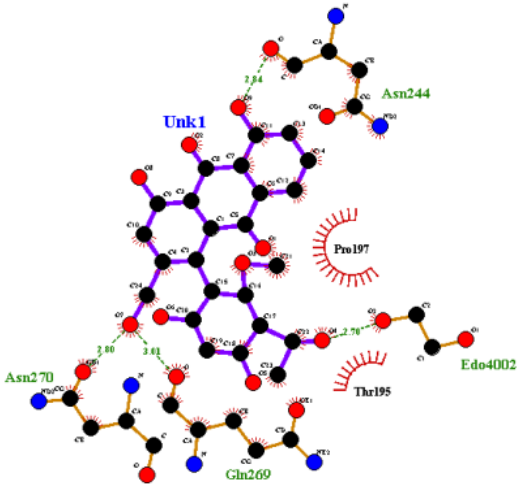

Phe107
Asn144
Glu26
Asn148
Glu155
Lys151
Asp25
Arg150
Unk1
Lys147
Lys27
Glu181
Asp179
Po43000


Lys151
Lys102
Asn148
Glu155
Glu181
Unk1
Arg150
Lys147
Asp179
Po43000
Glu26
Lys27
Asp25

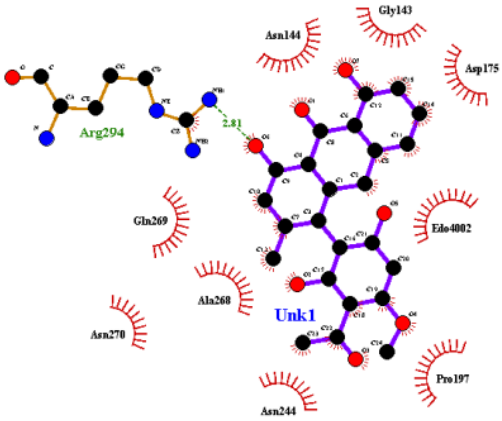
12


Asn144
Arg294
Ile142
Gly143
Ala268
Edo4002
Unk1
Asn196
Gln269
Ile197
Asn270
Thr195
Asn244

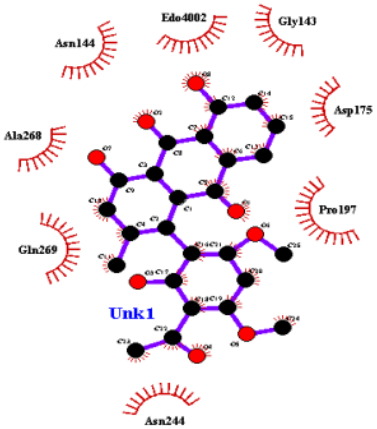
13


Asn244
Unk1
Pro197
Asn270
Edo4002
Thr195
Gln269

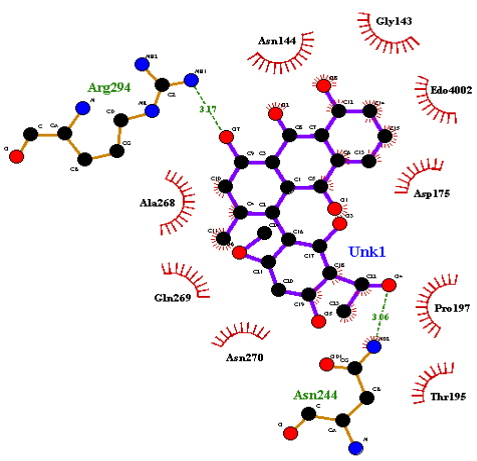
14


Gly143
Asn144
Asp175
Arg294
Gln269
Edo4002
Ala268
Unk1
Asn270
Pro197
Asn244

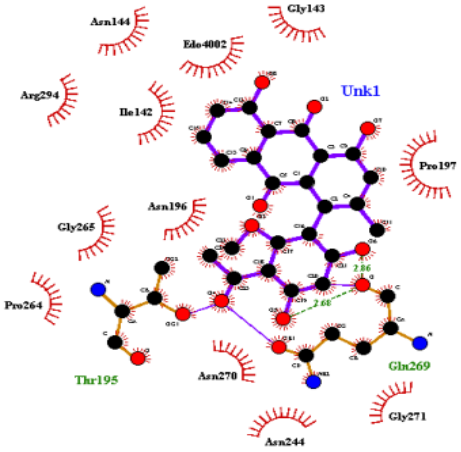
15


Edo4002
Gly143
Asn144
Asp175
Ala268
Pro197
Gln269
Unk1
Asn244

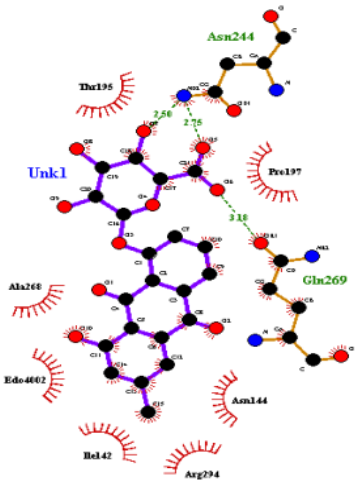
16



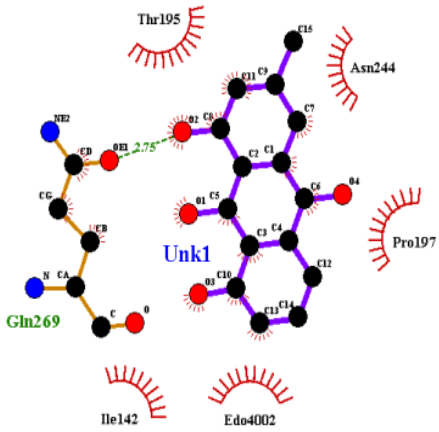
17



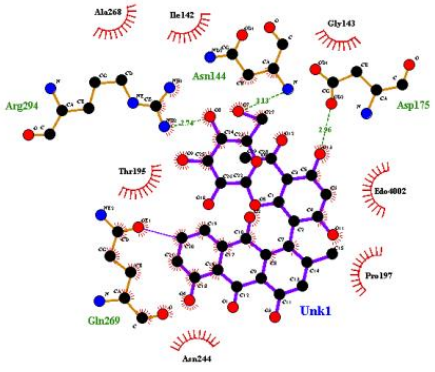
18



19



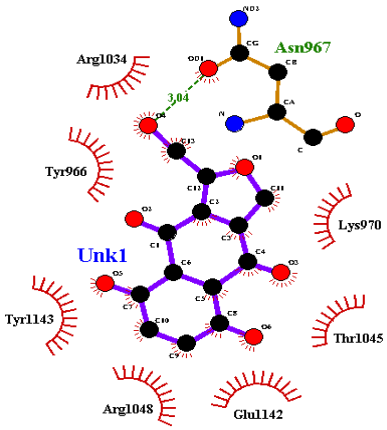
20



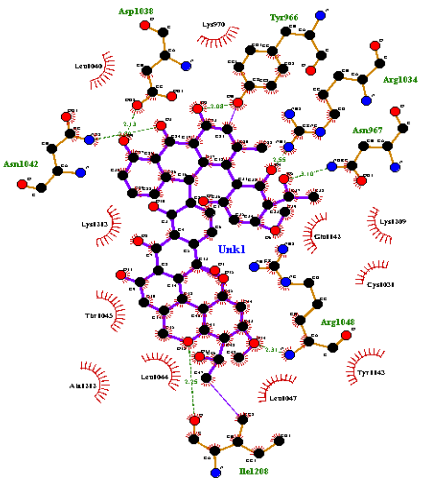
21

Figure 4.2.2.1: Lig plot showing interaction between Plasmodium orotidine 5' decarboxylase and Bulbine phytochemicals. **(1);** 5,8-dihydroxy-1-(hydroxymethyl)-4H,9H-naphtho[2,3-c]furan-4,9-dione, **(2);** 1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-6-[(9S)-1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methyl-10-oxo-9,10-dihydroanthracen-9-yl]-

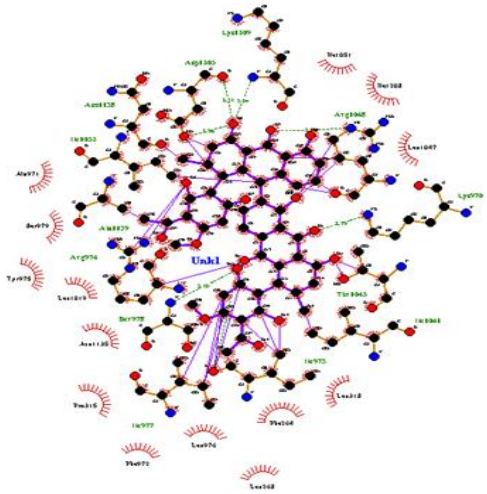
4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione, **(3);** 1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-6-[(9S)-1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methyl-10-oxo-9,10dihydroanthracen-9-yl]-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione, **(4);** PubChem CID:10208; **(5);** PubChem CID:68111;**(6);** PubChem CID:189763, **(7);** PubChem CID:438991 ; **(8);** PubChem CID:442731; **(9);** PubChem CID:442753; **(10);** PubChem CID:636652; **(11);** PubChem CID:5315852; **(12);** PubChem CID:5320386; **(13);** PubChem CID:1008440; **(14);** PubChem CID:10072444; **(15);** PubChem CID:10093576; **(16);** PubChem CID:11190093; **(17);** PubChem CID:11729754; **(18);** PubChem CID:12178518; **(19);** PubChem CID:25033784; **(20);** PubChem CID:91932460; **(21);** PubChem CID:102378267.



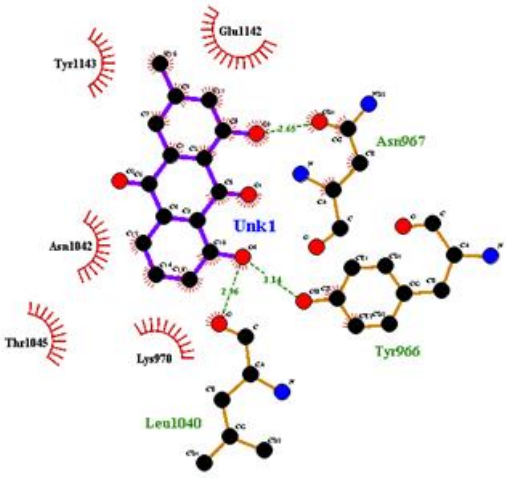
1



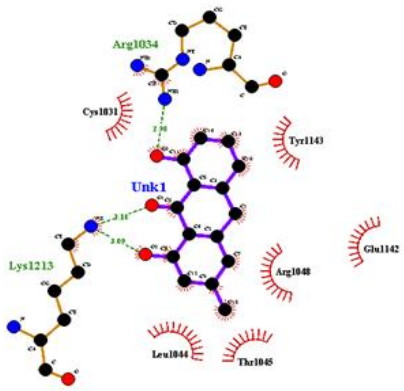
2



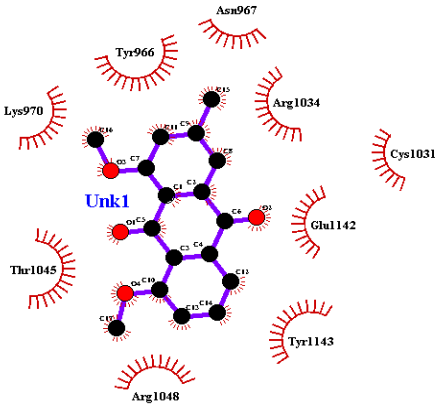
3



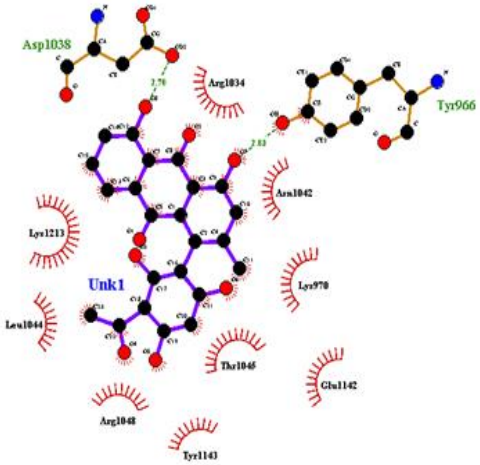
4


Arg1034
Cys1031
Tyr1143
Unk1
Glu1142
Lys1213
Arg1048
Leu1044
Thr1045

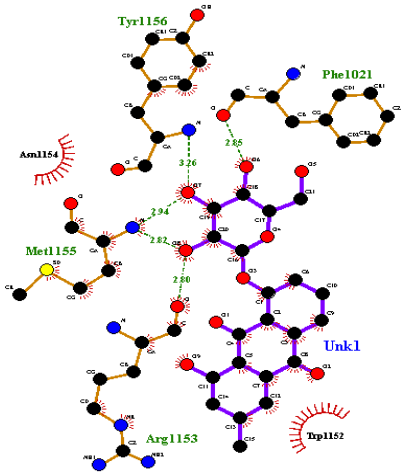
5


Asn967
Tyr966
Lys970
Arg1034
Cys1031
Unk1
Glu1142
Thr1045
Tyr1143
Arg1048

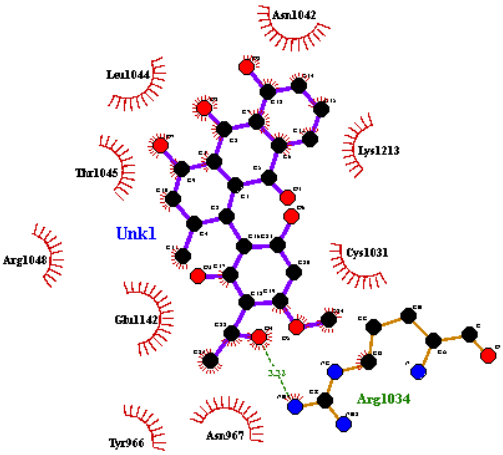
6


Asp1038
Arg1034
Tyr966
Asn1042
Lys1213
Unk1
Lys970
Leu1044
Thr1045
Glu1142
Arg1048
Tyr1143

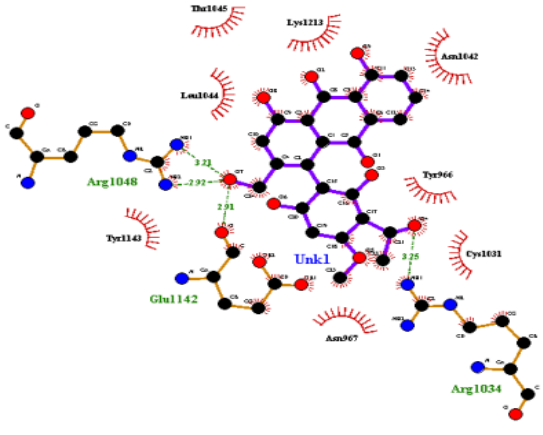
7


Tyr1156
Phe1021
Asn1154
Met1155
Unk1
Arg1153
Trp1152

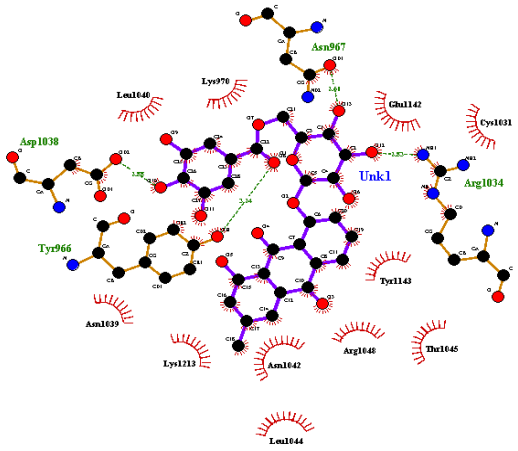
8


Asn1042
Leu1044
Lys1213
Thr1045
Unk1
Arg1048
Cys1031
Glu1142
Arg1034
Tyr966
Asn967

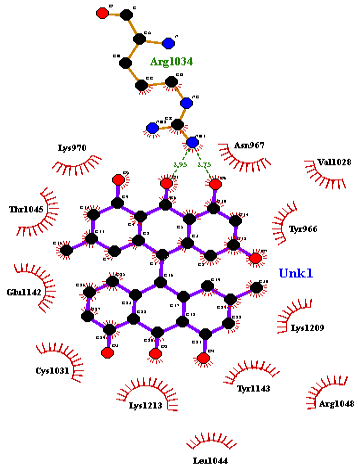
9


Thr1045
Lys1213
Asn1042
Leu1044
Arg1048
Tyr966
Tyr1143
Unk1
Cys1031
Glu1142
Asn967
Arg1034

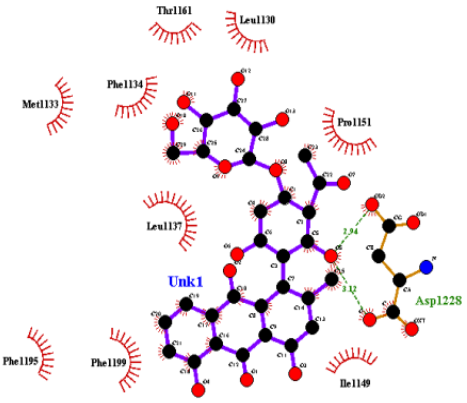
10


Asn967
Lys970
Leu1040
Glu1142
Cys1031
Asp1038
Unk1
Arg1034
Tyr966
Tyr1143
Asn1039
Lys1213
Asn1042
Arg1048
Thr1045
Leu1044

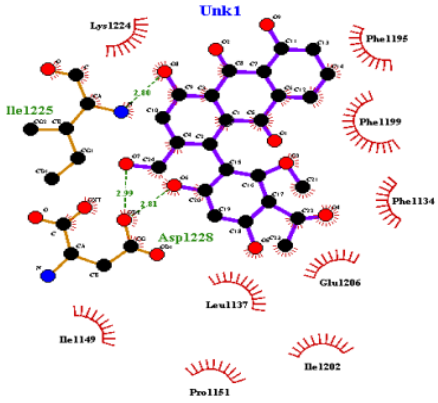
11


Arg1034
Lys970
Asn967
Val1028
Thr1045
Tyr966
Unk1
Glu1142
Lys1209
Cys1031
Tyr1143
Lys1213
Arg1048
Leu1044

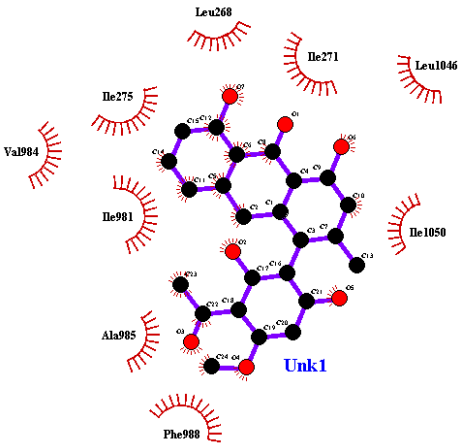
12


Thr1161
Leu1130
Phe1134
Met1133
Pro1151
Leu1137
Unk1
Asp1228
Phe1195
Phe1199
Ile1149

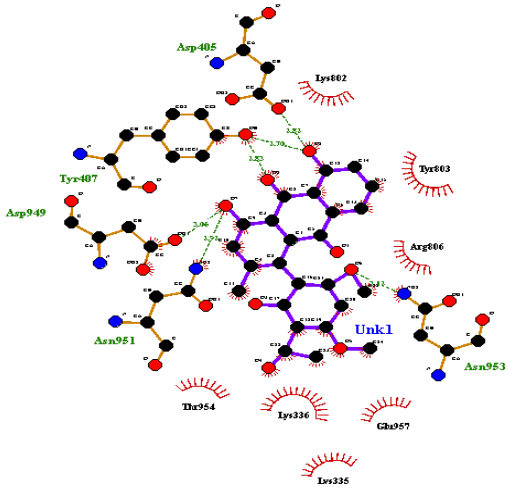
13


Unk1
Lys1224
Phe1195
Ile1225
Phe1199
Phe1134
Asp1228
Glu1206
Leu1137
Ile1149
Ile1202
Pro1151

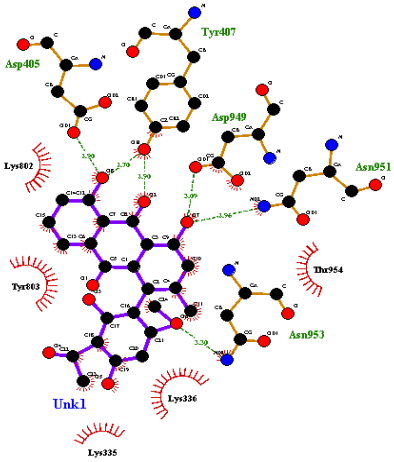
14



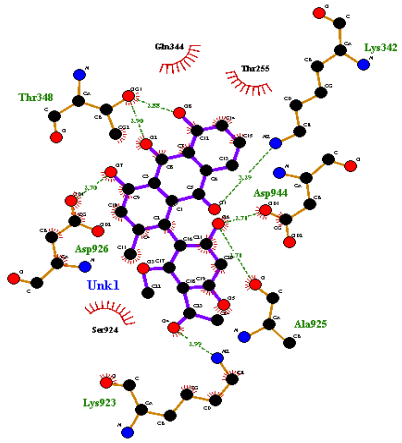
15



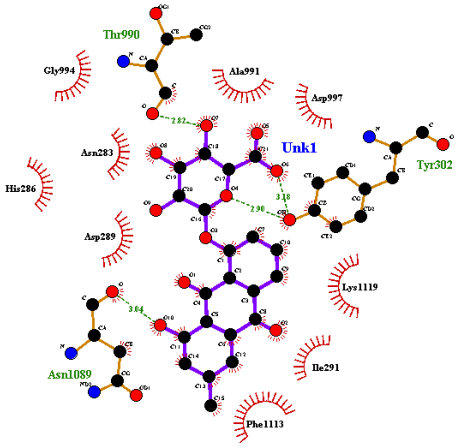
16



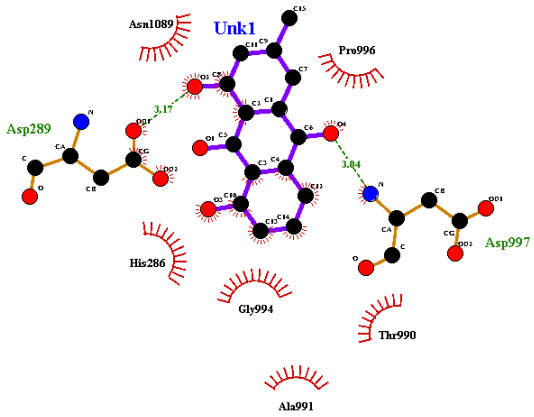
17



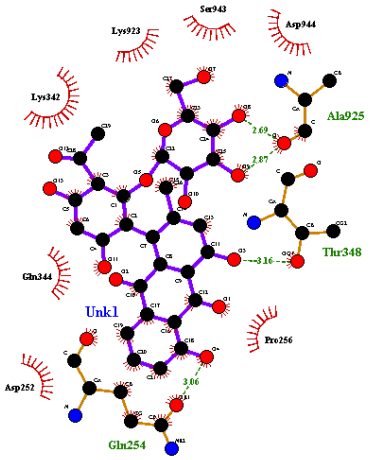
18



19



20



21

Figure 4.2.2.2: Lig plot showing interaction between ATPase and Bulbine phytochemicals. **(1);** 5,8-dihydroxy-1-(hydroxymethyl)-4H,9H-naphtho[2,3-c]furan-4,9-dione, **(2);** 1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-6-[(9S)-1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methyl-10-oxo-9,10-dihydroanthracen-9-yl]-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione, **(3);** 1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-6-[(9S)-1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methyl-10-oxo-9,10dihydroanthracen-9-yl]-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione,**(4);** PubChem CID:1020; **(5);** PubChem CID:68111; **(6);** PubChem CID:189763; **(7);** PubChem CID:438991 ; **(8);** PubChem CID:442731; **(9);** PubChem CID:442753; **(10);** PubChem CID:636652; **(11);** PubChem CID:5315852; **(12);** PubChem CID:5320386; **(13);** PubChem CID:1008440; **(14);** PubChem CID:10072444; **(15);** PubChem CID:10093576; **(16);** PubChem CID:11190093; **(17);** PubChem CID:11729754; **(18);** PubChem CID:12178518; **(19);** PubChem CID:25033784; **(20);** PubChem CID:91932460; **(21);** PubChem CID:102378267.

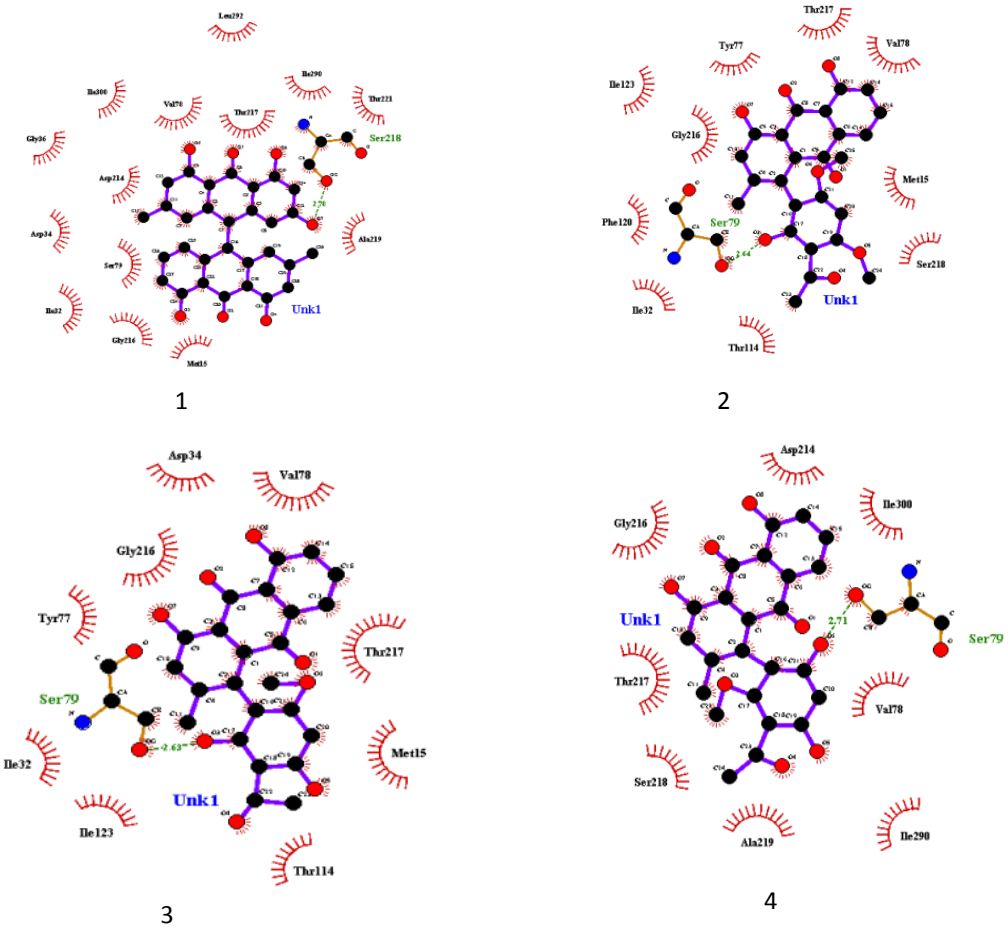
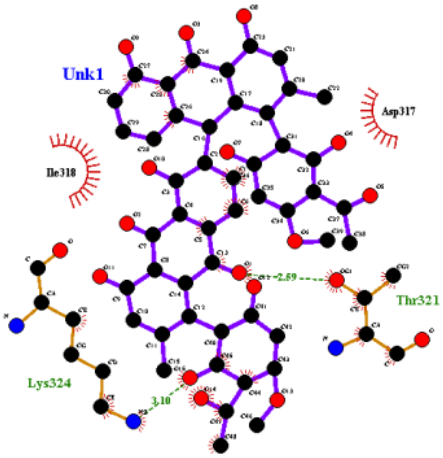
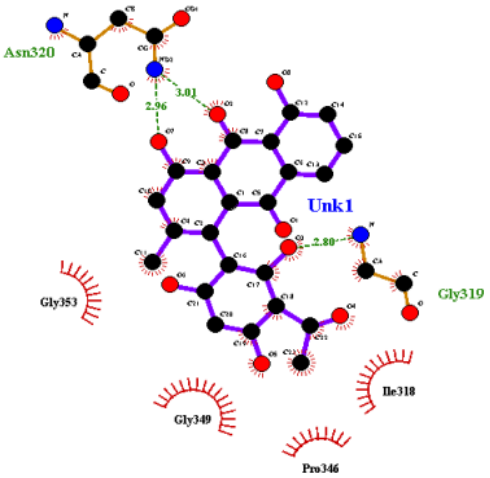


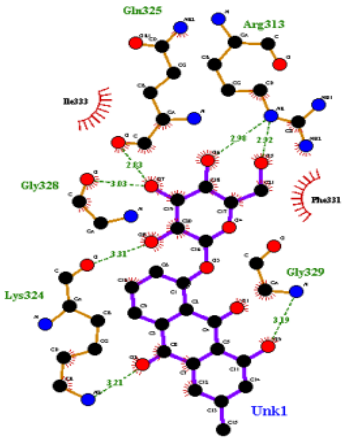
Figure 4.2.2.3: Lig plot showing interaction between Plasmepsin2 and Bulbine phytochemicals. **(1);** PubChem CID:5320386; **(2);** PubChem CID:11190093; **(3);** PubChem CID:11729754; **(4);** PubChem CID:12178518;


Unk1
Asp317
Ile318
Thr321
Lys324

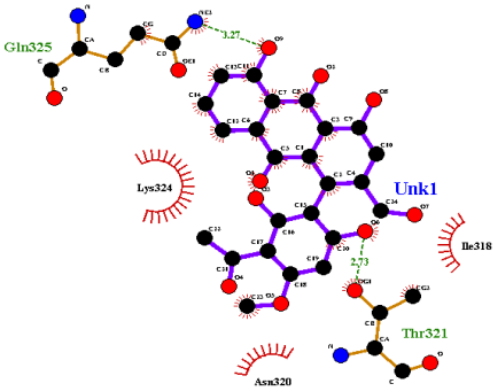
1


Asn320
Unk1
Gly319
Gly353
Ile318
Gly349
Pro346

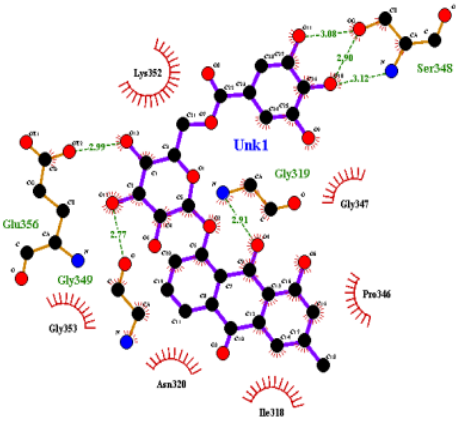
2

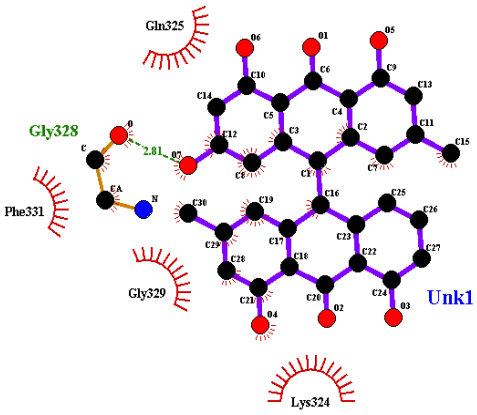

Gln325
Arg313
Ile333
Gly328
Phe331
Gly329
Lys324
Unk1

3

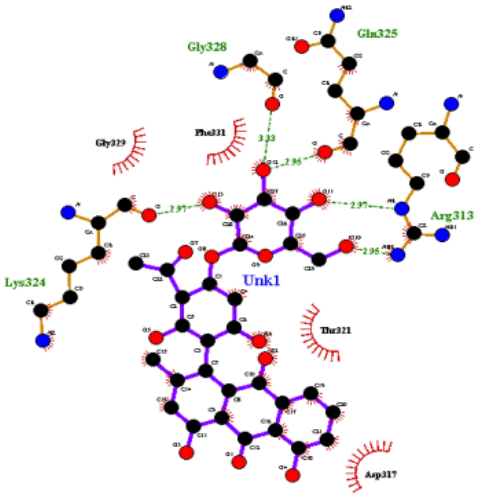

Gln325
Lys324
Unk1
Ile318
Thr321
Asn320

4

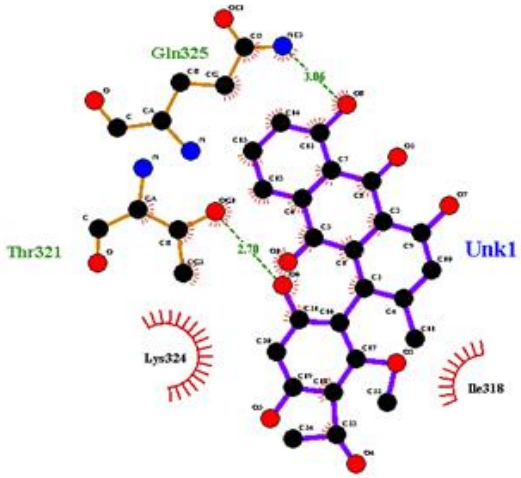

Lys352
Ser348
Unk1
Gly319
Gly347
Glu356
Gly349
Gly353
Pro346
Asn320
Ile318


Gln325
Gly328
Phe331
Gly329
Unk1
Lys324

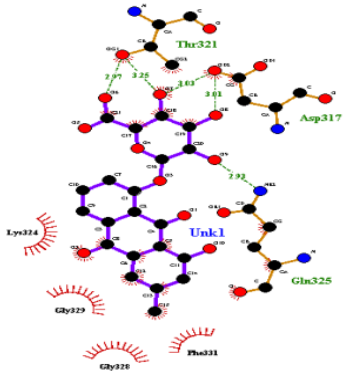
6



7

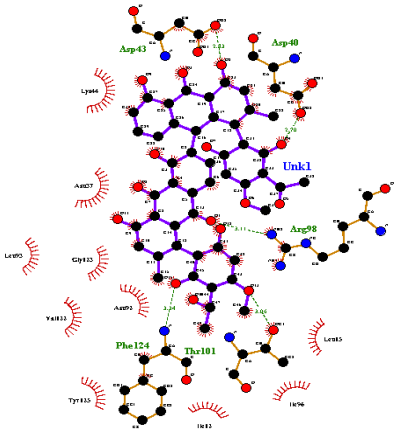


8

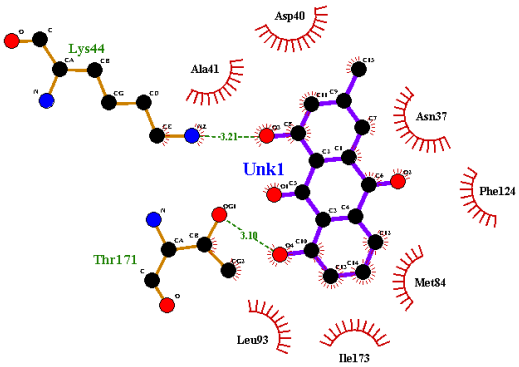


9

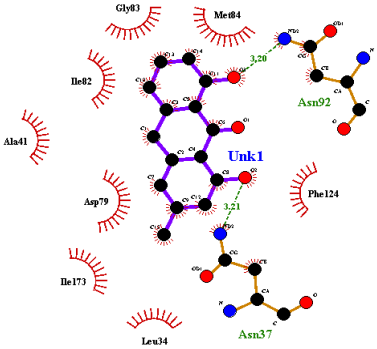
Figure 4.2.2.4: Lig plot showing interaction between Human orotidine 5' decarboxylase and Bulbine phytochemicals. **(1);** 1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-6-[(9S)-1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methyl-10-oxo-9,10dihydroanthracen-9-yl]-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione; **(2);** PubChem CID:438991; **(3);** PubChem CID:442731; **(4);** PubChem CID:636652; **(5);** PubChem CID:5315852; **(6);** PubChem CID:5320386; **(7);** PubChem CID:100724, **(8);** PubChem CID:12178518; **(9);** PubChem CID:25033784.


Asp43
Asp40
Unk1
Arg98
Phe124
Thr101

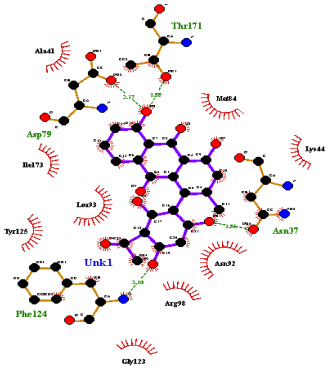
1


Asp40
Lys44
Ala41
Asn37
Unk1
Phe124
Thr171
Met84
Leu93
Ile173

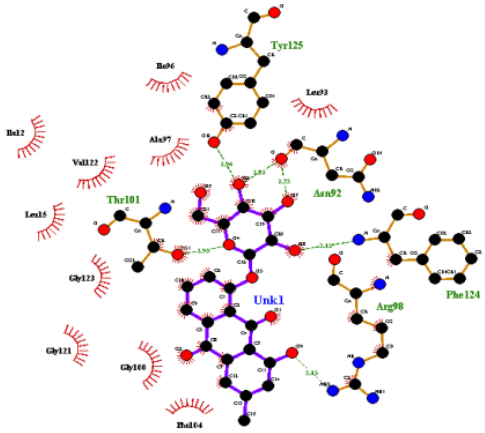
2


Gly83
Met84
Ile82
Asn92
Ala41
Unk1
Asp79
Phe124
Ile173
Asn37
Leu34

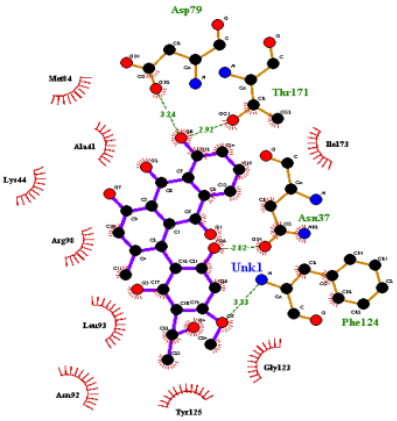
3


Thr171
Ala41
Met84
Asp79
Ile173
Lys44
Leu93
Tyr125
Asn37
Unk1
Asn92
Arg98
Phe124
Gly123

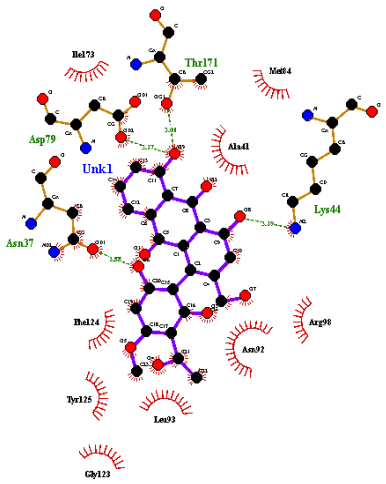
4


Tyr125
Ile96
Leu93
Ile12
Ala97
Val122
Asn92
Thr101
Leu15
Gly123
Unk1
Arg98
Phe124
Gly121
Gly100
Ile104

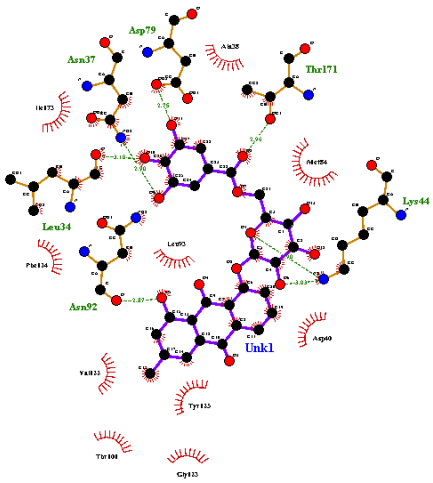
5


Asp79
Met84
Thr171
Ala41
Ile173
Lys44
Asn37
Arg98
Unk1
Phe124
Leu93
Gly123
Asn92
Tyr125

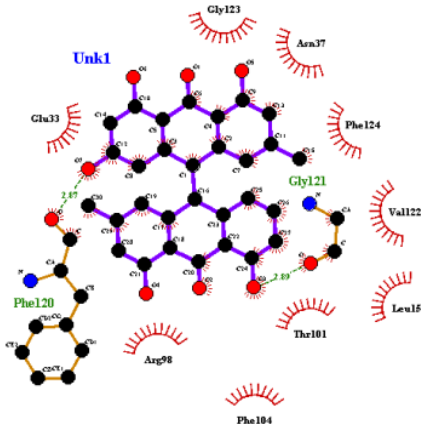
6


Ile173
Thr171
Met84
Asp79
Ala41
Unk1
Lys44
Asn37
Ile124
Arg98
Asn92
Tyr125
Leu93
Gly123

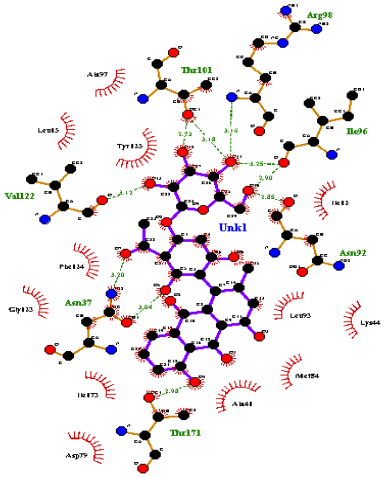
7


Asp79
Asn37
Thr171
Leu34
Lys44
Asn92
Unk1

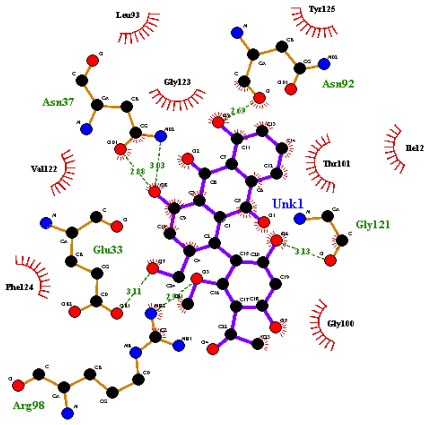
8


Gly123
Asn37
Unk1
Glu33
Phe124
Gly121
Val122
Phe120
Leu15
Thr101
Arg98
Phe104

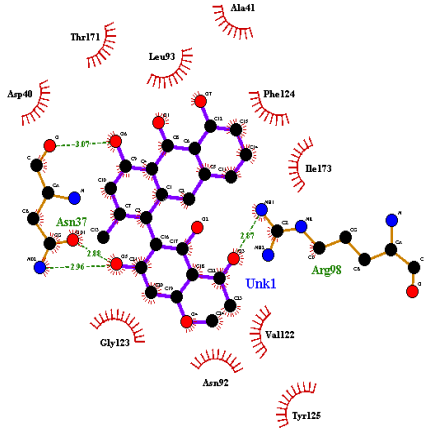
9


Arg98
Thr101
Ile96
Unk1
Asn92
Asn37
Thr171

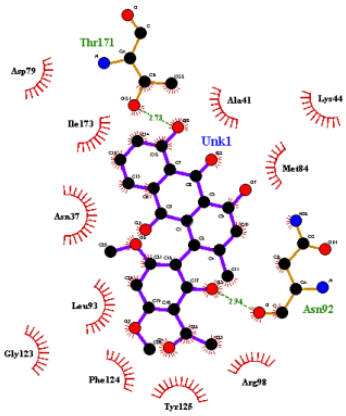
10



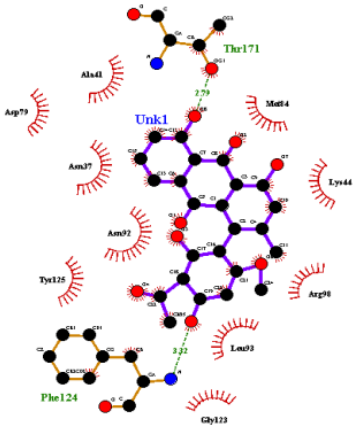
11



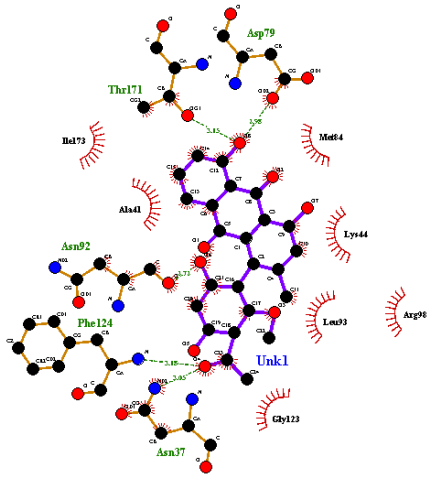
12



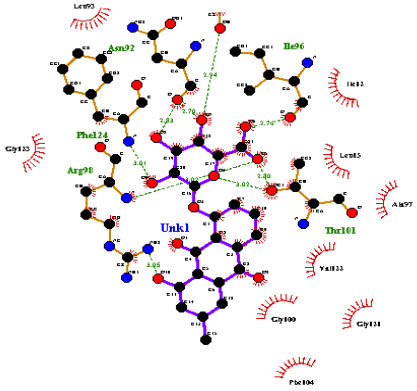
13



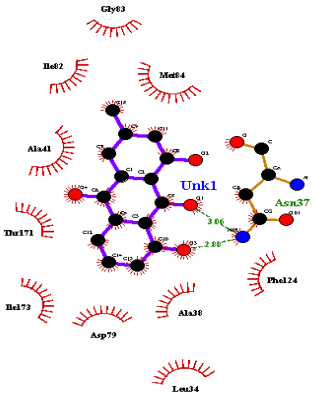
14



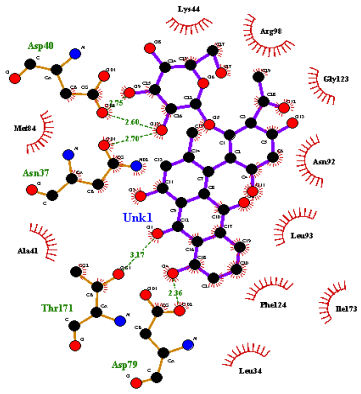
15



16

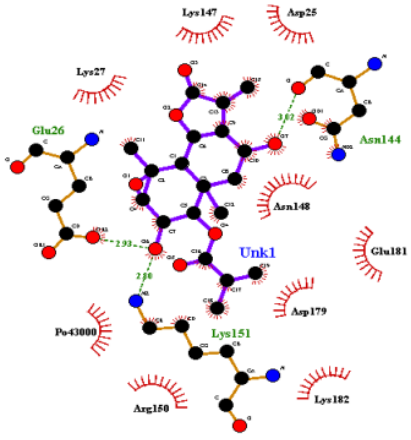


17

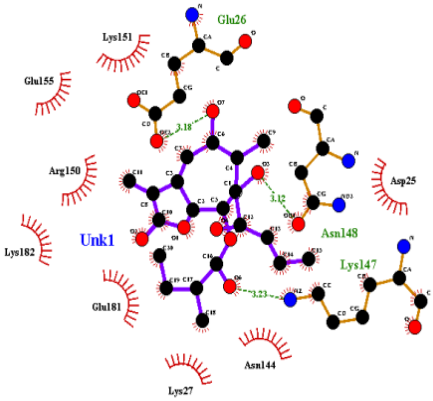


18

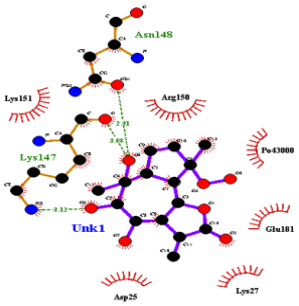
Figure 4.2.2.5: Lig plot showing interaction between HSP90) and Bulbine phytochemicals. **(1);** 1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-6-[(9S)-1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methyl-10-oxo-9,10dihydroanthracen-9-yl]-4,5-dihydroxy-2-methyl-9,10-dihydroanthracene-9,10-dione; **(2);** PubChem CID: 10208; **(3);** PubChem CID: 68111; **(4);** PubChem CID: 438991; **(5);** PubChem CID: 442731; **(6);** PubChem CID: 442753**;** **(7);** PubChem CID:636652 ; **(8);** PubChem CID: 5315852; **(9);** PubChem CID:5320386; **(10);** PubChem CID: 10008440; **(11);** PubChem CID:10072444; **(12);** PubChem CID: 10093576; **(13);** PubChem CID: 11190093; **(14);** PubChem CID:11729754; **(15);** PubChem CID:12178518; **(16);** PubChem CID:25033784; **(17);** PubChem CID:91932460; **(18);** PubChem CID:102378267



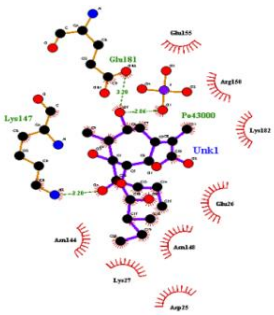
1



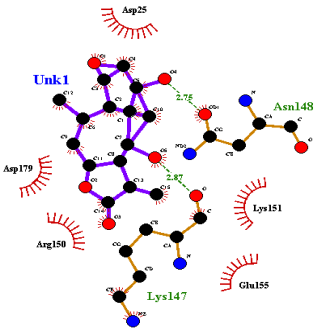
2


Asn148
Lys151
Arg150
Lys147
Po43000
Unk1
Glu181
Asp25
Lys27

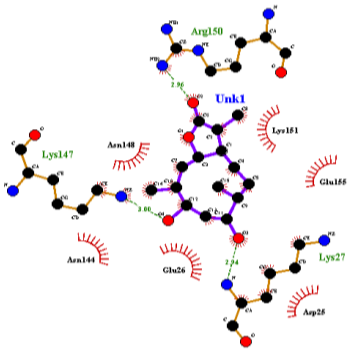
4


Glu155
Glu181
Arg150
Lys147
Po43000
Lys102
Unk1
Glu26
Asn144
Asn140
Lys27
Asp25

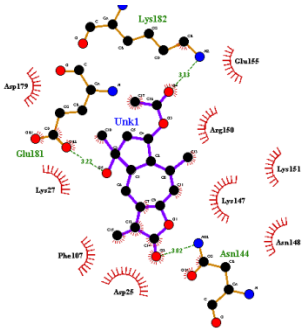
3


Asp25
Unk1
Asn148
Asp179
Lys151
Arg150
Glu155
Lys147

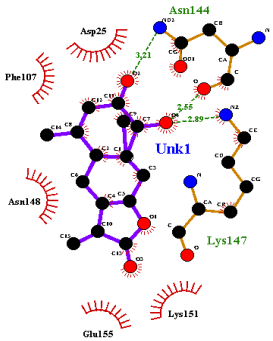
5


Arg150
Unk1
Lys151
Lys147
Asn148
Glu155
Asn144
Glu26
Lys27
Asp28

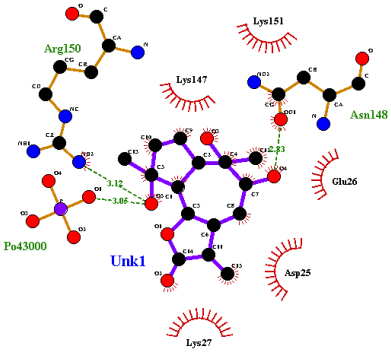
6


Lys182
Glu155
Asp179
Unk1
Arg150
Glu181
Lys151
Lys27
Lys147
Phe107
Asn144
Asn148
Asp28

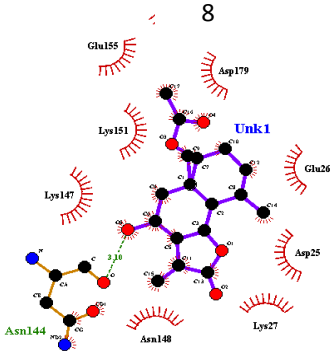
7


Asn144
Asp25
Phe107
Unk1
Asn148
Lys147
Lys151
Glu155

8

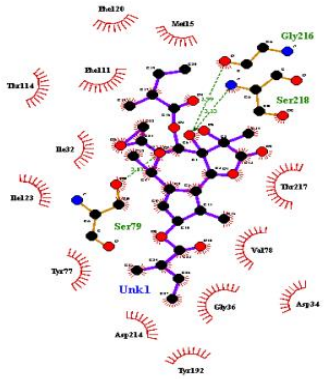

Lys151
Arg150
Lys147
Asn148
Glu26
Po43000
Unk1
Asp25
Lys27

9

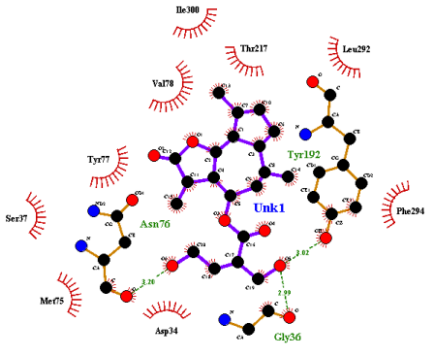

Glu155
Asp179
Lys151
Unk1
Glu26
Lys147
Asp25
Asn144
Asn148
Lys27

10

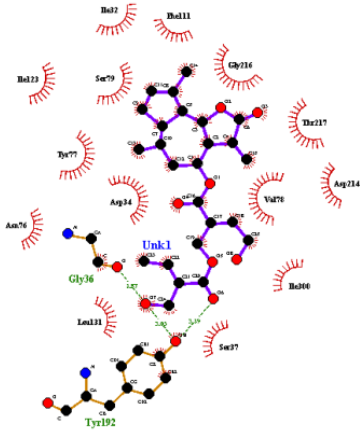
Figure 4.2.3.1: Lig plot showing interaction between Plasmodium orotidine 5' decarboxylase and sesquiterpene lactones. **(1);** PubChem CID: 639694, **(2);** PubChem CID: 21672237, **(3);** PubChem CID: 10098744, **(4);** PubChem CID: 6712627, **(5;)** PubChem CID: 98394739;6= PubChem CID: 101923143, **(7);** PubChem CID: 267247, **(8);** PubChem CID: 98050854, **(9);** PubChem CID: 14543458, **(10);** PubChem CID: 101650348.



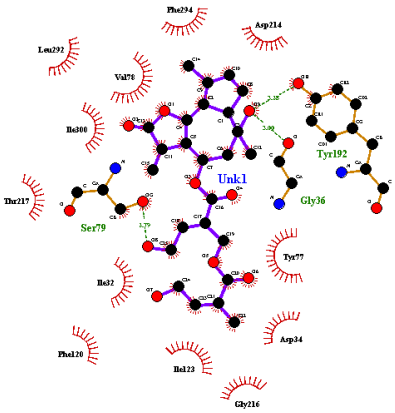
1



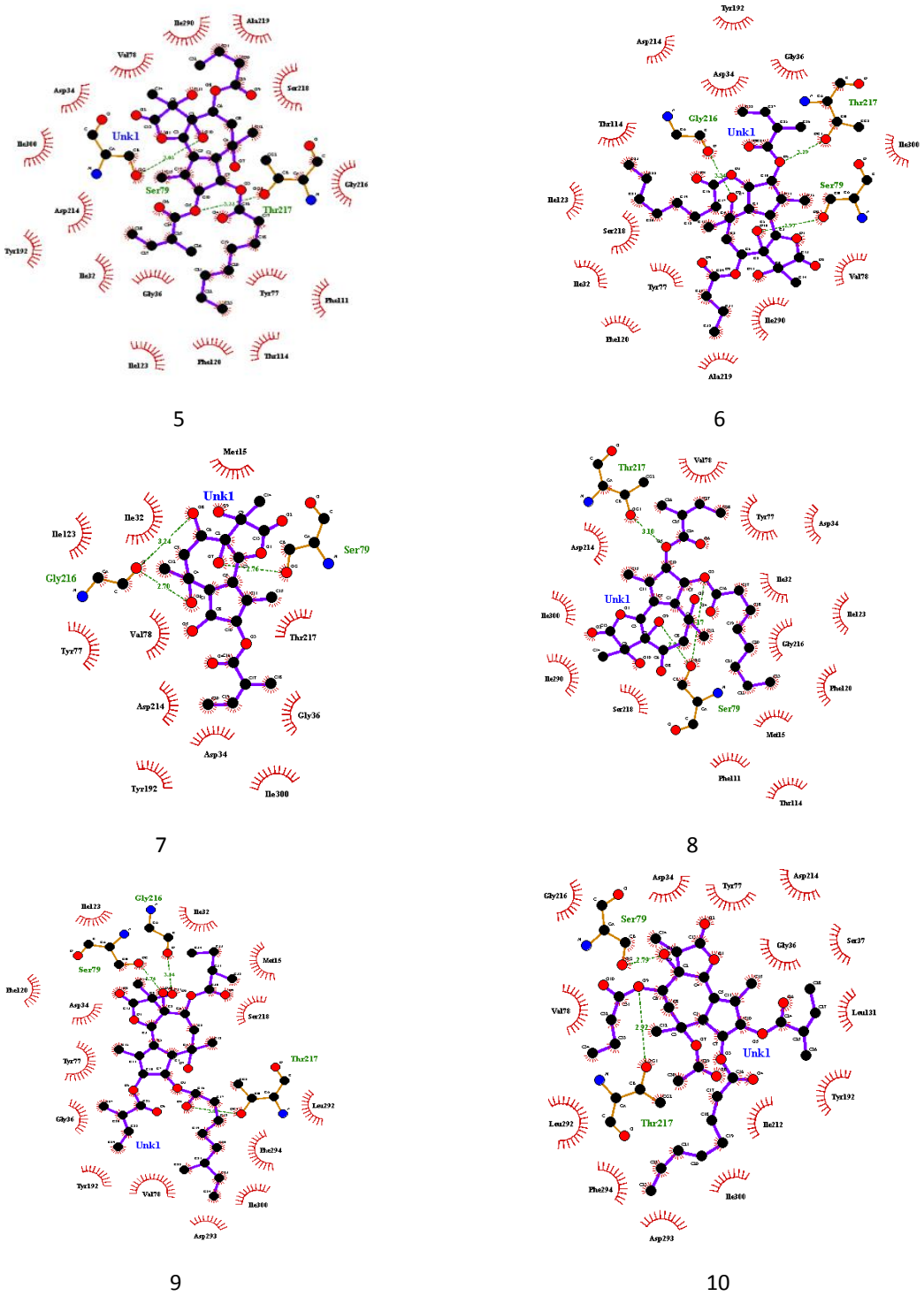
2



3



4



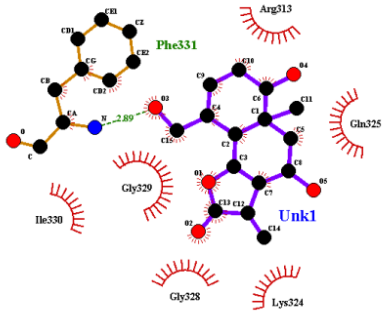
5 6

7 8

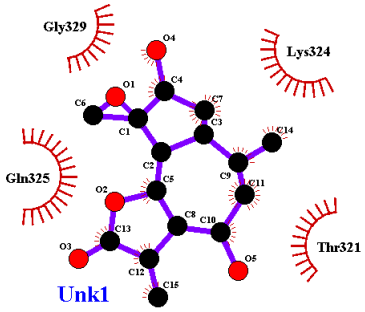
9 10

Figure: 4.2.3.2: Lig plot showing interaction between proteins (Plasmepsin2) and ligands. Ligand as **, (1);** PubChem CID: 102276755, **(2);** PubChem CID: 101667704, **(3);** PubChem CID: 44400048, **(4);** PubChem CID: 14779723, **(5);** PubChem CID: 6440697, **(6);** PubChem

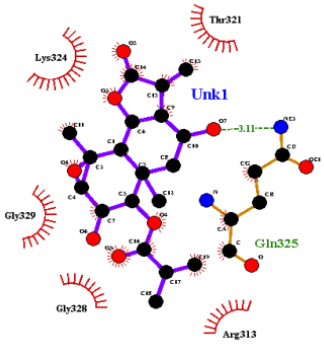
CID: 52943584, **(7);** PubChem CID: 101667702, **(8);** PubChem CID: 14845494, **(9);** PubChem CID: 125121058, **(10);** PubChem CID: 5322099.



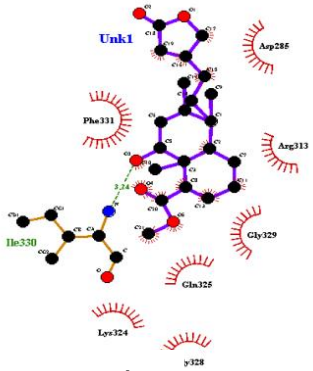
1



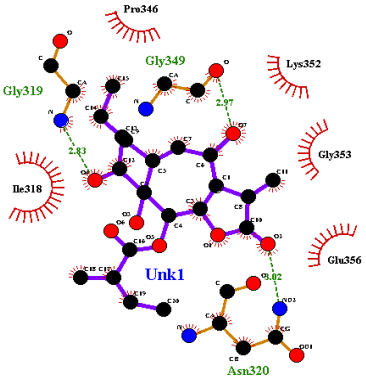
2



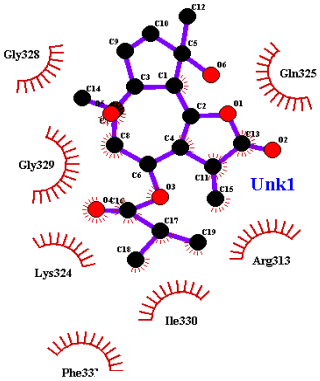
3



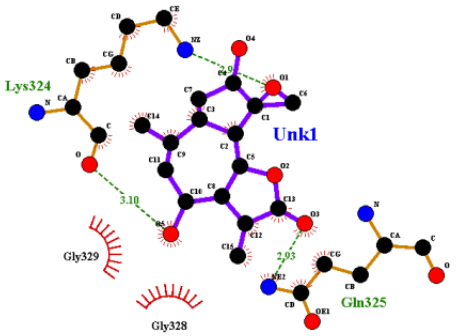
4



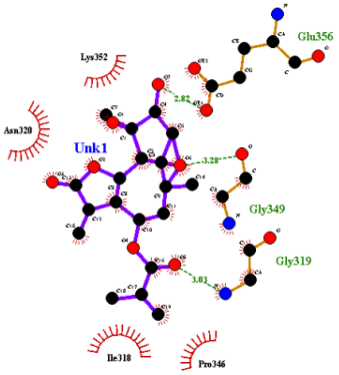
5



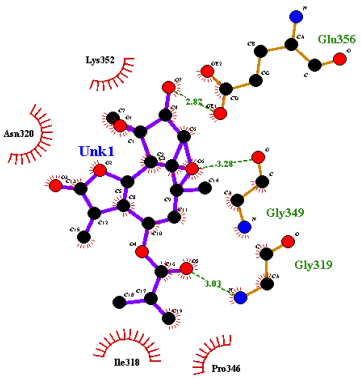
6



7

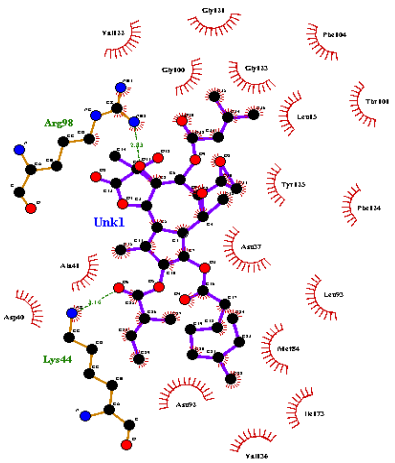


8

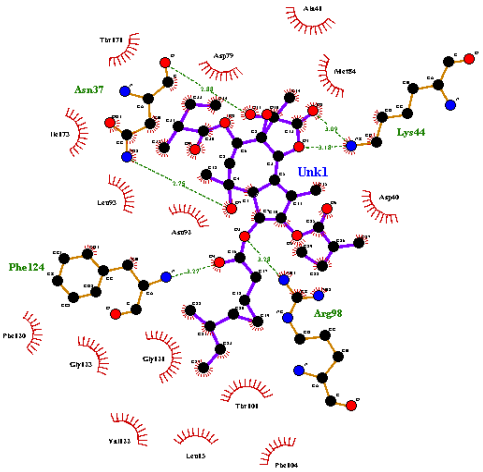


9

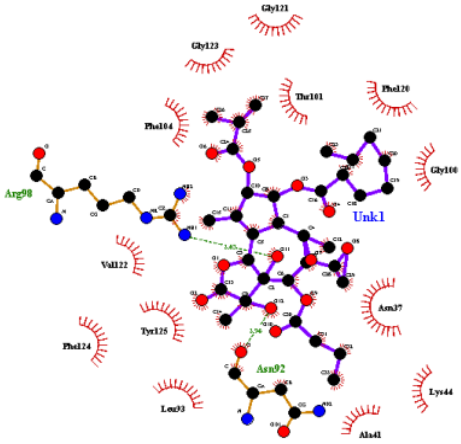
Fig 4.2.3.3: Lig plot showing interaction between Human orotidine 5' decarboxylase and sesquiterpene lactones. **(1);** PubChem CID: 483220, **(2);** PubChem CID: 102242189, **(3);** PubChem CID: 101705724; **(4)** PubChem CID: 176588, **(5);** PubChem CID: 639694, **(6);** PubChem CID: 101320302, **(7);** PubChem CID 11552280, **(8);** PubChem CID:44575369, **(9);** PubChem CID: 102057347.


Arg98
Unk1
Lys44

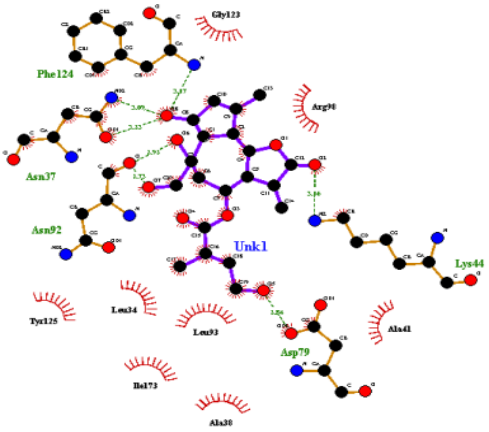
1


Asn37
Lys44
Unk1
Phe124
Arg98

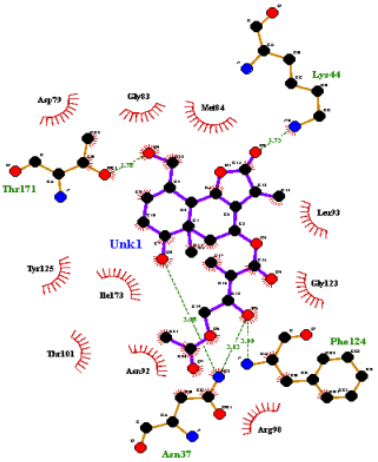
2


Arg98
Unk1
Asn92

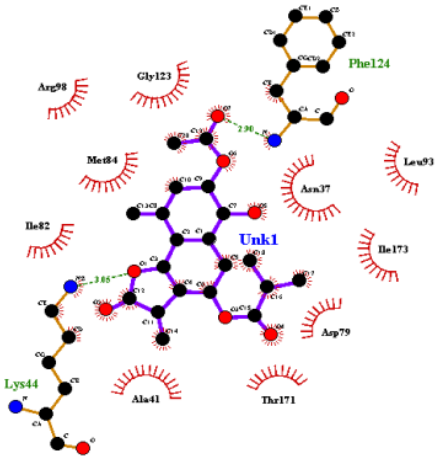
3


Phe124
Asn37
Asn92
Unk1
Lys44

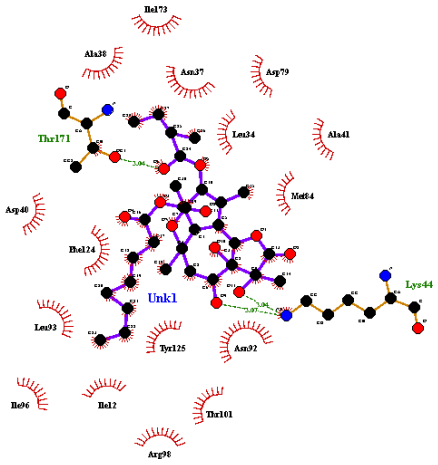
4


Lys44
Thr171
Unk1
Phe124
Asn37

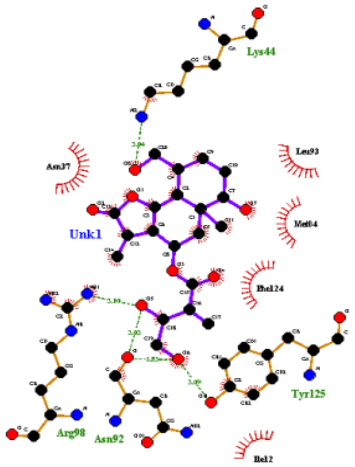
5


Phe124
Arg98
Gly123
Met84
Asn37
Ile82
Unk1
Ile173
Arg79
Lys44
Ala41
Thr171

6



7



8

Fig 4.2.3.4 Lig plot showing interaction between HSP90 and sesquiterpene lactone. **(1);** PubChem CID: 102276267, **(2);** PubChem CID: 102276755, **(3);** PubChem CID: 67976473, **(4);** PubChem CID:483222, **(5);** PubChem CID: 1142932, **(6);** PubChem CID: 101631678**, (7);** PubChem CID: 53325574, **(8);** PubChem CID: 483221.

The Lig Plot shows the amino acid of the target protein involved in the interaction with the ligand. The interaction profile of lead phytochemicals and the interface between heterodimers of the protein Human orotidine 5' decarboxylase, Plasmepsin 2, HSP90, P-type ATPase, Human orotidine 5' decarboxylase with lowest binding energy to its known standard inhibitor is shown. The amino acids in hydrogen bonding and hydrophobic interaction with the lead phytochemicals are given in table 4.1.3 to 4.1.15.

Number and types of amino acids of ligands involved in hydrophobic and hydrophilic interaction are tabulated in table 4.1.3 to 4.1.15.

Table 4.1.3 - Amino acids residues of Plasmodium orotidine5' decarboxylase involved in the hydrogen bonding and hydrophobic interaction with methylated flavonoid.

S.No	Protein-Ligand	Hydrogen bonding	Hydrophobic interaction
1	Plasmodium orotidine 5'	Arg150, Lys27, Arg294	Po43000,Glu181,Glu26,Asn14

	decarboxylase – 1*		4,Lys298,Asp25, Asn148, Lys147, Lys151
2	Plasmodium orotidine 5' decarboxylase – 2*	Arg150, Lys27	Asn148,Asn144,Arg294, Lys298Asp25,Glu26,Lys147,Lys151
3	Plasmodium orotidine 5' decarboxylase – 3*	Gly271,Gly267,Asn2701, Pge4000,Gly312	Leu273,Ala295,Arg294, Lys298,Tyr309
4	Plasmodium orotidine5' decarboxylase – 4*	Asn144	Lys298,Asp25,Arg294,Po43000,Glu181,Arg150Lys182,Glu15,Lys147,Asn148,Lys151,Ph07
5	Plasmodium orotidine5' decarboxylase – 5*	Asn244, Arg294	Gly143,Asn144,Asp175,Edo4002,Thr195,Pro197,Asn270,269, Ala268
6	Plasmodium orotidine5' decarboxylase – 6*	-	Lys151,Lys147,Asn148,Arg24, Asn144,Lys298, Lys27, Asp25, Glu181
7	Plasmodium orotidine5' decarboxylase – 7*	Asn144, Arg150	Arg294,Lys298,Asp25,Phe107,Lys147,Asn148,Lys151,Glu15, Glu181
8	Plasmodium orotidine 5' decarboxylase – 8*	-	Lys27,Lys298, Asn144, Asp25, Asn148,Lys151,Lys147,Arg10, Glu181
9	Plasmodium orotidine 5' decarboxylase – 9*	-	Asn148,Lys147, Asn144, Asp25, Lys298, Lys27,Glu181, Lys151
10	Plasmodium orotidine 5' decarboxylase – 10*	Arg150,Asn144, Lys147	Lys151,Phe107,Asp25, Glu26, Glu26, Glu155
11	Plasmodium orotidine 5' decarboxylase – 11*	-	Asn148,Lys147,Asp25,Arg294, Asn144,Lys298,Lys27,Glu181, Arg150
12	Plasmodium orotidine 5' decarboxylase – 12*	-	Asp175,Edo4002,Asn144,Arg294,Thr195,Gln269, Asn244
13	Plasmodium orotidine 5'	Asn144	Asn148,Lys151,Arg150,Lys14

	decarboxylase – 13*		7,Lys27,Asp25,Arg294,Lys298 , Phe107
14	Plasmodium orotidine 5' decarboxylase – 14*	-	Glu18,Asn148,Lys147,Arg294, Lys298, Asn144, Asp25, Lys27

Name of 1*-14* ligands are given in table 4.1.1 as serial no 1-14.

Table 4.1.4 - Amino acids residues of ATPase involved in the hydrogen bonding and hydrophobic interaction with methylated flavonoid.

S.No	Protein-Ligand	Hydrogen bonding	Hydrophobic interaction
1	ATPase – 1*	Arg1034	Asn1042, Asp1038, Asn1039, Tyr966, Thr1035, Arg1048, Gln1142, Leu1044, Ile1208, Al11212, Lys1209, Lys1213, Tyr1143
2	ATPase – 2*	Arg1034	Asn1039, Tyr966, Asn1022, Lys970, Leu1044, Tyr1143, Lys1209, Ala1212,Arg1214,Lys1213,Arg1048, Ile1208, Glu1142
3	ATPase – 3*	Arg1048, Tyr966	Lys970, Asn1042, Arg1034, Tyr1143, Arg1214, Lys1209, Lys1213, Glu1142
4	ATPase – 4*	Tyr966, Arg1048	Lys970, Tyr1143, Leu1044, Lys 1213, Asp1038, His1035, Asn1042, Arg1034
5	ATPase – 5*	-	Glu1142, Glu1045.Arg1034, Arg 963, Asn967, Cys1031, Tyr1031, Tyr1143, Arg1048, Lys1213, Leu 1044, Thr1045, Asn1042
6	ATPase – 6*	-	Ile1050, Ile981, Ile271, Ile272, Ile 977, Leu268, Ala313, Ile275, Val 1984, Ala985, Thr1054
7	ATPase – 7*	-	Ala1212, Lys1209, Tyr1143, Cys

			1031, Val1028, Arg1034, Asn967, Tyr966,Glu1142, Leu1044, Thr 1045, Lys1213, Arg1048
8	ATPase – 8*	Asn967	Val1028, Glu1142, Asn1042, Thr 1045, Leu1044, Lys1213, Arg1048, Tyr1143,Cys1031, Arg1034
9	ATPase – 9*	Tyr966, Arg1048	Lys970, Thr1045, Arg1034, Asn 1042, Lys1213, Arg1214, Tyr1143, Ala1212
10	ATPase – 10*	Ala313	Val984, Ile275, Ile981, Ile271, Phe 264,Gln267,Ile1041, Leu1046,Leu 268, Asn980
11	ATPase – 11*	-	Leu1046, Leu268, Phe264, Gln267 Ile1041, Ile271, Val984, Ile275,Ile 981
12	ATPase – 12*	-	Gln267, Ile271, Ile275, Ile981, Val 984, Ala313, Asn980, Leu268, Phe 264, Leu1046, Ile1041
13	ATPase – 13*	1048	Thr1045, Leu1044, Asn1042,Lys 1213,Glu1142,Val1028,Cys1031, Arg1034, Asn967, Tyr1143
14	ATPase – 14*	-	Leu1044, Thr1045, Tyr966, Asn967, Cys1031,Arg1034,Lys1213,Asn1042

Name of 1*-14* ligands is given in table 4.1.1 as serial no 1-14.

Table 4.1.5 - Amino acids residues of plasmepsin 2 involved in the hydrogen bonding and hydrophobic interaction with methylated flavonoid.

S No	Protein-Ligand	Hydrogen bonding	Hydrophobic interaction
1	Plasmepsin 2 – 1*	Gly36	Asp214, Tyr192, Asp34, Gly216, Phe120, Thr114, Ile32, Ser79, Tyr77, Phe111, Ile123,Val78, Thr217, Ile300, Leu292, Phe294
2	Plasmepsin 2 – 2*	Asp34	Tyr77, Leu131, Ser37, Gly36,

			Asn76, Val78, Ser79, Gly216, Phe120,Phe111, Ile123
3	Plasmepsin 2 – 3*	Thr217	Ile123, Phe120,Ser79, Ile32, Gly 216, Tyr77, Tyr192, Val78
4	Plasmepsin 2 – 4*	Tyr192	Asp214, Thr217, Ile300, Val78, Tyr77, Ile123, Asp34, Met15, Gly216, Phe120, Ser79, Ser218, Ile32
5	Plasmepsin 2 – 5*	Tyr192	Phe294, Val78, Gly216, Met15, Ile32, Ser79, Ile123, Asp34, Tyr77, Asn76
6	Plasmepsin 2 – 6*	-	Ile32, Phe120, Phe11 Thr114, Gly216, Ile123, Ser79, Tyr77, Ser37, Gly36, Asp34, Tyr192, Ile300,Val78, Leu292, Thr217
7	Plasmepsin 2 – 7*	Thr217, Ser79	Phe120, Gly216, Ile32, Asp34, Ser37, Tyr77, Tyr192, Gly36, Val78,Ile300, Leu292, Phe294
8	Plasmepsin 2 – 8*	-	Tyr192,Phe294,Asp214, Thr217,Ile300,Asp34,Gly216, Ile32,Ile123,Ser79,Tyr77, Val178, Asn76
9	Plasmepsin 2 – 9*	-	Gly216, Ile32, Met15, Phe120, Ser79, Tyr192,Asp34, Gly36, Ile123, Ser37, Tyr77, Ile300, Val78, Leu292, Thr217
10	Plasmepsin 2 – 10*	Ser218, Asp214	Gly216,Gly36,Val78, Tyr192,Tyr 77, Asp34, Ile123, Ser79, Phe120,Ile32 , Met15
11	Plasmepsin 2 – 11*	-	Thr217,Val78,Ile300, Tyr192,Gly 36, Ile133, Leu131, Asn39, Met75Asn76, Tyr77, Asp34, Gly216

12	Plasmepsin 2 – 12*	-	Asn76, Tyr77, Ser79, Asp34, Phe111, Ile123, Ile32, Thr217, Gly216Ile300, Val78, Phe294, Tyr192
13	Plasmepsin 2 – 13*	-	Pro243,Val78,Ser79, R37330, Ser118,Met15, Phe120, Thr114, Ile290,
14	Plasmepsin 2 – 14*	-	Phe111, Ile32, Ser79, Ile123, Asp34, Gly216, Ile300, Ile212, Phe294Tyr192, Asp214, Val78, Tyr77

Name of 1*-14* ligands is given in table 4.1.1 as serial no 1-14.

Table 4.1.6 - Amino acids residues of Human orotidine 5' decarboxylase involved in the hydrogen bonding and hydrophobic interaction with methylated flavonoid.

S No	Protein-Ligand	Hydrogen Bonding	Hydrophobic interaction
1	Human orotidine 5 decarboxylase – 1*	Lys324	Asn320, Ile318, Thr321, Asp317, Gln325, Gly329, Gly328
2	Human orotidine 5 decarboxylase – 2*	Ala316	Asp317, Pro346, Ile318, Gly349, Gly319
3	Human orotidine 5 decarboxylase – 3*	Gln325	Lys324,Thr321, Ile318, Asp317
4	Human orotidine 5 decarboxylase – 4*	Lys324, Thr321	Asp317, Gln325
5	Human orotidine 5 decarboxylase – 5*	Lys324	Asp317, Gln325, Gly329, Gly328 Thr321
6	Human orotidine 5 decarboxylase – 6*	Lys324	Ile318,Asn320, Gln325, Thr321
7	Human orotidine 5 decarboxylase – 7*	Lys324	Asp317,Thr321,Glu325, Gly328 Gly329
8	Human orotidine 5	Thr321	Ile318, Lys324, Gln325, Asp317

	decarboxylase – 8*		
9	Human orotidine 5 decarboxylase – 9*	Lys324, Arg313	Thr321,Gln325,Phe331, Gly329
10	Human orotidine 5 decarboxylase – 10*	Arg313	Phe331,Gly328,Gly329, Lys324 Gln325, Ile333, Asp285
11	Human orotidine 5 decarboxylase – 11*	Arg313	Gly328,Gly329,Lys324, Gln325 Asp285, Phe331
12	Human orotidine 5 decarboxylase – 12*	-	Arg313,Thr321,Lys324, Gly328 Gly329, Phe331, Gln325
13	Human orotidine 5 decarboxylase – 13*	-	Phe331,Asp285,Arg313, Gln325,Gly329, Ile330
14	Human orotidine 5 decarboxylase – 14*	-	Gly328,Arg313,Phe331,Glu325 Gly329, Lys324

Name of 1*-14* ligands is given in table 4.1.1 as serial no 1-14.

Table 4.1.7 - Amino acids residues of HSP90 involved in the hydrogen bonding and hydrophobic interaction with methylated flavonoidS

S. No	Protein-ligand	Hydrogen bond	Hydrophobic interaction
1	HSP90 – 1*	Asn92 , Arg98	Ile12, Thr101, Val122, Ile96, Tyr125, Leu15, Phe124, Asn37, Thr171, Ile173, Leu93, Gly123
2	HSP90 – 2*	Asn92	Tyr125,Phe124, Ile173, Thr171, Asp79, Ala41, Asn37, Arg98, Gly123, Val122, Leu15, Thr101
3	HSP90 – 3*	Lys44, Gly83	Ile173,Asn37,Leu93, Thr171,Ala41,Met84, Ile82, Asp88, Asn92, Asn140
4	HSP90 – 4*	Lys44	Phe124, Arg98, Asn92, Thr101, Val122, Gly123, Tyr125,Asn37, Thr171,Met84,Asp79,Ala38,Gly 83, Ala41

5	HSP90 – 5*	Lys 44	Ala41, Gly83, Asn92, Arg98, Tyr125, Leu93, Phe124, Asn37, Asp79, Thr171, Met84
6	HSP90 – 6*	-	Arg98, Thr101, Val122, Tyr125, Gly123, Ile96, Asn92, Leu93, Val 136, Met84, Ile173, Ala41, Asn37
7	HSP90 – 7*	Lys44, Thr171	Met84, Asp79, Ala41, Ile173, Gly123,Phe124, Tyr125,Asn37, Leu93, Asn92, Gly83
8	HSP90 – 8*	Lys44	Asp79, Thr171, Ala41, Val122, Gly123,Tyr125,Phe124, Leu93, Arg98, Asn92 ,Gly83, Met84, Ile 82, Asn37
9	HSP90 – 9*	-	Met84, Val136, Leu93, Gly123, Leu15,Val122,Thr101, Phe124, Tyr125, Asn92, Arg98, Asn97, Ile173, Asp79, Ala41
10	HSP90 – 10*	Phe124, Asp79	Thr171, Met84, Ala41, Asn92, Arg98, Gly123, Asn37, Ile173
11	HSP90 – 11*	Asn92	Phe124, Val122, Tyr125, Gly123,Asn37, Thr171, Ala41, Gly83, Met84
12	HSP90 – 12*	-	Asn92,Leu93, Gly123, Phe124, Asn37,Ile173,Asp79,Thr171,Ala 41
13	HSP90 – 13*	-	Met116,Ala107,Phe120,Phe104, Leu104, Leu15, Thr101, Val122, Gly121, Gly100
14	HSP90 – 14*	-	Val136, Thr171, Ile173, Leu93, Asn92, Gly123, Tyr125, Thr101, Phe124, Asn37

Name of 1*-14* ligands is given in table 4.1.1 as serial no 1-14.

Table 4.1.8 - Amino acids residues of Plasmodium orotidine 5' decarboxylase involved in the Hydrogen bonding and hydrophobic interaction with Bulbine frutescens phytochemicals.

S. No	Protein-ligand PCID	Hydrogen bond	Hydrophobic interaction
1	Plasmodium orotidine 5 decarboxylase – 1*	Asn144, Asp25, Asn148, Lys147	Arg150, Lys151
2	Plasmodium orotidine 5 decarboxylase – 2*	Lys147, Lys27	Asp175, Po43000, Glu180, Asn148,Asp25,Asn144, Ile142, Gln269, Edo4002
3	Plasmodium orotidine 5 decarboxylase – 3*	Edo4002,Asn244,Tyr246,Lys204	Pro197,Lys193,Ser199,Ala200,Gln269
4	Plasmodium orotidine 5 decarboxylase - 10208	Glu181	Asp25,Asn148, Asn144, Phe107, Lys147, Lys151, Arg150, Glu155
5	Plasmodium orotidine 5 decarboxylase - 68111	Glu181	Glu155,Arg150, Lys151, Lys147, Asp25, Asn146, Asn144, Phe107
6	Plasmodium orotidine 5 decarboxylase- 189763	-	Glu155,Lys151,Lys147, Phe107, Asp25, Asn148, Glu181, Asn144
7	Plasmodium orotidine 5 decarboxylase- 438991	Lys151,Lys182,Glu155, Arg150	Po43000,Glu26,Asn148, Lys147, Asp25
8	Plasmodium orotidine 5 decarboxylase- 442731	Glu180,Asn144,Po43000	Tyr178,Lys174,Pro197,Edo4002, Gln269 Gly143, Asp175
9	Plasmodium orotidine 5 decarboxylase- 442753	Asn148, Asn144,	Phe107, Asp25, Lys27, Glu181, Glu26,Lys147
10	Plasmodium orotidine 5	Asn148,Asn144,Ly	Lys151, Glu181, Phe107, Asp25,

	decarboxylase - 63665	s147	Lys298,Lys27
11	Plasmodium orotidine 5 decarboxylase-5315852	Glu155,Arg150,Lys 147, Asn144	Phe107, Glu26, Asn 148, Lys151,Asp179,Glu181,Po43000 , Lys27, Asp25
12	Plasmodium orotidine 5 decarboxylase-5320386	Lys151,Asp25,Asp 179,Glu155	Lys182,Glu181,Arg150,Po43000 ,Lys27, Glu26, Lys147, Asn148
13	Plasmodium orotidine 5 decarboxylase-10008440	Edo4002,Arg294,Al a268,Asn244	Asn144, Ile142, Gly143, Asn196, Pro197,Thr195, Asn270, Gln269
14	Plasmodium orotidine 5 decarboxylase-10072444	Asn244,Asn270,Gl n269,Edo4002	Thr195
15	Plasmodium orotidine 5 decarboxylase-10093576	Arg294	Asn144, Gly143, Asp175, Edo4002, Pro197, Asn244, Asn270, Ala268,Gln269
16	Plasmodium orotidine 5 decarboxylase-11190093	-	Gly143, Asp175, Pro197, Asn244, Gln296 Ala268, Asn144, Edo4002
17	Plasmodium orotidine 5 decarboxylase-11729754	Arg294, Asn244	Asn144, Gly143, Edo4002, Asp175, Pro197, Thr195, Asn 270, Gln269,Ala268
18	Plasmodium orotidine 5 decarboxylase-12178518	Gln269, Thr195	Gly143, Pro197, Gly271, Asn244,Asn270,Pro264, Gly265, Asn196, Ile142, Arg294, Asn144, Edo4002
19	Plasmodium orotidine 5 decarboxylase-25033784	Asn244, Gln269	Pro197, Asn144, Arg294, Ile142, Edo4002, Ala268, Thr195
20	Plasmodium orotidine 5 decarboxylase-91932460	Gln269	Thr195, Asn244, Pro197, Edo4002,Ile142

21	Plasmodium orotidine 5 decarboxylase-102378267	Asn144, Asp175, Arg294, Gln269	Gly143, Pro197, Edo4002, Asn244, Thr195, Ala268, Ile142

Name of 1*-3* ligands is given in table 4.1.2 as serial no 1-21.

Table 4.1.9 - Amino acids residues of ATPase involved in the Hydrogen bonding and hydrophobic interaction with Bulbine frutescens phytochemicals**.**

S.No	Protein-ligand PCID	Hydrogen bond	Hydrophobic interaction
1	P-Type ATPase – 1*	Asn 967	Tyr1143, Arg 1048, Glu1142, Tyr 966, Lys 970, Thr1045, Arg1034
2	P-Type ATPase – 2*	Tyr966,Asp1048,Arg1034,Asn967,Arg 1048, Ile 1208, Asn 1042	Lys970,Lys1209,Lys1031,Tyr1143,Leu1047,Leu1044,Ala1212,Thr1045,Lys1213,Leu1040
3	P-Type ATPase – 3*	Lys1209,Asp1205, Arg1043,Lys970,Ile 1041,Thr1045,Ile973,Ile977,Ser973Arg 974, Ala1139, Ile1052, Asn1038	Ile1051, Ile1208, Leu1047, Leu318,Phe264,Ile268, Leu976,Phe972,Pro315,Asn1135,Leu1019, Tyr975Ser979, Ala971
4	P-Type ATPase -10208	Asn967,Tyr966,Leu 1040	Glu1142, Lys970, Thr1045, Asn1042, Tyr1143
5	P-Type ATPase -68111	Arg1034, Lys1213	Tyr1143,Glu1142, Arg1048, Thr1045,Leu1044, Cys1031
6	P-Type ATPase -189763	-	Asn967, Arg1034, Cys1031, Glu1142,Tyr1143, Arg1048, Thr1045, Lys970,Tyr966
7	P-Type ATPase -438991	Tyr966, Asp1038	Arg1034, Asn1042, Lys970, Glu1142,Thr1045, Tyr1143, Arg1048, Leu1044,Lys1213

8	P-Type ATPase -442731	Phe1021,Tyr1156, Met1155, Arg1153	Asn1154, Trp1152,
9	P-Type ATPase - 442753	Arg1034	Asn1042, Lys1213, Cys1031, Asn967,Tyr966, Glu1142, Arg1048, Thr1045,Leu1044
10	P-Type ATPase - 636652	Arg1034, Glu1142, Arg1048	Tyr966, cys1031, Asn967, Tyr1143,Leu1044, Thr1045, Lys1213, Asn1042
11	P-Type ATPase -5315852	Asn967,Arg1034,Asp1038, Tyr966	Glu1142, Cys1031, Thr1143, Thr1045,Arg1048, Asn1042, Lys1213, Asn1039,Leu1040, Lys970, leu1044
12	P-Type ATPase -5320386	Arg1034	Asn967,Tyr966, Lys1209, Arg1048,Tyr1143, Leu1044, Lys1213, Cys1031,Glu1142, Thr1045, Lys970, Val1028
13	P-TypeATPase -10008440	Asp1228	Pro1151, Ile1149, Phe1199, Phe1195,Leu1137, Phe1134, Met1133, Thr1161,Leu1130
14	P-Type ATPase-10072440	Asp1228, Ile1225	Phe1195,Phe1199,Phe1134, Glu1206,Leu1137, Ile1202, Pro1151, Ile1149,Lys1224
15	P-Type ATPase-10093576	-	Ile1050, Phe988, Ala985, Ile981, Val1984, Ile275, Leu268, Ile271,Leu1046
16	P-Type ATPase-11190093	Asn953,Asn951,Asp949,Tyr407, Asp405	Lys802, Tyr803, Arg806, Glu957, Lys335,Lys336, Thr954
17	P-Type ATPase-11729754	Tyr407,Asp949,Asn951,Asn953,Asp405	Thr954, Lys336, Lys335, Tyr803, Lys802
18	P-Type ATPase-12178518	Lys342,Asp944, Ala925,Lys923,Asp	Gln344, Thr255, Ser924

		926, Thr348	
19	P-Type ATPase-25033784	Tyr302,Asn1089,Thr990	Ala991, Asp997, Lys1119, Ile291, Phe1113,Asp289, His286, Asn283, Gly994
20	P-Type ATPase-91932460	Asp997, Asp289	Pro996, Thr990, Ala991, Gly994, His286,Asn1089
21	P-TypeATPase-102378267	Ala925,Thr348,Gln254	Asp944, Pro256, Asp252, Gln344, Lys342,Lys93, Ser943

Name of 1*-3* ligands is given in table 4.1.2 as serial no 1-21.

Table 4.1.10 - Amino acids residues of plasmepsin2 involved in the Hydrogen bonding and hydrophobic interaction with Bulbine frutescens phytochemicals**.**

S. No	Protein-ligand PC	Hydrogen-bonding	Hydrophobic interaction
1	Plasmepsin2- 5320386	Ser218	Thr221, Ala219, Met15, Gly216, Ile32, Ser79, Asp34, Asp214, Gly36, Val178,Leu292, Ile300, Thr217, Ile290
2	Plasmepsin2- 1119009	Ser79	Val178, Met15, Ile14, Ser218, Thr114, Phe120, Ile32, Gly216, Ile123, Tyr77,Thr217
3	Plasmepsin2- 1172975	Ser79	Asp34, Val178, Thr217, Met15, Thr114, Ile123,Tyr77, Gly216
4	Plasmepsin2- 1217851	Ser79	Asp214, Ile300, Val178, Ile290, Ala219,Ser218, Thr217, Gly216

Table 4.1.11 - Amino acids residues of Human orotidine 5’ decarboxylase involved in the Hydrogen bonding and hydrophobic interaction with Bulbine frutescens phytochemicals

S.No	Protein-ligand	Hydrogen bonding	Hydrophobic interaction
1	Human orotidine 5 decarboxylase - 3*	Lys324, Thr321	Ile318, Asp317
2	Human orotidine 5 decarboxylase - 438991	Gly319, Asn320	Gly353, Gly349, Pro346, Ile318
3	Human orotidine 5 decarboxylase - 442731	Arg313,Gly329,Lys324,Gly328, Gln325,	Ile333, Phe331
4	Human orotidine 5 decarboxylase - 636652	Gln325, Thr321	Lys324, Asn320, Ile318
5	Human orotidine 5 decarboxylase -5315852	Ser348,Gly319,Glu356, Gly349	Lys352, Gly353, Asn320, Ile318, Gly347, Pro346
6	Human orotidine 5 decarboxylase - 5320386	Gly328	Gln325,Phe331,Gly329,Lys324
7	Human orotidine 5 decarboxylase - 1000844	Gln325, Gly328, Arg313, Lys324	Phe331,Gly329,Asp317,Thr321
8	Human orotidine 5 decarboxylase-12178518	Gln325, Thr321	Lys324, Ile318
9	Human orotidine 5 decarboxylas - 25033784	Thr321, Asp317, Gln325	Lys324,Gly329,Gly328, Phe331

Name of 3* ligands is given in table 4.1.2 as serial no 1-21.

Table 4.1.12 - Amino acids residues of HSP90 involved in the Hydrogen bonding and hydrophobic interaction with Bulbine frutescens**.**

S.No	Protein-ligand	Hydrogen bond	Hydrophobic interaction
1	HSP90 – 3*	Asp40,Asp98, Thr101,Phe124,Asp43	Leu15, Ile96, Ile12, Tyr125, Asn92, Y11122, Gly123, Leu93,Asn37, Lys44
2	HSP90 - 10208	Lys44, Thr171	Ala41, Asp40, Asn37, Phe124, Met84, Ile173, Leu93
3	HSP90 - 68111	Asn92, Asn37	Met84, Gly83,Ile82, Ala41, Asp79,

			Ile173 Leu34 ,Phe124
4	HSP90 - 438991	Thr171,Asn37, Phe124, Asp79	Met84, Lys44, Asn92, Arg98, Gly123, Tyr125, Leu93, Ile173, Ala41
5	HSP90 - 442731	Asn92,Phe124,Arg98, Thr101, Ile96,Tyr125	Phe104, Gly100, Gly121, Gly123, Leu15, Val122, Ie12, Leu96
6	HSP90 - 442753	Asp79,Thr171, Asn37, Phe124	Ile173, Gly123, Tyr125, Asn92, Leu93, Arg98, Lys414, Ala41, Met84
7	HSP90 - 636652	Thr171,Asp79, Asn37, Lys44	Met84, Ala41, Arg98, Asn92, Leu93, Gly123,Tyr125, Phe124, Ile173
8	HSP90 - 5315852	Thr171,Lys44, Asp79, Asn37, Leu34, Asn92	Al138, Met84, Asp40, Tyr125, Gly123, Thr101, Y1112, Phe124, Ile173, Leu93
9	HSP90 - 5320286	Gly121, Phe120	Gly123, Asn37, Phe124, Val122, Leu15, Thr101, Phe104, Arg98, Glu33
10	HSP90-10008440	Arg98, Ile96, Asn92, Thr171,Asn37,Val122, Thr101	Lys44, Leu93, Ile12,Asp79, Ile173, Gly123,Phe124, Tyr125, Leu15, Ala97, Ala41, Met24
11	HSP90-10072444	Gly121, Arg98, Glu33, Asn37, Asn92	Ile12,Thr101,Gly100, Phe124,Val122,Gly123Leu93, Tyr125
12	HSP90-10093576	Arg98, Asn37	Ala41, Phe124, Ile173, Arg98, Val122, Asn92,Tyr125,Gly123, Asp40, Thr171, Leu93
13	HSP90-11190093	Asn92, Thr171	Ala41, Lys44, Met84, Arg98, Tyr125, Phe124,Gly123, Leu93, Asn37, Ile173, Asp79
14	HSP90-11729754	Thr171, Phe124,	Met84, Lys44, Arg98, Leu93, Gly123, Tyr125,Asn92, Asn37, Asp79, Ala41
15	HSP90-12178518	Asp79,Thr171,Asn92, Phe124,Asn37	Met84, Lys44, Leu93, Arg98, Gly123, Ala41,Ile173
16	HSP90 - 2503378	Ile96, Thr101, Arg98, Phe124,Asn92,Tyr125	Ile12, Leu15, Ala97 Y1 1122, Gly121, Gly100Pbe104, Gly123, Leu93
17	HSP90-91932460	Asn37	Gly83, Met84, Ile82, Ala41, Thr171,

			Ile173,Asp79, Ala38, Phe124, Leu34
18	HSP90-102378267	Asp40,Asn37, Thr171, Asp79	Lys44, Arg98, Gly123, Asn92, Leu93, Phe124,Ile173, Leu34, Ala41, Met84

Name of 3* ligands is given in table 4.1.2 as serial no 1-21.

Table4.1.13 - Amino acids residues of Plasmodium orotidine 5' decarboxylase involved in the Hydrogen bonding and hydrophobic interaction with sesquiterpene lactone.

S no	Protein-Ligand PCID	Hydrogen bonding	Hydrophobic-interaction
1	Plasmodium orotidine 5 decarboxylase - 639694	Asn144, Lys151, Glu26	Asn148, Glu181, Asp179, 182, Arg150, Po43000, Lys27, Lys147, Asp25
2	Plasmodium orotidine 5 decarboxylase - 21672237	Glu26, Asn148, Lys147	Asp25,Asn144,Lys27,Glu181Lys182, Arg150, Glu155, Lys151
3	Plasmodium orotidine 5 decarboxylase -100987444	Asn148, Lys147	Arg150, Po43000, Glu181, Lys27, Asp25, Lys151
4	Plasmodium orotidine 5 decarboxylase - 6712627	Po43000, Glu181, Lys147	Glu155, Arg150, Lys182, Glu 26, Asn148, Asp25, Lys27, Asn144
5	Plasmodium orotidine 5 decarboxylase -98394739	Asn48, Lys147	Lys151, Glu155, Arg150, Asp179, Asp25
6	Plasmodium orotidine 5 decarboxylase -101923143	Arg150, Lys147, Lys27	Lys151, Glu155, Asp25, Glu26Asn144, Asn148
7	Plasmodium orotidine 5 decarboxylase -267247	Lys182, Glu181, Asn144	Glu155,Arg150,Lys151,Lys147, Asn148, Asp25,Phe107, Lys27,Asp179
8	Plasmodium orotidine 5 decarboxylase -98050854	Lys147, Asn144	Lys151, Glu155,Asn148, Phe107, Asp25
9	Plasmodium orotidine 5	Arg150,Po43000,Asn14	Lys151, Lys147, Glu26,

	decarboxylase -14543458	8	Asp25,Lys27
10	Plasmodium orotidine 5 decarboxylase -101650348	Asn144	Glu26, Asp25, Lys27, Asn148, Lys147, Lys151, Glu55, Arg150Asp179

Table 4.1.14 - Amino acids residues of Plasmepsin 2 involved in the Hydrogen bonding and hydrophobic interaction with sesquiterpene lactone

S.No	Protein-Ligand PCID	Hydrogen-bonding	Hydrophobic interaction
1	Plasmepsin 2 - 532099	Gly216,Ser218,Ser79	Thr217, Va178, Gly36, Asp34, Try192, Asp214, Tyr77,Ile123,Ile32,Thr114,Phe111,Phe120,Met15
2	Plasmepsin 2-6440697	Tyr192,Gly36, Asn76	Phe294,Asp34,Met75,Ser37,Tyr77Va178, Thr217, Ile300, Leu292
3	Plasmepsin 2-14779723	Gly36, Tyr192	Leu131, Asn76, Asp34, Tyr77, Ser79, Ile123, Ile123, Phe111, Gly216, Thr217, Asp214, Val178, Ile300, Ser
4	Plasmepsin2-14845494	Tyr192, Gly36, Ser79	Tyr77, Asp34, Gly216, Ile123,Phe120, Ile300, Val1798, Leu292,Phe 294, Asp214,Ile32,Thr217
5	Plasmepsin2-44400048	Thr217, Ser79	Gly216, Phe111, Tyr77, Thr114, Phe120, Ile123, Gly36, Ile32, Tyr 192,Asp214,Ile300,Asp34,Val178Ile290, Ala219, Ser218
6	Plasmepsin2-52943584	Thr217,Gly216, Ser79	Ile300, Va178, Ile290, Ala219, Phe120, Tyr77,

			Ile32, Ser218, Ile 123, Thr114, Asp214, Tyr192,Asp 34, Gly36
7	Plasmepsin2-101667702	Ser79, Gly216	Thr217, Gly36, Asp34, Ile300,Tyr192,Asp214,Va178, Tyr77, Ile123Ile32, Met15
8	Plasmepsin2-101667704	Ser79, Thr217	Va178, Tyr77, Asp34, Ile32,Ile123,Gly216,Phe120, Met15, Phe111,Thr114, Ser218, Ile290, Ile300, Asp14
9	Plasmepsin2-102276755	Gly216,Ser79,Thr217	Ile32, Met15, Ser218, Leu292,Phe294, Ile300, Asp293, Va178, Tyr192, Gly36, Tyr77, Asp34, Phe120Ile123
10	Plasmepsin2-125121058	Ser79, Thr217	Leu131, Tyr192, Ile212, Ile300,Asp293, Phe294, Leu292, Va178Gly216, Asp34, Tyr77, Asp214, Gly36, Ser37

Table 4.1.15 - Amino acids residues of Human orotidine 5' decarboxylase involved in the Hydrogen bonding and hydrophobic interaction with sesquiterpene lactone

S. No	**Protein-Ligand**	**Hydrogen bonding**	**Hydrophobic interaction**
1	Human orotidine 5' decarboxylase - 483220	Phe331	Arg313, Gln325, Lys324, Gly328,Gly329, Ile330
2	Human orotidine 5' decarboxylase -176588	-	Lys324, Thr321, Gly329, Gly325
3	Human orotidine 5' decarboxylase - 639694	Gln325	Arg313, Gly328, Gly329, Lys324,Thr321

4	Human orotidine 5' decarboxylase-11552280	Ile330	Asp285, Arg313, Gly329, Gln325,Gly328, Lys324, Phe331
5	Human orotidine 5' decarboxylase-44575369	Asn320, Gly349, Gly319	Pro346, Lys352, Gly353, Glu356Ile318
6	Human orotidine 5' decarboxylase-101320302		Gly328, Gly329, Lys324, Phe331, Ile330, Gln325
7	Human orotidine 5' decarboxylase-101705724	Gln325, Lys324	Gly329, Gly328
8	Human orotidine 5' decarboxylase-102057347	Glu356, Gly349, Gly319	Lys352, Asn320, Ile318, Pro346
9	Human orotidine 5' decarboxylase-102242189	Thr321	Lys324,Glu325, Asp317,Ile318

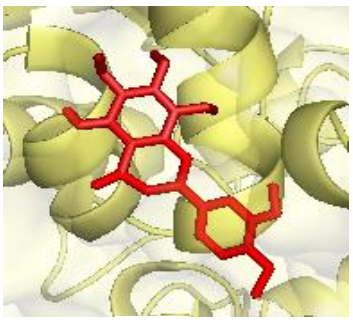
Table 4.1.16 - Amino acids residues of HSP90 involved in the Hydrogen bonding and hydrophobic interaction with sesquiterpene lactone.

S no	Protein-ligand	Hydrogen bonding	Hydrophobic interaction
1	HSP 90-102276267	Arg98, Lys44	Y11136,Ile173,Met84,Leu93, Asn37,Tyr125,Pbe124, Leu15,,Thr101,Gly123, Pbe104, Gly100, Gly121, Y1 1122, Al141,Asp40, Asn93
2	HSP90 -102276755	Lys44,Asn37,Arg98, Pbe124	Asp40,Thr101,Pbe104, Leu15,Pbe104,Leu15, Gly21,Y11122,Gly123, Pbe120,Asn93Leu93, Ile173,Tbr171,Asp79Al141,Met84
3	HSP 90 - 67976473	Arg98, Asn92	Asn37, Lys44, Ala41, Leu93 Tyr125, Phe104, Gly123,Gly 121, Thr101, Phe120, Gly100, Va1122, Phe124

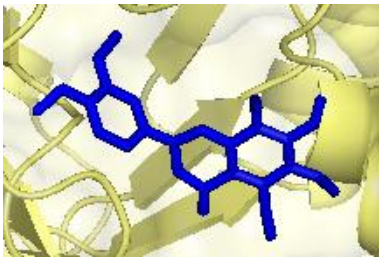
4	Hsp90 - 11429323	Asp79,Lys44,Phe124, Asn37, Asn92	Gly123, Arg98, Ala41, Leu93 Ala38, Ile173, Leu34, Tyr125
5	Hsp90 - 483222	Lys44,Thr171,Phe124, Asn37	Leu93, Gly123, Arg98, Asn92, Thr101, Ile173, Tyr125, Asp 79, Gly83, Met84
6	Hsp90 - 101631678	Phe124, Lys44	Leu93, Asn37, Ile173, Asp79, Thr171, Ala41, Ile82, Met84 Gly23, Arg98
7	Hsp90 - 483221	Lys44,Tyr125,Arg98,Asn 92	Leu93, Met84, Phe124, Ile12, Asn37

4.3 Surface structure of the protein and ligand interaction

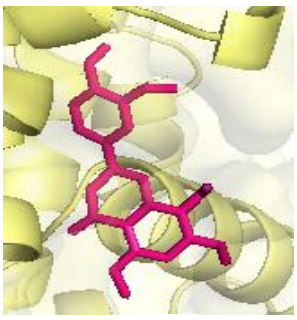
The binding of the modified ligands with active sites of the targeted proteins. The interaction between the protein and ligand showed the position of the orientation of a ligand when it is bound to the proteins. The surface structure of the protein and ligand determined through the Pymol software after the modified pdbqt files of protein and ligand prepared. The surface structure showed the interaction between the protein and ligands. The best binding affinity of ligands showed the better interaction with targeted proteins. The binding affinity was found in the range of (-6.0kcal/mol) to (-10.7kcal/mol). The surface structure of the targeted proteins and best lead compounds as shown in figure (4.3.1) to (4.3.13).



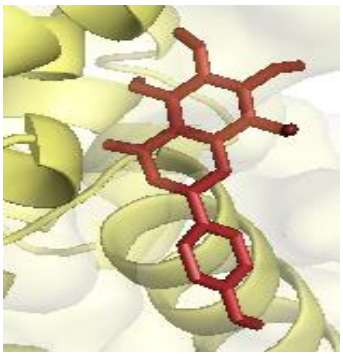
1



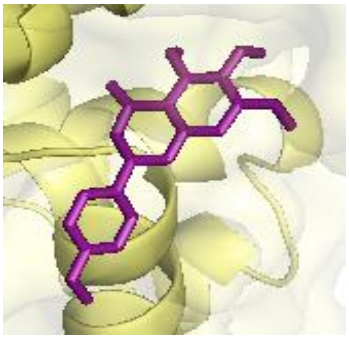
2



3

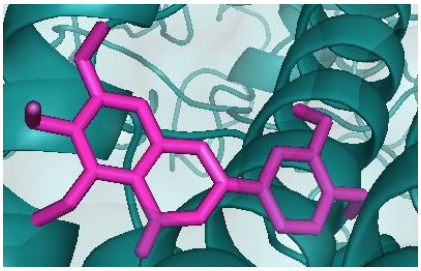


4



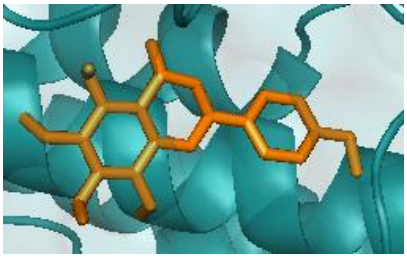
5

Figure 4.3.1: Surface structure of the lead methylated flavonoids ligand docked against plasmodium omp decarboxylase (1) ; (2S)-2-(3,4-dimethoxyphenyl)-5-hydroxy-6,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(2);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7,8 tetramethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(3);** (2S)-2-(3,4-dimethoxyphenyl)-5,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(4);** (2S)-5,6,7,8-tetramethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(5);** (2S)-5,6,7-trimethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one.

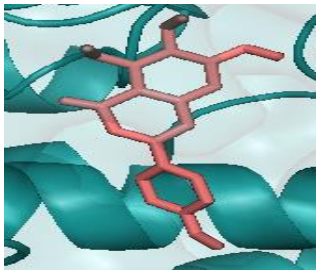


1

2



3



4

Figure 4.3.2: Surface structure of the lead methylated flavonoids docked against ATPase. (1); (2S)-2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-3,4-dihydro-2H-1-

benzopyran-4-one**, (2) ;** (2S)-2-(3,4-dimethoxyphenyl)-5,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(3);** (2S)-5,6,7,8-tetramethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(4);** (2S)-5,6,7-trimethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one.

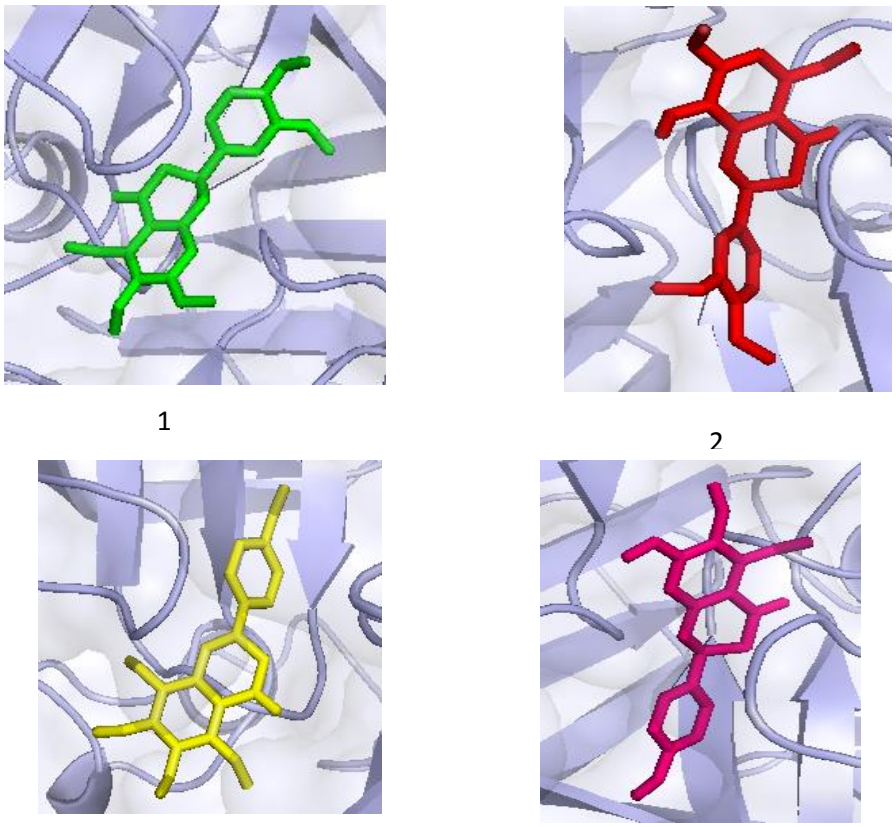
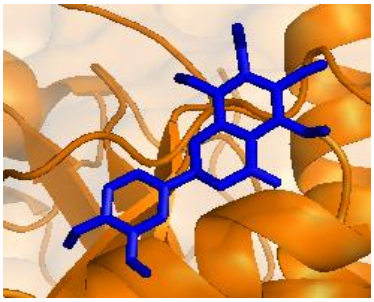
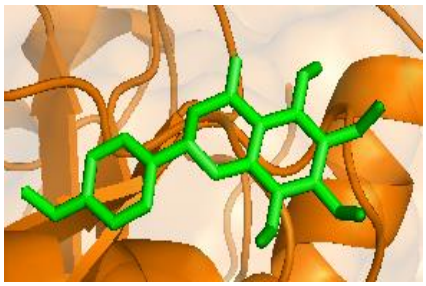


Figure 4.3.3: Surface structure of the lead methylated flavonoids docked against plasmepsin 2. (1); (2S)-2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(2);** (2S)-2-(3,4-dimethoxyphenyl)-5,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(3);** (2S)-5,6,7,8-tetramethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one; **(4);** (2S)-5,6,7-trimethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one.

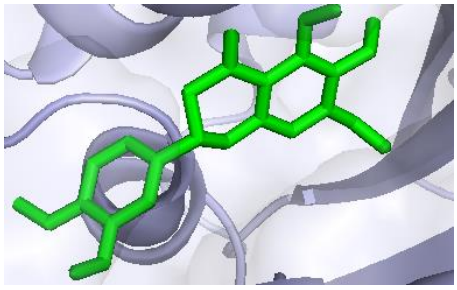


1

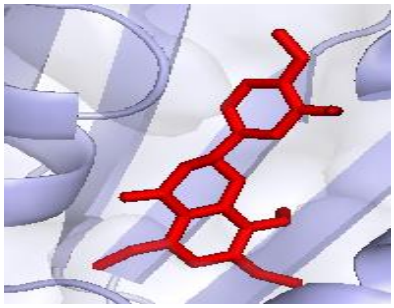


2

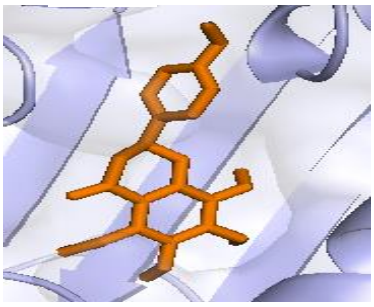
Figure 4.3.4: Surface structure of lead methylated flavonoids docked against human omp decarboxylase. **(1);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(2);** (2S)-5,6,7,8-tetramethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one.



1



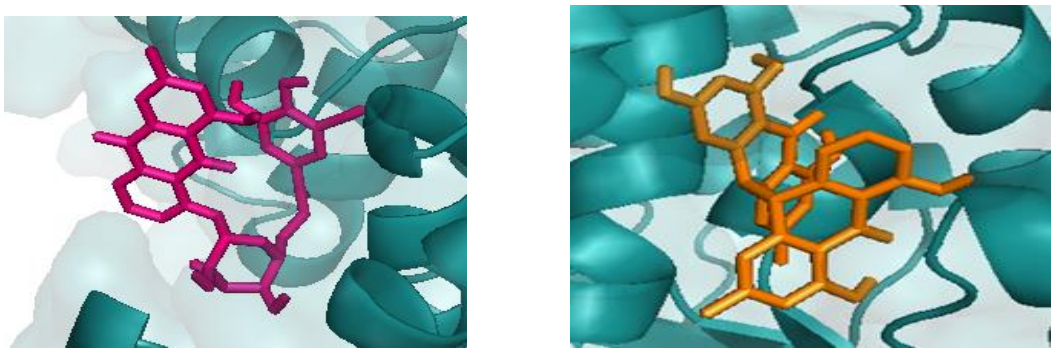
2



3

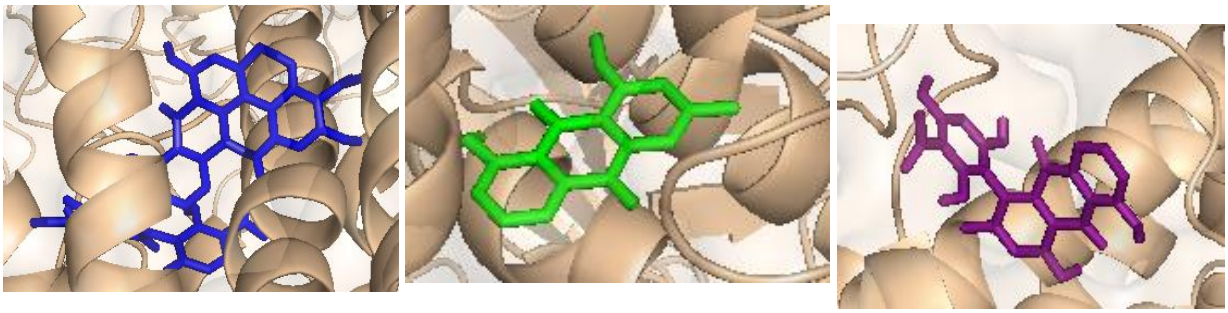
4

Figure 4.3.5: Surface structure of the methylated flavonoids docked against HSP90. **(1);** (2S)-2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(2);** (2S)-2-(3,4-dimethoxyphenyl)-5,7,8-trimethoxy-3,4-dihydro-2H-1-benzopyran-4-one, **(3);** (2S)-5,6,7,8-tetramethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one, **(4);** (2S)-5,6,7-trimethoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one.

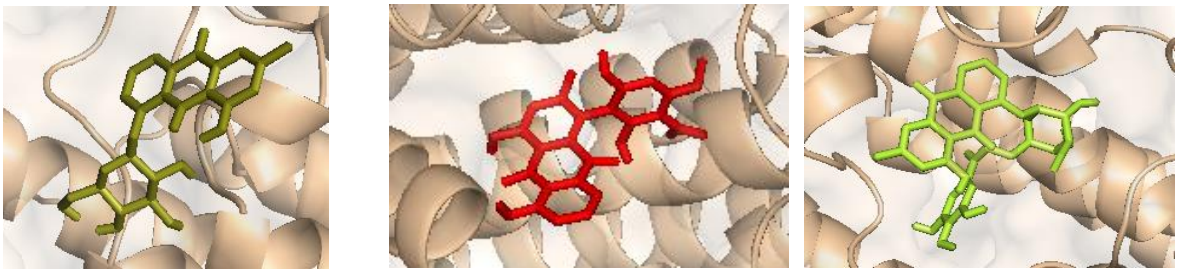


1 2

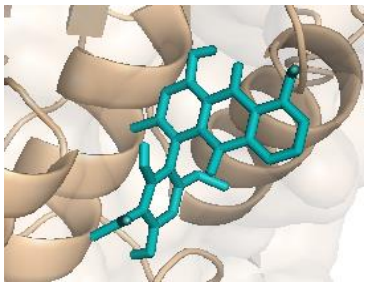
Figure 4.3.6: Surface structure of lead Bulbine phytochemicals docked against plasmodium 5' decarboxylase. **(1)** PubChem CID:5315852; **(2)** PubChem CID:5320386



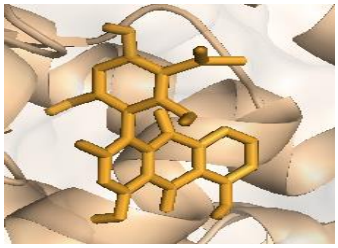
1 2 3



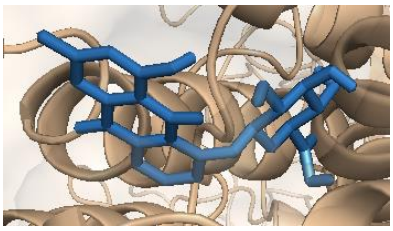
4 5 6



7

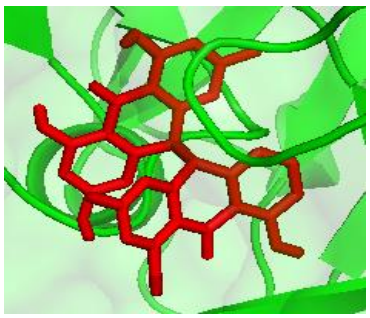


8



9

Figure 4.3.7: Surface structure of the lead Bulbine phytochemicals docked against ATPase. (1) PubChem CID: ; **(2)** PubChem CID:10208 ; **(3)** PubChem CID:438991;**(4)**PubChem CID: 442731; **(5)** PubChem CID:442753; **(6)** PubChem CID:5315852; **(7)** PubChem CID:11729754; **(8)** PubChem CID:12178518; **(9)** PubChem CID:25033784.



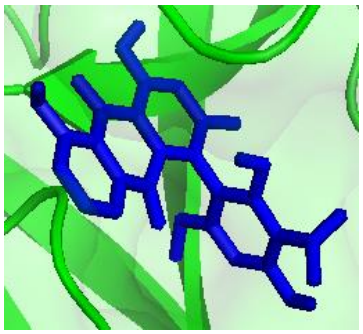


Figure 4.3.8: S 1 **ce structure of the lead Bulbir** 2 **hytochemicals docked against plasmepsin 2. Ligand as, (1)** PubChem CID:5320386; **(2)** PubChem CID:11729754.

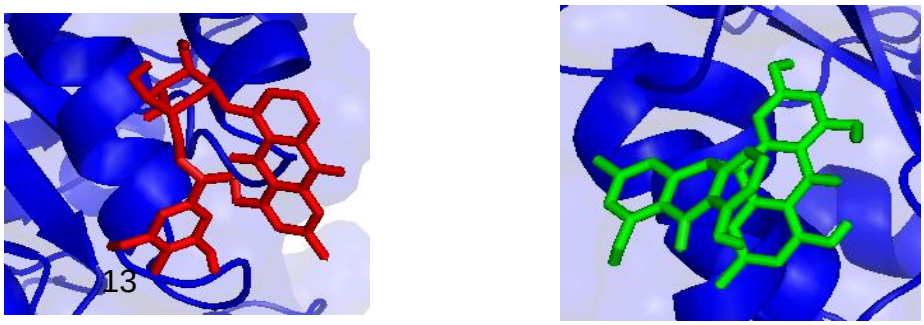


Figure 4.3.9: Sur[1] structure of the lead Bulbin[2] ytochemicals docked against human orotidine 5' decarboxylase. **(1)** PubChem CID:5315852; **(2)** PubChem CID:5320386.

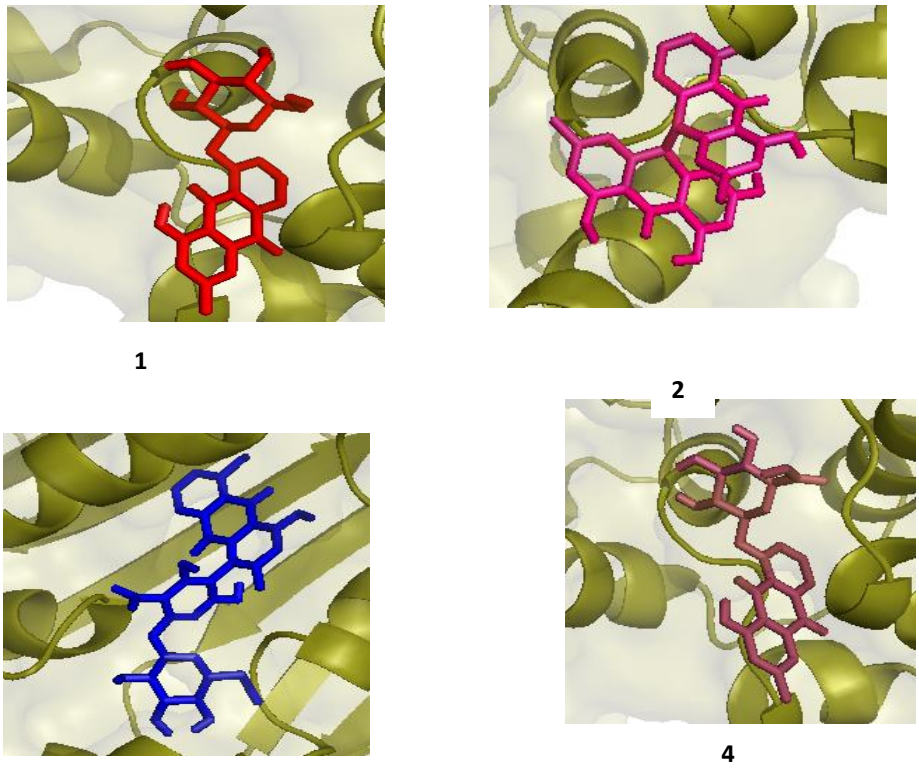
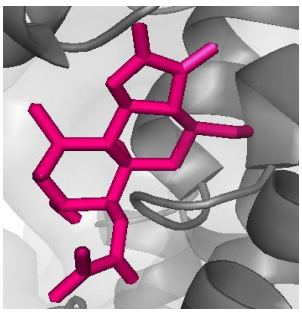
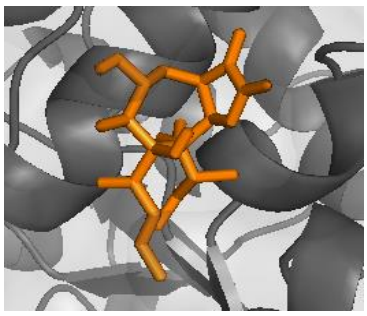


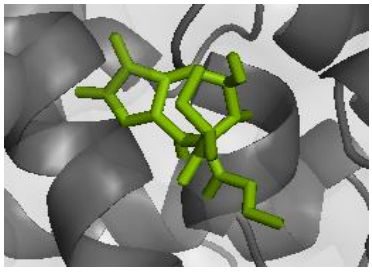
Figure 4.3.10:-[3] face structure of the lead Bulbine phytochemicals docked against HSP90. **(1)** PubChem CID:442731; **(2)** PubChem CID:5320386; **(3)** PubChem CID:10008440; **(4)** PubChem CID:25033784.



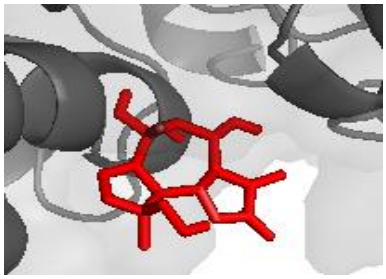
1



2

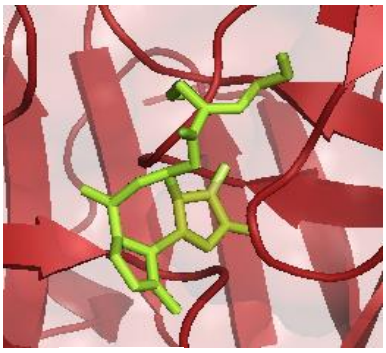


3

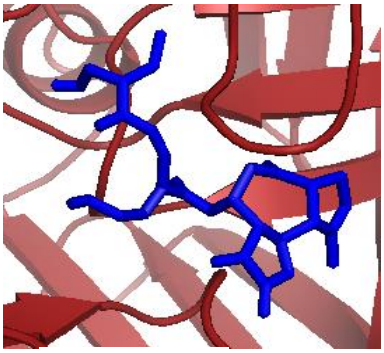


4

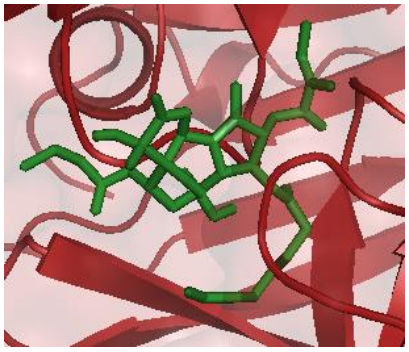
Figure 4.3.11: Surface structure of the lead sesquiterpene lactones docked against plasmodium orotidine 5' decarboxylase. **(1)** PubChem CID: 639694; **(2)** PubChem CID: 6712627; **(3)** PubChem CID: 21672237; **(4)** PubChem CID: 100987444.



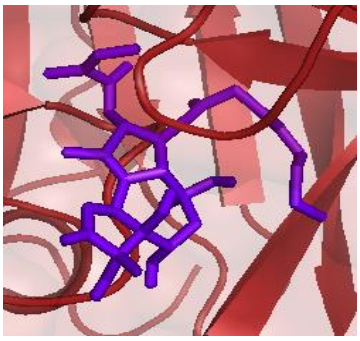
1



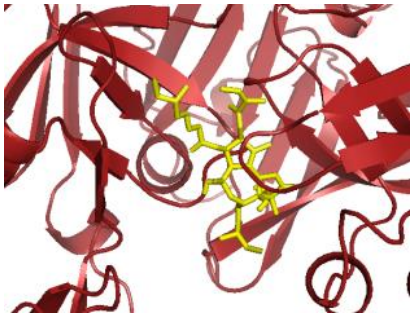
2



3

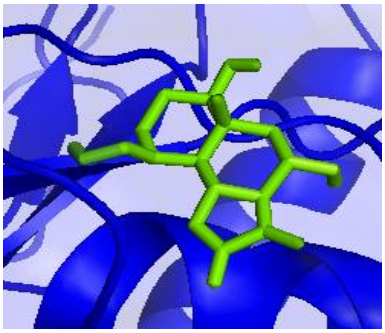


4

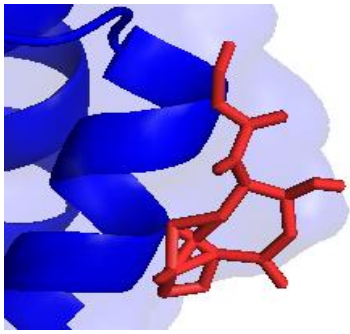


5

Figure 4.3.12: Surface structure of the lead sesquiterpene lactones docked against plasmepsin 2., **(1)** PubChem CID: 6440697; **(2)** PubChem CID: 14779723; **(3)** PubChem CID: 4440048; **(4)** PubChem CID: 101667704; **(5)** PubChem CID: 102276755.

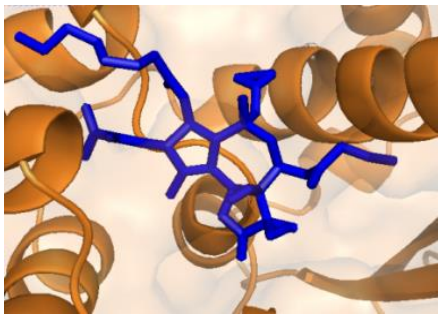


1

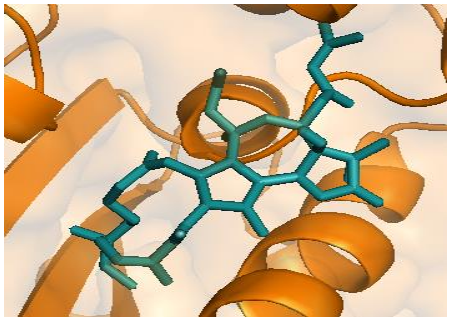


2

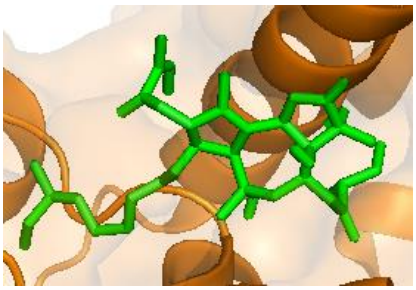
Fig 4.3.13: Surface structure of the lead sesquiterpene lactones docked against Human orotidine 5' decarboxylase. **(1)** PubChem CID: 483220; **(2)** PubChem CID: 1022421.



1



2



3

Figure 4.3.14: Surface structure of the lead sesquiterpene lactones docked against HSP90, (1) PubChem CID: 67976473**; (2)** PubChem CID: 102276267; **(3)** PubChem CID: 102276755

Results of laboratory techniques

4.4 *In Vitro* anticancer activity (MTT assay)

To study the antiproliferative activity of 002, we seeded 10000 cells per well in 96 well plate in three in number, Incubated for 24hrs at 37^{o}C after the cells adhere on a plate for 24hrs treated with the drug at different concentration range. The cells were incubated for 24 hrs, 48 hrs, 78hrs time intervals for identification of IC50 at the respective intervals. Afer incubation removes media and adds 100ul of MTT 5 mg/ml. Incubate the cells for 4 hrs and remove MTT and add DMSO of 100ul in each well. Incubate for 5 minutes In a dark room and read the plate for absorption at 590nm using a spectrophotometer.

To assess the antiproliferative activity of compound 002, against colon adenocarcinoma cell lines (HT-29) MTT assay was used and the result was shown in figure 4.4.1. The result showed that 002 exhibited moderate anticancer efficacy at test concentration (100nM- 50uM). About 50% growth inhibition activity was observed at higher test concentration (05.uM) against colon cancer cell line. The log(x) values were plotted in terms of molar concentration. The IC$_{50}$ values can be determined from the graph which distinctively shows the percentage inhibition with respect to the concentration.

.

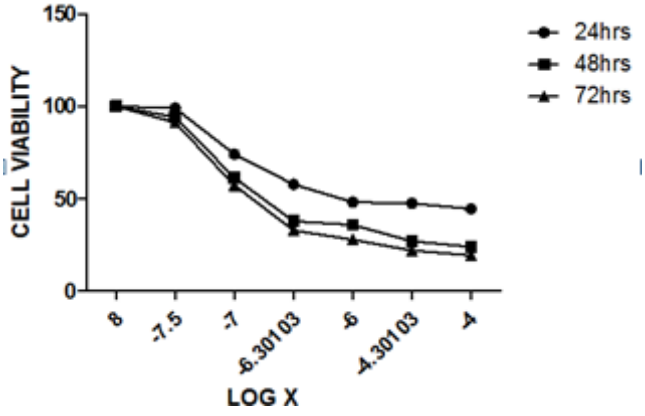


Fig 4.4.1-Graphical representation of compound 002 treatment on MTT assay in log(x) v/s% viability of cells

4.5 DPPH radical scavenging activity

The antioxidant activity of 00a was measured at different concentration (1,2,5,10,20,30 µl/ml) by using DPPH assay and compared with standard antioxidant compound BHA (30 µl/ml). The result showed that the antioxidant activity increased with increasing concentration of the sample (Figure 4.5.1). The radical scavenging activity of 002 was found to be 75-80% at different concentration (1-20 µl/ml). It was found at higher concentration.

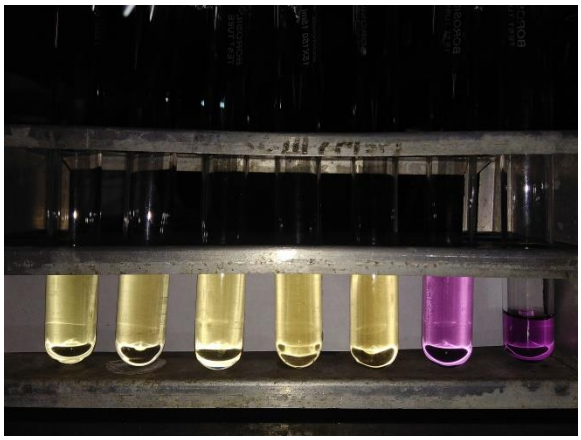


Figure 4.5.1: Free radical scavenging activity of 002 using DPPH assay

4.6 Disc diffusion method (Antibacterial activity)

Antimicrobial activity was determined by using Kirby-Bauer disc diffusion method against Gram-negative *Escherichia coli*. The LB agar plates containing *Escherichia coli* bacterial strains were retained and the paper disks saturated with the phytochemicals were placed in the media. After 24hour incubation, the zone of

inhibition (ZOI) was observed in the plates with standard antibiotic disks of penicillin (10 µg/disc) and norfloxacin (10 µg/disc). However, the efficient zone of inhibition could not be seen in the agar plates with the phytochemicals (Bauer et al.,1966). The figures 4.6.1 (a) and (b) show a zone of inhibition in the bacterial colonies with disks of penicillin and norfloxacin respectively. Norfloxacin showed a greater zone of inhibition than penicillin. There was no ZOI observed in the control i.e. DMSO as shown in the figure 4.6.1 (c).

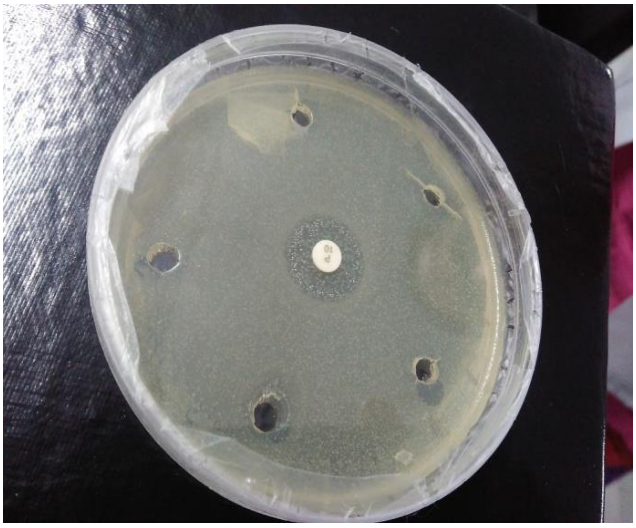


Figure 4.6.1- (a) culture containing

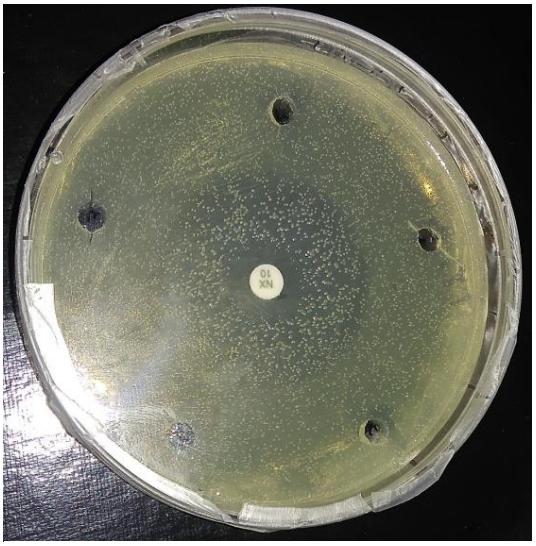


Fig 4.6.1-(b) culture containing Norfloxacin

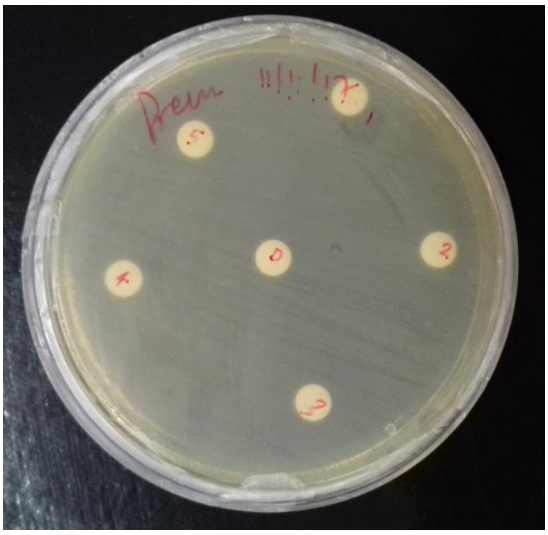


Fig 4.6.1- (c) Culture containing DMSO and test compound at different concentration.

4.6 DISCUSSION

Different proteins are involved in malaria which changes the microenvironment of the parasite. These proteins play a significant role in homeostasis maintenance and some vital process such as proliferation, differentiation, and apoptosis in malarial parasite. OMP decarboxylase, Pf ATPase6, plasmepsin2, and HSp90 are the targeted proteins in the malarial life cycle, which are involved in drug resistance. Our study explores potential phytoconstituents that inhibit various proteins in malarial parasite.

Two aspartic proteases, Plasmepsin 1 and Plasmepsin 2 are encoded by a malarial parasite in Plasmepsin 2, which helps in hemoglobin degradation and novel target for an antimalarial drug. From previous data, a human erythrocyte is invaded by a malarial parasite in the asexual stage of infection. When it enters in erythrocyte, parasite uses human hemoglobin as a food source and digesting it with the help of series of protease. Plasmepsin is also called as aspartic protease, which has an important role in hemoglobin degradation, so it is targeted as antimalarial drug development. In vitro, it is shown that antimalarial activity against *Plasmodium falciparum* exhibited by myricetin and fisetin. Cysteine protease and aspartic protease of plasmepsin considered as an important antimalarial drug target (Huangtao et al 2014).

In the present study, we found that phytochemical sesquiterpene lactone has potential in silico inhibitory activity against Plasmepsin 2 with docking score (-9.4), in comparison to standard inhibitor RS370, sesquiterpene showed 101 % of binding activity.

We investigated the role of iron and artemisinin on PfATP6, in search of a plausible mechanism of action, via density functional theory calculations, docking, and molecular dynamics simulations. Results show that artemisinin is activated by iron which is inhibited by *Plasmodium falciparum* ATPase by closing phosphorylation. Previous study chloroquine and pyrimethamine-sulfadoxine (SP), are a serious

hindrance to the control of malaria. So now it is used for clinical care and for the avoidance of drug resistance (Yeung et al 2004)

Endoperoxide bridge of artemisinin is left by intraparasitic heme, now this cleaved endoperoxide bridge transform to carbon centered free radical, which act as alkylating agent and this react with both heme and parasite protein. A previous study with *P. falciparum* suggested that a sarcoplasmic and endoplasmic reticulum Ca^{2+} ATPase(SERCA)-type protein encoded by a gene denoted *pfatp6* might be the major chemotherapeutic target of these drugs (Eckstein-Ludwig et al2003).

In the present study, we found that phytochemical flavonoids have potential in silico inhibitory activity against PATPase6 with docking score (-7.3), in comparison to standard inhibitor Artemisinin, flavonoid showed 128 % of binding activity.

HSP90 involved in growth and development of malarial parasite, regulating its sexual development in human erythrocyte, and have a crucial role in regulating ring to trophozoite stage transition in the parasite. It shows highest ATPase activity than that of human HSP90. The previous report HSP90 inhibitorsSNX-0723 in combination inhibitor PIK inhibitor synergistically reduce the liver stage parasite load (Tarun *et al* 2008). Identified an amino alcohol carbazole as a pfHSP90 selective inhibitor by virtually docking a large set of antimalarial compounds, previously found in phenotype screen into a pfHSP90 specific pocket. (Barta, et al 2011).

In the present study we found that phytochemical flavonoids have potential in silico inhibitory activity against HSP90 with docking score (-6.9, in comparison to standard inhibitor Tanespimycin, flavonoid showed 133 % of binding activity.

In OMP decarboxylase malarial parasite exhibit rapid nucleic acid synthesis during their intraerythrocytic growth phase. In Plasmodium purine and pyrimidine metabolic pathway are distinct from those of their human host. Thus targeting purine and pyrimidine metabolic pathway, for novel drug development. The previous report showed that TDHO, atovaquone, pyrazafurin arrest growth of *Plasmodium falciparum*, an only moderate decrease in UTP, CTP, TTP were observed 5 fluoroacetates also arrest the growth of *Plasmodium falciparum* with major of

fluorouridine mono, di, triphosphate and most significant inhibition of denovo biosynthesis of pyrimidine nucleotide (Kristen et al 1994).

In the present study, we found that phytochemical sesquiterpene lactone has potential in silico inhibitory activity against omp decarboxylase with docking score (-6.9), in comparison to standard inhibitor XMP, flavonoid showed 142 % of binding activity.

5 • CONCLUSION

Conclusion

Plants product are available to have their importance in Indian medicinal systems. These plant products have been reported by a various traditional system of medicine for treatment of the various disorders. The present studied showed that the phytoconstituents present in the natural product have targeted in various stages in the malarial life cycle. The data predicted from the present study shows the protein-ligand interaction property of phytochemicals. Research is going on for the progress of natural anti-malarial drugs.

List of publication

swagata Das, Prareeta Mahapatra, Priyanka Kumari. Prem Prakash Kushwaha, and Shashank Kumar. (2018) Phytochemicals as Hope for the Treatment of Hepatic and Neuronal Disorders Phytochemistry, In Volume 2: Pharmacognosy, Nanomedicine, and Phytochemicals as foes, Eds: Egbuna Chukwuebuka, Shashank Kumar, Ifemeje Jonathan Chinenye, Jaya Vikas Kurhekar, CRC press, USA.(Communicated).

References

Barta, T. E.; Veal, J. M.; Rice, J. W.; Partridge, J. M.; Fadden, R. P.; Ma, W.; Jenks, M.; Geng, L.; Hanson, G. J.; Huang, K. H.; Barabasz, A. F.; Foley, B. E.; Otto, J.; Hall, (2008).S. E. Discovery of benzamide tetrahydro-4H-carbazol-4-ones as novel small molecule inhibitors of Hsp90. *Bioorganic Medicinal Chemistry Letters*. **18**:517−3521.

Dallakyan, S. & Olson, A.J., (2015).Small-molecule library screening by docking with PyRx. *Chemical Biology: Methods and Protocols*.***126*:** 243-250.

Eckstein-Ludwig, U., R. J. Webb, I. D. Van Goethem, J. M. East, A. G. Lee, M. Kimura, P. M. O'Neill, P. G. Bray, S. A. Ward, and S. Krishna. (2003). Artemisinins target the SERCA of *Plasmodium falciparum*. *Nature*. **424**:957-961.

Huangtao Jin a, Zhongyu Xu a, Kunqiang Cui a, Tianshu Zhang a, Weiqiang Lu b, ⁎, Jin Huang a, (2014) Dietary flavonoids fisetin and myricetin: dual inhibitors of Plasmodium falciparum falciparum-2 and plasmepsin II**.** *Fitoterapia* **94**:55-61

Kong DX, Li XJ, & Zhang HY: (2009).Where is the hope for drug discovery? Let history tell the future. *Drug Discovery Today*. **14**: 115-119.

Kumar, S.; Chashoo, G.; Saxena, A.K.; Pandey, A.K.(2013). *Parthenium hysterophorus*: a probable source of anticancer, antioxidant and anti-HIV agents. *BioMed Research International*. **2013**:810734-45.

Kumar, S.; Kumar, R.; Dwivedi, A.; Pandey, A.K.(2014) *In vitro* antioxidant, antibacterial, and cytotoxic activity and *in vivo* effect of *Syngonium podophyllum* and *Eichhornia crassipes* leaf extracts on isoniazid-induced oxidative stress and hepatic marker.s *Biomed Research International.* **2014**:459452-63.

Kristen K. Seymour, Stephen D. Lyons, Leonidas Phillips, Karl H. Rieckmann,t & Richard I. Christopherson,(I994). Cytotoxic Effects of Inhibitors of de Novo Pyrimidine Biosynthesis upon *Plasmodium falciparum*. *American Chemical Society* Vol. **33:** 5268-5274.

Lahlou, M. (2013). The success of natural products in drug discovery. *Pharmacol Pharma*, **4**: 17-31.

Liu KCC, Yang S, Roberts MF, Elford BC,& Phillipson JD.(1992). Antimalarial activity of *Artemisia annua*flavonoids from whole plants and cell cultures. *Plant Cell Reports*. **11**, 637-640.

Moon, R. P., D. Bur, H. Loetscher, A. D'Arcy, L. Tyas, C. Oefner, F. Gruenninger-Leitch, D. Mona, K. Rupp, A. Dorn, H. Matile, U. Certa, C. Berry, J. Kay, and R. G. Ridley. (1998). Studies on plasmepsin I and II from the malarial parasite Plasmodium falciparum and their exploitation as drug targets. Advance in. *Experimental. Medicine and Biology*. **436**:397–406.

Newman DJ, Cragg GM. (2007). Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products*.**70**, 461-477.

Nichols, S. E.; Baron, R.; Ivetac, A.; McCammon, J. A.(2011) Predictive power of molecular dynamics receptor structures in virtual screening. *Journal of Chemical. Information and Modeling.* **51**:1439−1446.

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011) Open Babel: An open chemical toolbox. *Journal of Cheminformatics*.**3:**33.

Patwardhan B. (2005), Ethnopharmacology and drug discovery. *Journal of Ethnopharmacology*. **100**: 50-52.

Phillipson JD, O'Neill MJ. Antimalarial and amoebicidal natural products. In: Hostettmann K, Lea PJ,(1987). Biologically Active Natural Products. *Oxford: Clarendon Press,* **31**: 49–64.

Rosenthal, P. J. (1998). Proteases of malaria parasites: new targets for chemotherapy. *Emerging. Infectious Disease*. **4**:49–57.

Sebisubi, F.M.; Tan, G.T.(2011) Natural products with antimalarial activity. Journal of Pharmacognosy and Phytochemistry,**1**:50-52.

Steck EA. (1972). *The Chemotherapy of Protozoan Diseases*. Division of Medicinal Chemistry, Walter Reed Army Institute Research,Washington..

Tyas, L. R., P. Moon, H. Loetscher, B. M. Dunn, J. Kay, R. G. Ridley, and C. Berry. (1998). Plasmepsin I and II from the malarial parasite Plasmodium falciparum. Advance in. *Experimental Medicine and Biology*. **436**:407–411.

Tarun AS, Peng X, Dumpit RF, Ogata Y, Silva-Rivera H, Camargo N, Daly TM, 600 Bergman LW, Kappe SH.(2008). A combined transcriptome and proteome survey of 601 malaria parasite liver stages. *Proceeding of the National Academy of Science United States of America.* **105**:305-310.

W. Bauer, W. M. Kirby, J. C. Sherris, and M. Turck, (1966). “Antibiotic susceptibility testing by a standardized single disk method,” *The American Journal of Clinical Pathology*, **45**:493–496.

Warhurst DC.(1985) New drugs and their potential use against drug-resistant malaria. Ann Ist Super Sanita.**21**:327–336.

Yeung, S., W. Pongtavornpinyo, I. M. Hastings, A. J. Mills, and N. J. White**.** (2004). Antimalarial drug resistance, artemisinin-based combination therapy, and the contribution of modeling to elucidating policy choices*. American Journal of Tropical. Medicine and. Hygiene*. **71**:179-186.