IN SILICO DOCKING STUDIES OF BIOACTIVE NATURAL PRODUCTS AS PUTATIVE *Pf*-DHFR ANTAGONISTS

A DISSERTATION

Submitted in partial fulfillment of the requirements for the award of the degree of

MASTER OF TECHNOLOGY

in

ADVANCED CHEMICAL ANALYSIS

By

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CANDIDATE'S DECLARATION

I hereby declare that the work presented in this project report entitled **"In silico docking studies of bioactive natural products as putative Pf-DHFR antagonists"** is an authentic record of work carried out by me during the period August 2011 to April 2012 at "Indian Institute of Technology, Roorkee" under the supervision of **Dr.Anuj Sharma**, Assistant professor, Chemistry Department, IIT Roorkee. This is being submitted for the partial fulfillment for the award of Master's Degree in Technology (Advance Chemical Analysis) of Indian Institute of Technology, Roorkee (Uttaranchal). This has not been submitted by me anywhere else for the award of any other degree/diploma.

Place: Roorkee **(ANURADHA)**

Date: 13.06.12 Anuradha

CERTIFICATE

This is to certify that the above declaration made by the candidate is correct to the best of my knowledge and belief.

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Place : Roorkee

Date: 13.06.2012

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(ANURADHA)

ABSTRACT

Malaria is one of the serious hurdles to public health with about one million deaths annually. Present chemotherapies are inadequate because of the genesis and emergence of new drug resistance strains. So there is a urgent need to recognize new inhibitors against known or novel targets. Herein we are presenting docking studies of bioactive natural plant products against Pf-DHFR. For this purpose we have built a library of 205 active molecules of plant origin having some folklore history as being used as antimalarial. Three docking engines AutoDock 4.2, MVD 5.0 (Molegro Virtual Docker) and iGEMDOCK 2.1 were used for Screening. AutoDock and iGEMDOCK runs resulted in the energy scores from -4.6 to -12.07 Kcal/mol and -63 to -156.2 kcal/mol respectively. Our analysis shows Ochrolifuanine and Chrobisiamine as most promising hit with K_i in nanomolar range. We also focused on the common structure features of ligand and the residues of protein which remain almost conserved throughout the analysis. We hoped this work would aid in antimalarial combat strategies and can serve to provide new therapeuticals in a rapid manner.

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ABBREVIATIONS

1.	ADT	AutoDock Tools
2.	AM1	Austin Model 1
3.	DHFR	Dihydrofolate reductase
4.	DTMP	Deoxythymidine monophosphate
5.	DUMP	Deoxyuridine-monophosphate
6.	GA	Genetic Algorithm
7.	LGA	Lamarckian Genetic Algorithm
8.	MINDO	Modified Intermediate Neglect of Differential Overlap
9.	MM	Molecular Mechanics
10.	MNDO	Modified Neglect of Differential Overlap
11.	MOPAC	Molecular Orbital Package
12.	MVD	Molegro Virtual Docker
13.	NADPH	Nicotinamide Adenine Dinucleotide Phosphate
14.	PDB	Protein Data Bank
15.	PM3	Parameterized Model series-3
16.	QSAR	Quantitative structure–activity relationship
17.	RMSD	Root Mean Square Deviation
18.	SPDBV	Swiss PDB Viewer
19.	VS	Virtual Screening

CHAPTER 1

INTRODUCTION

Diseases have been treated with medications for thousands of years. The effects of these drugs were usually discovered over centuries by trial and errors. Many drugs used now a day have been discovered by such observations. However as the cellular and molecular mechanism behind many diseases is increasingly understood, new avenue for rational drug development emerges and a systematic search for drugs began. Overtime, newly development techniques and an ever increasing knowledge led to new, but complementary, strategies for drug discovery.

1.1 Malaria: A global challenge

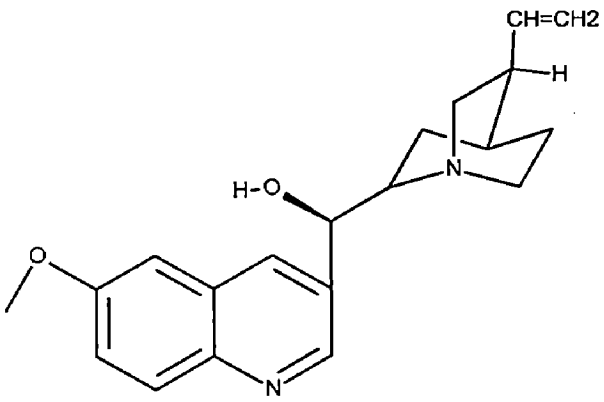
Malaria is a transmissible disease, caused by a unicellular parasite Plasmodium. There are five malarial parasites- *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, Plasmodium knowlesi. P. falciparum* is the most dangerous parasite with the highest morbidity rates, and generally is the only species that may cause death in humans [1]. The *malaria parasite* depends on both humans and mosquitoes to carry out its deadly life cycle. These parasites are transmitted from one person to another via the bite of female anopheles mosquitoes in the human body. *Plasmodium* develops in the gut of the mosquito and is passed on in the saliva of an infected insect each time it takes a new blood meal. When an infected mosquito bites a human, the parasite rapidly goes to the liver within 30 minutes. There the parasite starts reproducing rapidly in the liver and then enter into red blood cells, multiply and spread in the host's blood. The malaria parasite spends part of its life cycle in red blood cells, feeds on their hemoglobin and then breaks them apart and thus Destruction of R.B.Cs leads to fever, flu- like symptoms, such as fever, vomiting, headache, tiredness and nausea etc [3]. Malaria is complex but it is a curable and preventable disease. Lives can be saved if the disease is detected early and adequately treated. The World Malaria Report 2011 summarizes that in 2010 more than 216 million cases of malaria were reported, and about 6, 55,000 people died from this disease. Most of cases came from African and

Asian region. Malaria is the fifth leading cause of death worldwide and almost half the world's (3.3 million) population is at risk [2].

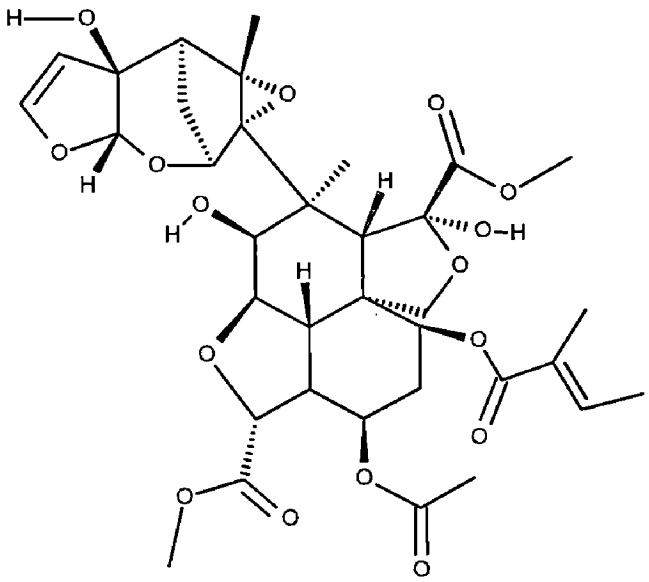
Irrespective of a large range of synthetic antimalarial drugs, the world is facing problems in the complete eradication of malaria. One of the main causes for the comeback of malaria is that the most widely used drug against malaria, Chloroquine has been rendered useless by drug resistance. Now scientists and researchers are looking for nature to find out a solution to this disease along with improvements in the existing synthetic and semi-synthetic drugs in use [3, 4].

1.2 Natural Products: A source of Drug lead

Plants have always been a rich source of lead compounds (e.g. *Morphine, Cocaine, Quinine, Tubocourarine, Ticotine, and Quanine).* Nearly half of all human pharmaceuticals now in use were originally derived from natural sources. Perhaps, one of the most famous examples is **Quinine**, which is originally extracted from the bark of *Cinchona* species and purified in 1820. **Azadirachtin,** an antimalarial, is taken from the *plant Azadiracta indica* [6].

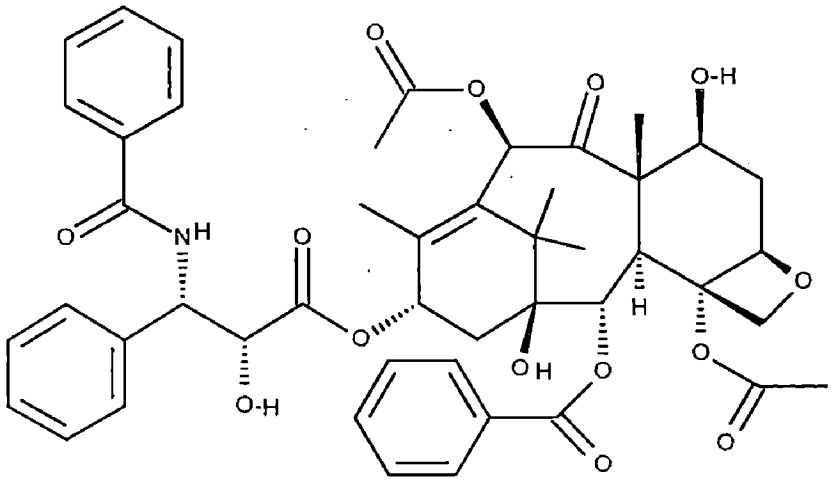


Quinine

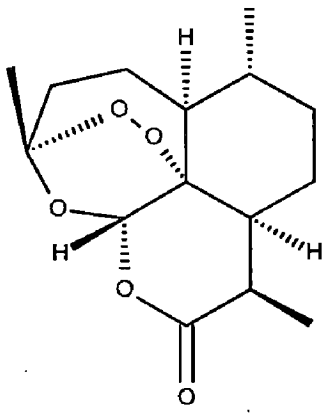


Azadirachtin

The anticancer agent **Paclitaxel (Taxol)** is derived from the *Yew tree* and another important anti-malarial drug **Artemisinin**, a natural product, was first isolated from the leaves of the shrub sweet wormwood *Artemisia annua* in 1971.



Paclitaxel (Taxol)



Artemisinin

Plants provide a large bank of rich, complex and highly varied structures which are unlikely to be synthesized in laboratories. Even today, the number of plants that have been extensively studied is relatively very few and the vast majority has not been studied at all [7, 8, 9].

Moreover these drugs have advantages over other synthetic drugs, as there is minimum chance of toxicity i.e. least side effects. These are easy to available, thus cheap in cost.

Certain aspects of mechanism of action of these natural antimalarial drugs are still not fully understood; even then a huge world population is using these natural drugs. In absence of vaccine these natural compounds and their derivatives are crucial for the control of malaria.

Dihydrofolate reductase -thymidylate synthase (DHFR-TS) from *Plasmodium falciparum* is a main target for antifolate antimalarial. Since the Dihydrofolate reductase (DHFR) activity of *Plasmodium* species (Pf-DHFR) is essential for the parasite, Hence inhibition of DHFR and its role in combating malaria has provided us the rationale to carry out structure-based drug design studies [9, 10, 11].

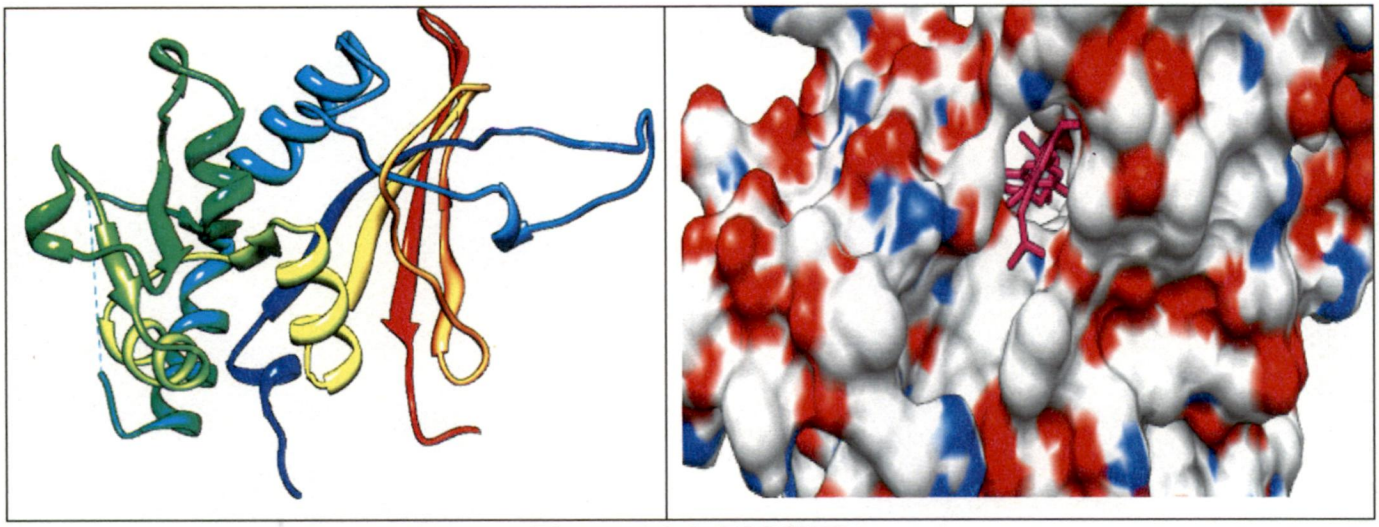
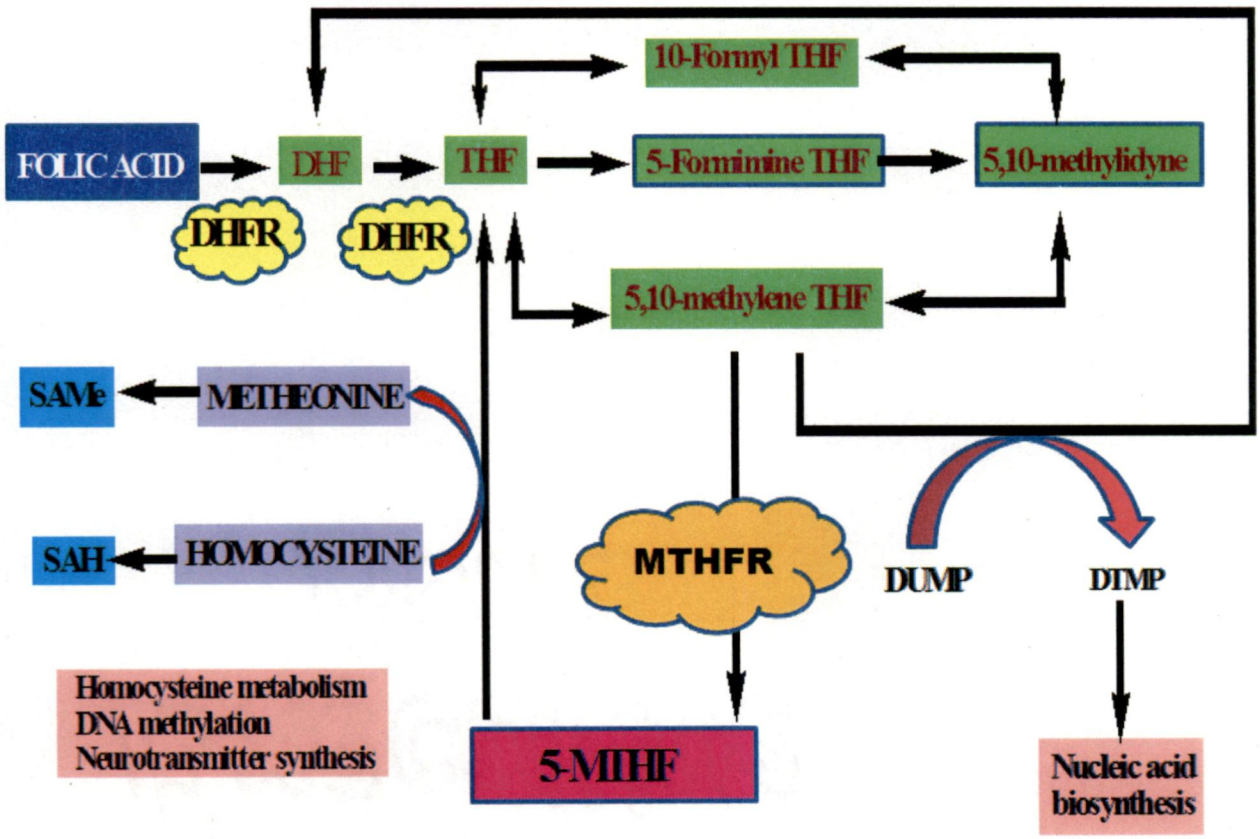


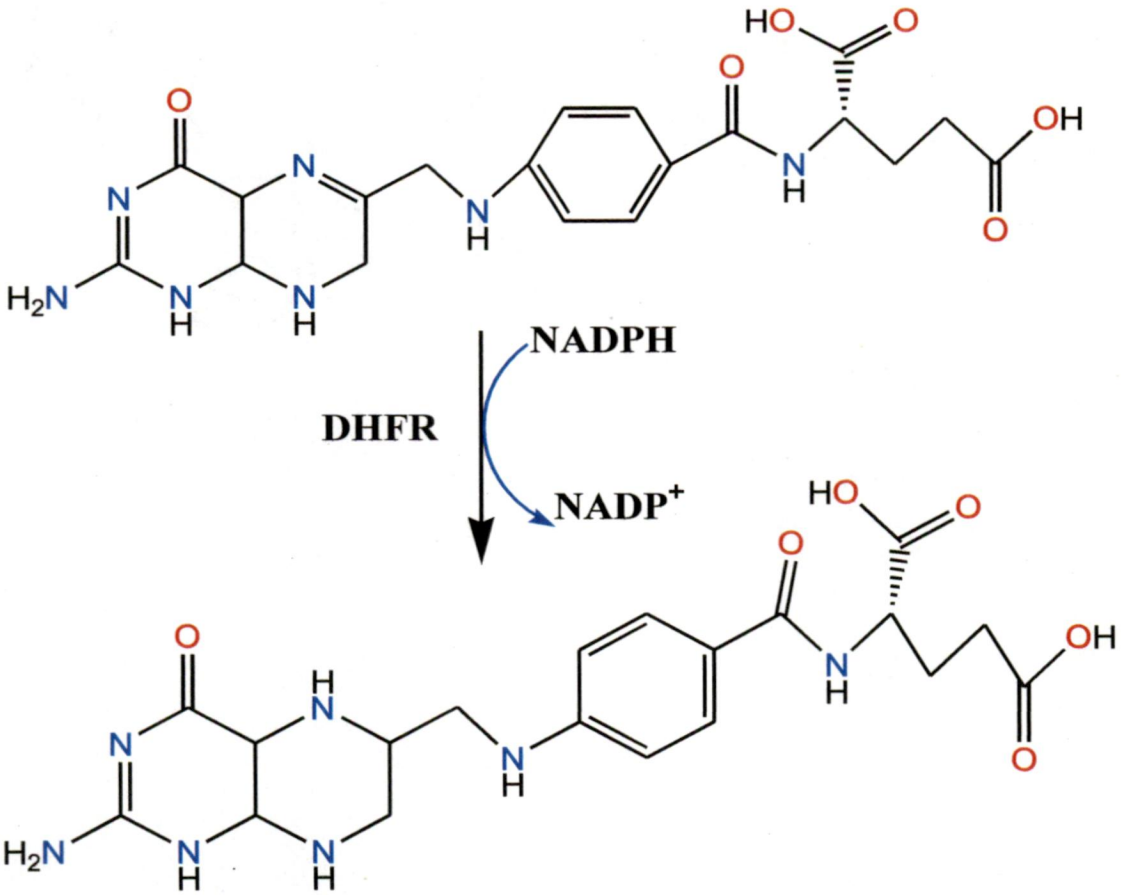
Figure-1.2.1: Pf-DHFR

1.3 DHFR As a target

Bifunctional enzyme Dihydofolate reductase (DHFR), found in almost all living cells, play an important role in the survival of malaria parasite. It generates tetrahydrofolate (THF), various cofactors of which are involved in the transfer reactions of the one carbon unit used in the biosynthesis of nucleic and amino acids, including methylation of DUMP to DTMP [12]. It is a well defined target of many drugs like methoterxate cycloguanil, pyrimethamine etc, DHFR inhibitors act by halting synthesis of DNA, RNA, and proteins, thereby arresting cell growth. DHFR is an important target for drug development against cancer and a variety of infectious diseases caused by bacteria, protozoa, and fungi. Despite of possibility to develop mutations, the availability of target based screening models and detailed crystal structure makes DHFR still an attractive target for malaria [13, 14].



Activation of folic acid and interconversion of reducer folate metabolites DHF, Dihydrofolate; DHFR, Dihydrofolate reductase; THF, tatrahydrofolate; MTHFR, Methyltetrahydrofolate reductase; 5-MTHF, Methyltetrahydrofolate; SAMe, S-adenosylmethione; SAH, 5-adenosylhomocysteine; DUMP, deoxyuridine monophosphate; DTMP, deoxythymidine monophosphate



Scheme 1.3.1: Reduction of Dihydrofolate to Tetrahydrofolate

1.4 Rational Drug Design

The development of new drugs with potential therapeutic applications is one of the most complexes and difficult process in the pharmaceutical industry, this process is time consuming, require more efforts and millions of dollars. The goal of medicinal chemist is to design and synthesize new active molecules. Trial and error screening is a very costly and less efficient process. Computational studies have been developed to unravel the mechanism of action of the antimalarial drugs and to give ideas for the development of new derivatives of improved efficiency.

Computer aided Drug Design is the inventive process of finding new medications based on the knowledge of the biological target. Drug design involves design of small molecules that are complementary in shape and charge to the biomolecular target to which they interact. It includes structure-based design, molecular docking, ligand-based approaches,

pharmacophore elucidation, peptidomimetics and 3D-QSAR. The drug is most commonly an organic molecule that activates or inhibits the function of a Biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient [4, 5,].

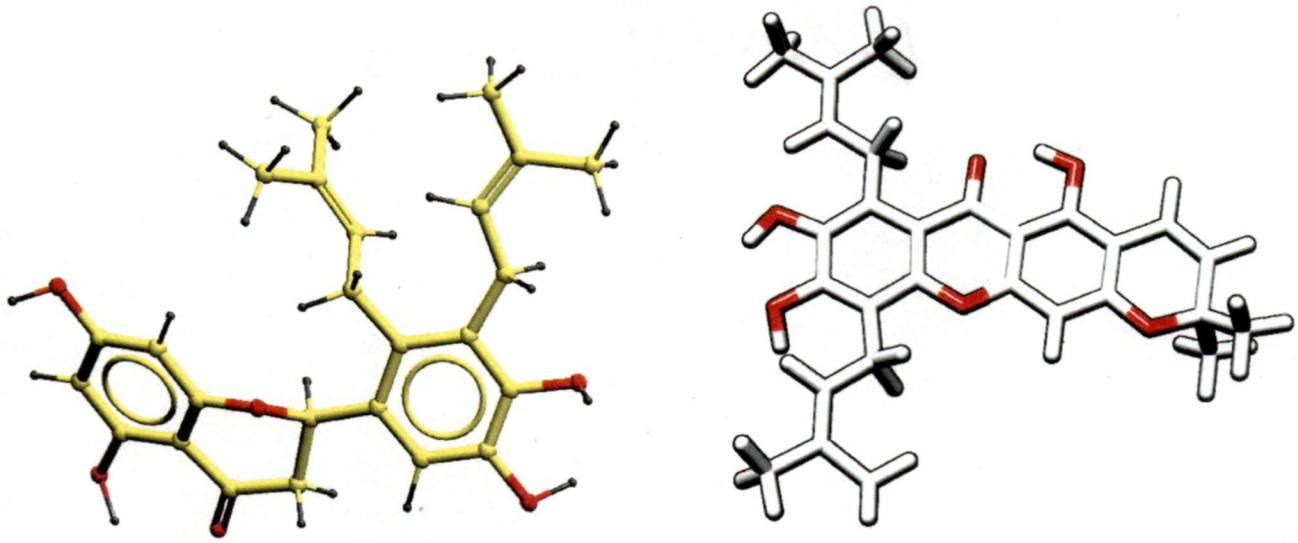


Figure 1.4.1: 3-D models

There are two major types of drug design.

1. **Ligand-based drug design**: The analysis is based on the comparison of the stereo chemical and physiochemical features of a set of known and active/inactive molecules (ligands); lead structures are designed on the bases of the pharmacophore model obtained by such analysis.
2. **Structure-based drug design**: Structure based drug design consider the three dimensional features of the receptor site obtained through methods such as X-ray crystallography and NMR spectroscopy.

The development of molecular modeling programs and their application in pharmaceutical research has been formalized as a field of study known as computer assisted drug design (CADD). Computer-assisted drug design uses computational chemistry to discover new drugs and related biologically active molecules [15]. The aim of study is to predict whether a given molecule will bind to a target and if so how strongly it will bind.

Medicinal chemists are facing many challenges; one is the rational design of new therapeutic agents for treating human diseases. Of the many fascinating and ever expanding roles computers play within biology, chemistry and physic certain key modeling technologies have been kept in mind during the architecture of the presented software system. Major applications of bioinformatics involve sequence alignment, genome assembly, protein structure prediction, protein-protein and protein-ligand docking, molecular dynamics simulations and energy minimization. The process of developing concepts of molecular modeling started with the quantum approach. Molecular modeling has widened the horizons of pharmaceutical research by providing tools for finding new lead compounds [16]. The aim of molecular modeling is to understand the fundamental relationship between chemical and physical properties of a molecule, its chemical and three dimensional structures.

This approach yield excellent results on the ab-initio level, but the size of the molecular system which can be handled is remained rather limited. Moreover, the development of more accurate and reliable algorithms greatly increased computational methods permit studies to be performed with the necessary reliability and accuracy [17].

Molecular dynamics (MD) is a computational method for obtaining all information about the internal molecular motion of a protein. MD is a simulation of the time-dependent behavior of a molecular system. Molecular mechanics is useful for calculating geometry and energy of molecule. The goal of molecular mechanics is to understand the fundamental relationship between the chemical and physical properties of a molecule, its chemical structure and three dimensional structures that the molecule adopts.

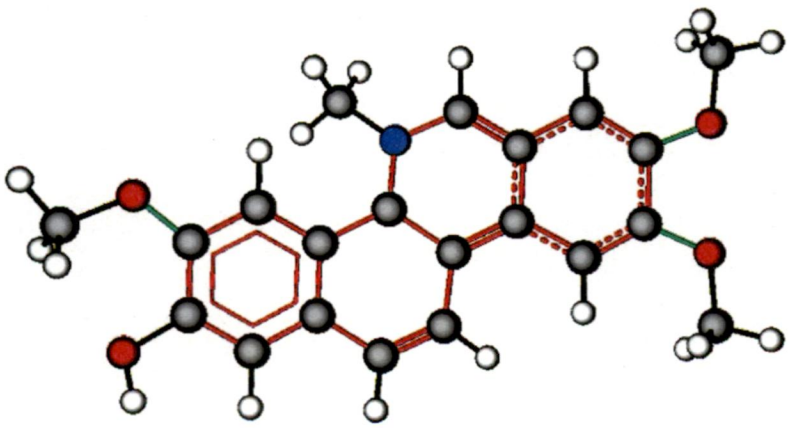


Figure 1.4.2: Ball and stick diagram

The virtual screening (VS) is a computational methodology used in drug discovery. It deals with the quick search of the large number of libraries of chemical structure in order to identify those structures which are most likely to bind to a drug target. VS can be considered as a powerful computational tool for reducing the size of a natural or chemical library that will be further experimentally tested.

Molecular Docking simulations represent a widely employed computational tool in drug discovery field, which tries to predict the structure of the intermolecular complex formed between two or more constituents, where the receptor is usually a protein or a nucleic acid molecule (DNA or RNA) and the ligand is either a small molecule or another protein. It can also be defined as a simulation process where a ligand position is estimated in a predicted or pre-defined binding site.

This approach can be effective if we have a good idea of the expected binding mode. However, X-ray structures have revealed that even very similar inhibitors can adopt different binding modes. Usually, docking programs search along the conformational degrees of freedom of a small molecule (ligand) while the protein is treated as a rigid body.

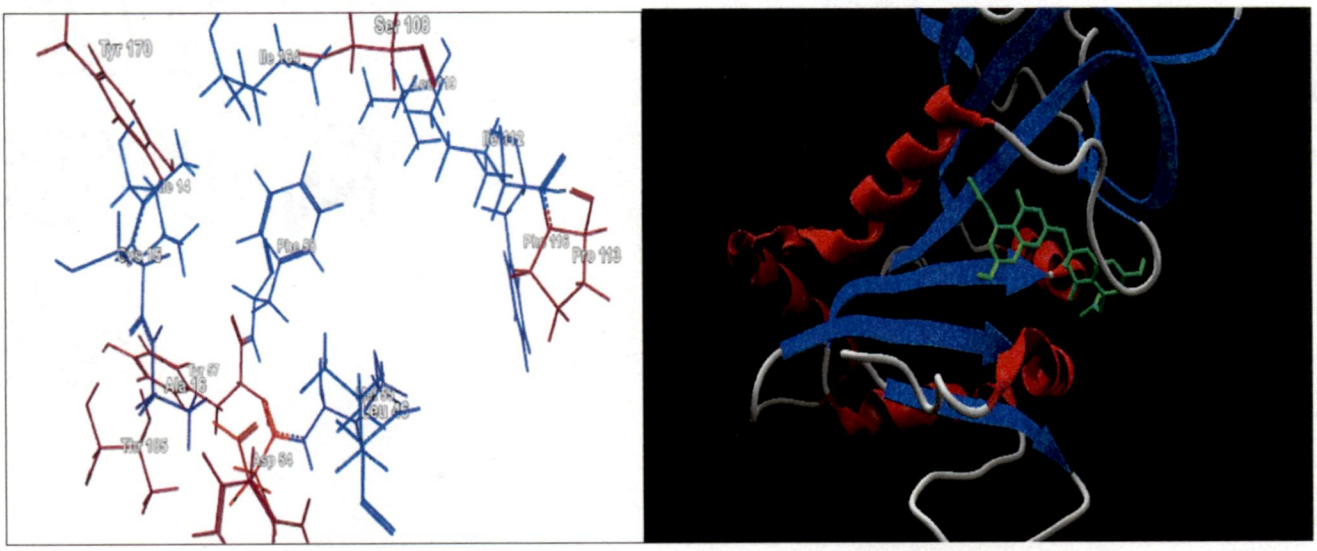


Figure 1.4.3: Binding pocket residues of Pf-DHFR showing hydrophobic (blue) and hydrophilic (red) characteristic

A standard docking protocol consists of a step-wise process. First, a proper search algorithm predicts the various conformations of the ligand within the target binding site. In the second step, each docked pose is evaluated and ranked assessing the intermolecular interaction tightness throughout an estimation of the binding free-energy [3]. Many forces are involved in

the intermolecular interactions such as hydrophobic interaction, hydrogen bonding and electrostatic interactions. Hydrophobic interactions usually provide major driving force, while specificity of binding is controlled by hydrogen bonding and electrostatic interactions.

Molecular docking simulations may be used for reproducing experimental data through docking validations algorithms, where protein-ligand or protein-protein conformations are obtained *in silico* and compared to structures obtained from X-ray crystallography or nuclear magnetic resonance. Furthermore, docking is one of main tools for virtual screening procedures, where a library of several compounds is "docked" against one drug target and returns the best hit.

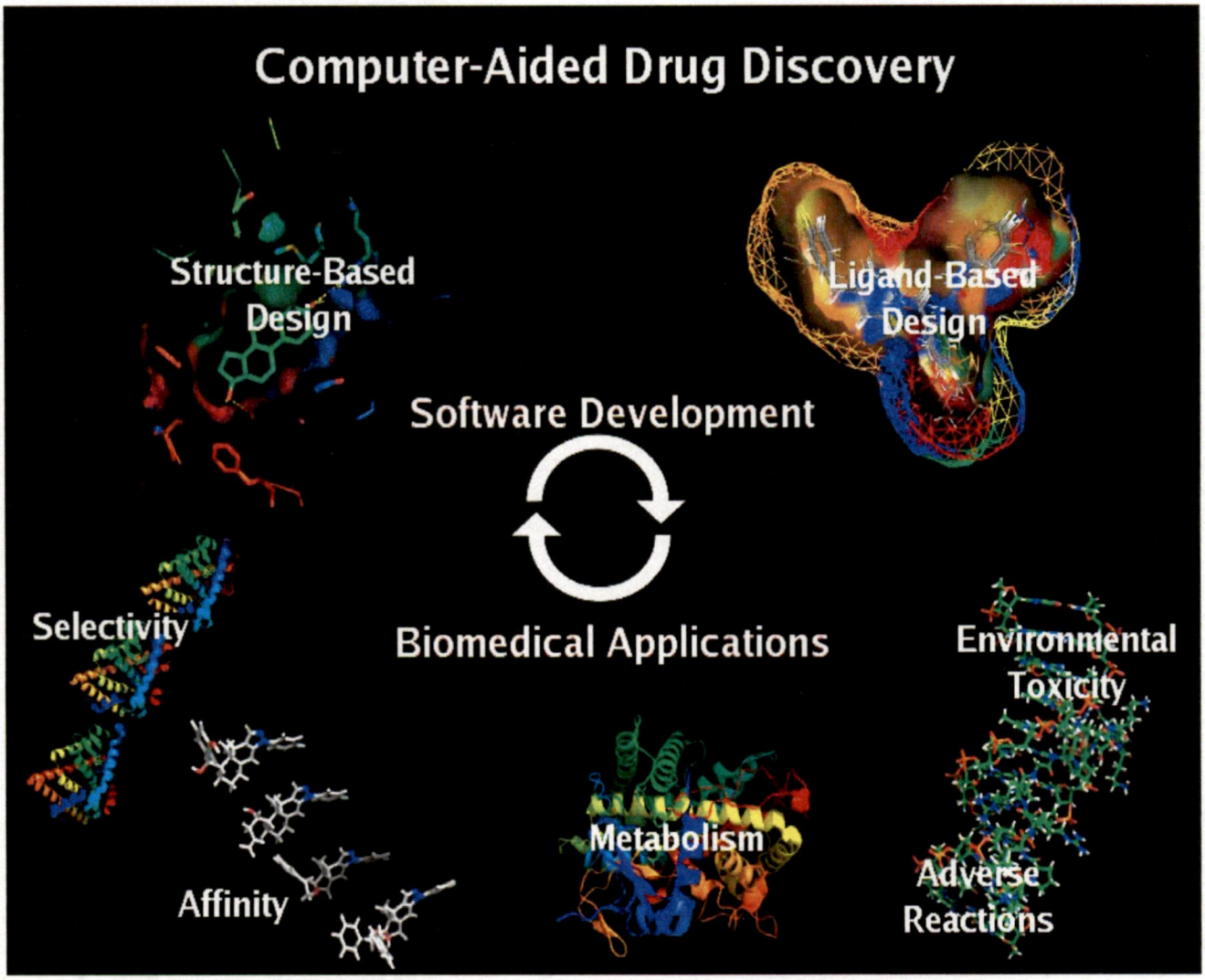


Figure 1.4.4: Different aspects of Computer Aided Drug Discovery (http://people.pharmacy.purdue.edu/~mlill/)

Local docking and Global docking: In Local Docking, the binding site in the receptor is known, and the docking refers to finding the position of the ligand in that binding site while in global docking the binding site is unknown. First, there is need to search for the binding site and the position of the ligand in the binding site can then be performed.

Types of interactions:

Protein-Protein Docking Interactions

Protein-protein interactions occur between two proteins that are approximately the same size. The docking site between the two molecules tends to be planer than those in protein-ligand interactions. Protein-protein interactions are usually more rigid; the interfaces of these interactions do not have the ability to alter their conformation in order to improve binding and ease movement. Conformational changes are limited by steric constraints and thus are said to be rigid.

Protein Receptor-Ligand Docking

Protein receptor -ligand docking is used to check the structure, position and orientation of a protein when it interacts with small molecules like ligands. Protein receptor-ligand motifs fit together tightly, and are often referred to as a lock and key mechanism. Protein –ligand binding usually occurs through non-covalent interaction. Most docking approaches keep the receptor rigid and the ligand flexible during docking.

Ligand and protein flexibility are crucial for protein-ligand docking. Binding events ligands and their receptors in biological systems form the basis of physiological activity and pharmacological effects of chemical compounds.

Rigid Docking: During the Docking process both ligand and receptor are kept rigid.
Flexible Docking: flexibility is allowed for the receptor, or the ligand, or both.

(a) Rigid Receptor with a Flexible ligand

During docking, the ligand is allowed to be flexible but receptor remains rigid and it is assumed that the configuration of protein cannot change. This type of docking allows the ligand to structurally rearrange in response to the receptor.

(b) Flexible Receptor with a Flexible ligand

In this type of docking all degree of freedom of the ligand and the protein are taken into account. Usually this type of docking is very complex [10, 11].

Applications

A binding interaction between a small molecule (ligand) and an enzyme (protein) may result in activation or inhibition of the enzyme. If the protein is a receptor, ligand binding may result in agonism or antagonism. Docking is most commonly used in the field of drug design. Most drugs are small organic molecules, and docking may be applied to:

- Hit identification – docking combined with a scoring function can be used to quickly screen large databases of potential drugs in silico to identify molecules that are likely to bind to protein target of interest.
- Lead optimization – Lead optimization is the synthetic modification of a biologically active compound, to fulfill all stereoelectronic, physicochemical, pharmacokinetic and toxicologic requirement for clinical usefulness. Docking can be used to predict in where and in which relative orientation a ligand binds to a protein.
- Bioremediation - Protein-ligand docking can also be used to predict pollutants that can be degraded by enzymes [4, 5].

1.5 AIM OF THE THESIS

The project described in this thesis is aimed at the *in Silico* identification of bioactive natural plants products interacting with *Pf*-DHFR and able to inhibit complex formation and biologically dependent functions. All the selected compounds have some folklore history as being used as antimalarial (as crude extracts). The work describe herein is also an effort to provide them a rational and scientific background. All experiments were performed using docking softwares AutoDock 4.2, MVD 5.0 and iGemDock 2.1. Top scored molecules have shown some novel scaffolds with μM to nM inhibition. We hope that these findings will aid in antimalarial combat strategies and serve to provide new therapeuticals in rapid manner.

Chapter 1 is a brief introduction of the problem and its different aspects while chapter 2 provides a clear cut description of all the selected candidates along with the current state of the problem. Chapter 3 deals with tool and techniques used for docking studies suitable for *Pf*-DHFR. Final portion of the thesis includes result and discussion based on extensive analysis. As a first step towards the development of an effective procedure, a data set of 205 bioactive natural compounds and known Pf-DHFR binders were employed. We found that the adopted method and the scoring function were successful in predicting the ranks of active and inactive compounds into binding site of the ensemble protein.

CHAPTER-2

LITERATURE SURVEY

2.1 Structure based drug design:

The RCSB protein data bank (PDB), founded in 1971 which provides more than 40,000 experimentally determined biological structures such as protein, nucleic acid and macro-molecular complex. Recently, the founding members such as RCSB PDB, the Macro-molecular Structure Database at the European Bioinformatics Institute (MSD-EBI) and the Protein Data Bank Japan (PDB) at Osaka University, have established collaboration in order to ensure a single and uniform database of PDB data through worldwide Protein Data Bank (wwPDB). The BioMagRes-Bank (BMRB) at the University of Wisconsin-Madison became a member in 2006 and represents a deposition site for primary experimental data.

The studies on natural products are being focused on their uses in cancer and other diseases. A crucial factor in understanding a major probability of successful identification of interesting new lead structures from natural products in respect to synthetic compounds is that they contain a broader range of structural diversity and a large number of chiral centers. Even though, combinatorial synthesis is now producing molecules that are drug-like in terms of size and property, these molecules, in contrast to the small chemical substances produced by different organisms, have not evolved to interact with biomolecules. The interest for natural products in drug discovery appears to be recently increasing [7, 9]. Earlier Application of the widely-used molecular docking computational method has been published for the VS of chemical libraries of natural compounds against *Pf*-DHFR. Recently, some molecular modelling studies have predicted possible binding mode of the inhibitor molecules against Pf-DHFR.

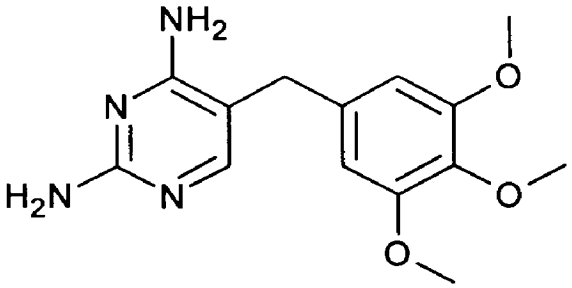
***Pf*-DHFR**

2.2 DHFR Inhibitors

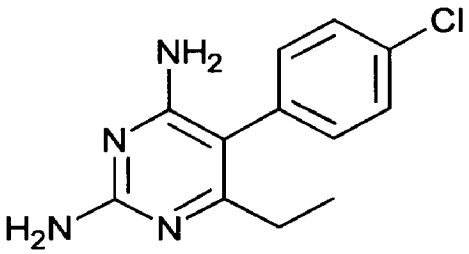
A three-dimensional structure model of the dihydrofolate reductase (DHFR) domain of the bifunctional DHFR-thymidylate synthase of Plasmodium falciparum was used as a basis for computational screening.

The dihydrofolate reductase (DHFR) of *P. falciparum* is one of the few well defined targets in malarial chemotherapy. The enzyme catalyzes the NADPH dependent reduction of dihydrofolate to tetrahydrofolate. Protein-ligand interactions were studied using DHFR protein 1J3I, extracted from PDB to evaluate the strength of affinity of various molecules towards ligand binding site and to study computational dock scores. DHFR is essential for tetrahydrofolate (THF) biosynthesis, plays a central role in promoting cell growth and is the target of several anticancer and antibiotic drugs. Enzymes with essential roles are sensitive targets for drug therapy. Dihydrofolate reductase was the first enzyme to be targeted for cancer chemotherapy. Pf-DHFR is a well-characterized target for antimalarial antifolate drugs, such as pyrimethamine and cycloguanil [15, 16].

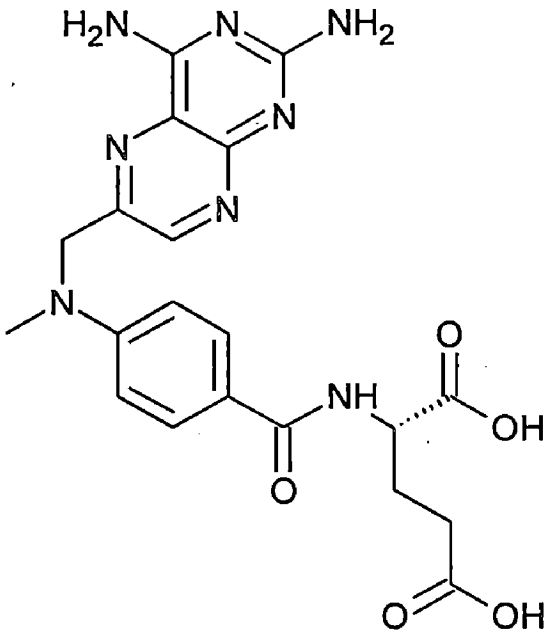
Methotrexate is a cancer chemotherapeutic agent and effective against leukemia and choriocarcinoma. It forms an inactive ternary complex with DHFR and NADPH and is a nonspecific inhibitor of the DHFR of bacteria and cancerous cells. **Trimethoprim**, an antibiotic, has selective affinity for the bacterial DHFR enzyme. **Pyrimethamine** is a diaminopyridine derivative which inhibits the DHFR of *Plasmodium* without inhibiting that of human. **Pentamidine** is a diamine used in the treatment of African Trypanosomiasis, Leshmaniasis and Pneumocystis Pneumonia. It shows inhibition of the DHFR of parasites [17, 18].



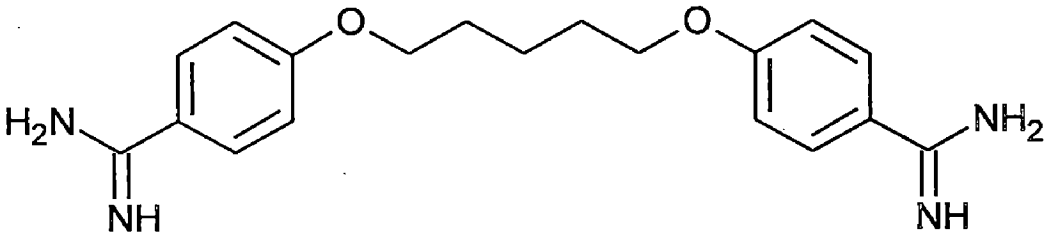
Trimethoprime



Pyrimethamine



Methotrexate



Pentamidine

Figure 2.2: 2-D and 3-D models of DHFR inhibitors

2.3 DHFR and Docking studies

Nutan Prakash et al. performed docking studies on *Plasmodium falciparum* and *Plasmodium vivax* for the inhibition of dihydrofolate reductase using Argus Lab & Hex software. When the receptor (DHFR) was docked with the drug Proguanil the energy value obtained was (-6.59) using Argus Lab and (-174.54) using hex. The most feasible position for the drug to interact with the receptor was found having energy -9.56 K.cal/mole using Argus Lab and -201.92 Kcal/mole using HEX Tool [19].

Docking study of hybrid phenyl thiazolyl-1,3,5-triazine on wild type Pf-DFHR-TS was performed by Gahtori Prashant et. al. and concluded that a significant correlation was exist between in vitro results and in silico prediction (r^2=0.543) [20].

In vitro antimalarial activity and docking analysis of 4-aminoquinoline-clubbed 1,3,5-triazine derivatives on Pf-DHFR-TS was performed by Bhat, H. R. Ghosh S. K. Prakash A. Gogoi K. and Singh U. P. and molecules found considerable bioactivity against the malaria parasite. Hydrophobic interaction was identified as the only major interacting force playing a role between ligand-receptor interaction and minor with hydrogen bonds. The study provided a novel insight into the necessary structural requirement for rationale-based antimalarial drug discovery [21].

2.4 Natural Products with antimalarial activity:

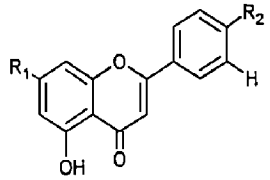
Herbal medicines for the treatment of various diseases including malaria are an important part of the cultural diversity. Two major antimalarial drugs widely used today came originally from indigenous medical systems i.e. *Quinine* and *Artemisinin*, from Peruvian and Chinese ancestral treatments, respectively.

Kraft et al. (2003) performed in vitro study on extracts from some of plants species including *Artemisia afra, Vernonia colorata, V. natalensis (Asteraceae), Parinari curatellifolia (Chrysobalanaceae), Clutia hirsuta, Flueggea virosa, (Euphorbiaceae), Adenia gummifera (Passifloraceae)* and *Hymenodictyon flori-bundum, (Rubiaceae)* and concluded that flavanoids (Acacetin (**1**): IC_{50}= 5.5 µg/ml, Genkwanin (**2**): IC_{50}= 5.5 µg/ml and 7-Methoxyacacetin (**3**): IC_{50}= 4.3 µg/ml) extracted from *Artemisia afra* proved to be the most active against the *Plasmodium falciparum* [22, 23].

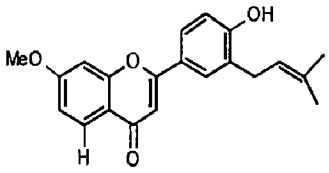
Yenesew et al. (2004) extracted some flavanones (compounds **4, 5, 6, 7**) and flavanoids (compound **8**) from stem bark of *Erythrina abyssinica* which showed anti-plasmodial activity against *Plasmodium falciparum* and IC_{50} values for these compounds was 13.6, 5.8, 5.4, 4.9, 5.8 and 10.3µg/ml respectively [24].

Kuria et al. (2002) searched new antimalarial drugs from *Ajuga remota benth (labiatae)*, (compound **9** and **12**) with IC_{50} value 23 and 8.2µg/ml [25].

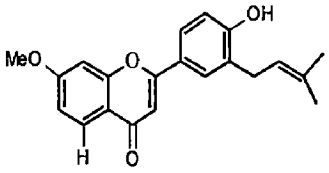
Murata et al. (2008) isolated new triterpenoid compounds (**10, 11**) from the stem bark of *Ekebergia capensis* with antimalarial activity against *Plasmodium falciparum* [26].



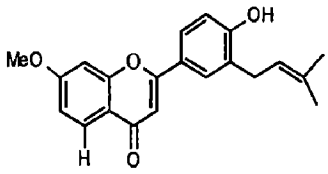
1. Acacetin; R_1=OH, R_2=OCH_3
2. Genkwanin; R_1=OCH_3, R_2=OH
3. 7-Methoxy-acacetin; R_1=OCH_3, R_2=OCH_3



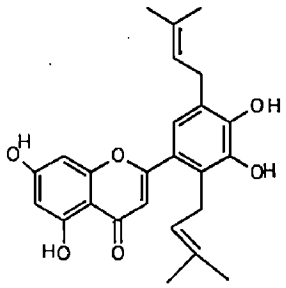
4. 5-deoxyabysssinin II



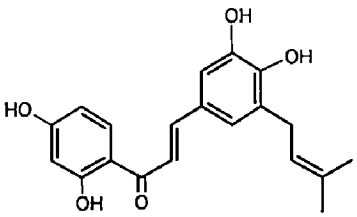
5. Abyssinin III



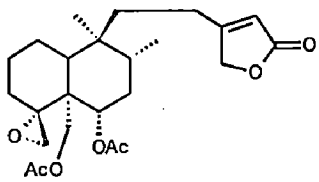
6. Abyssinin IV



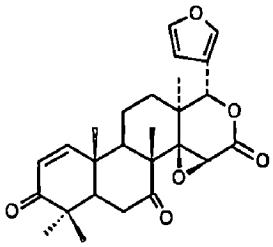
7. Sigmoidin



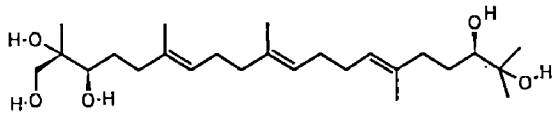
8. 5-Prenylbutein



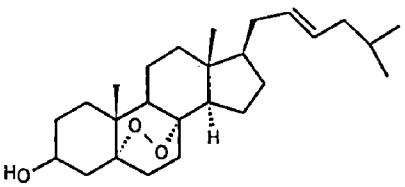
9. Diterpene-ajugarin



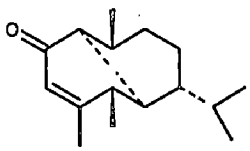
10. Deacetoxy-7-oxogedunin



11. 2-hydroxymethyl-2,3,22,23-tetrahydroxy 2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene

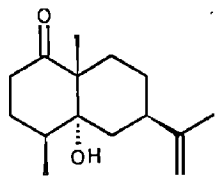


12.Triterpene-ergosterol 5-8 endoperoxide

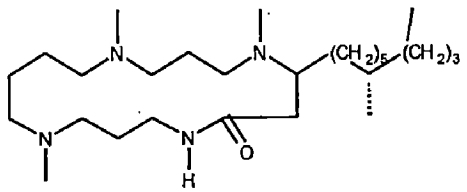


13. Sesquiterpene-mustakone

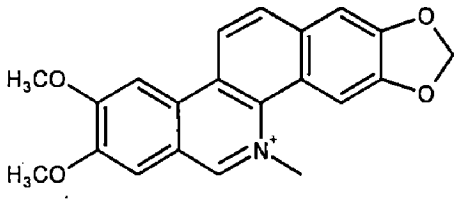
Rukunga et al. (2007) extracted alkaloids (compounds) from *Albizia gummifera* which were active against *plasmodium berghei* and in 2008 found two sesquiterpenes (**12**), mustakone (**13**) and corymbolone (**14**), isolated from the chloroform extract of the rhizomes of *Cyperus articulatus* were active against *plasmodium*[27, 28].



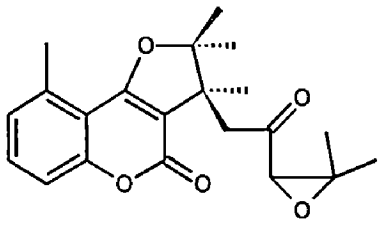
14. Corymbolone



15. Budmunchiamine-K1; R_1 = CH3, R_2 = CH_3, R_3 = H
16. Budmunchiamine-K2; R_1 = CH_3, R_2 = CH_3, R_3 = OH
17. Budmunchiamine-K3; R_1 = CH_3, R_2 = H, R_3 = H
18. Budmunchiamine-K4; R_1 = CH_3, R_2 = H, R_3 = OH
19. 9-normethylBudmunchiamine; R_1= H, R_2 =CH_3 R_3 = H

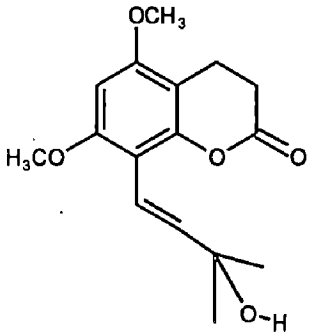


20. Nitidine



21. 2-Epicycloisobrachy-coumarinone-epoxide; $R_1 = CH3$, $R_2 = H$

22. Cycloisobrchy-coumarinone-epoxide; $R_1 = H$, $R_2 = CH_3$



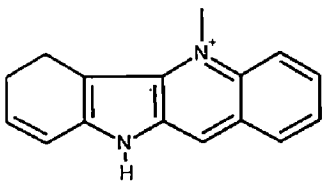
23. Coumarin

Spermine alkaloids (compounds **15, 16, 17, 18, 19**) were isolated from *Albizia- gummifera* and found active against *Plasmodium species.*

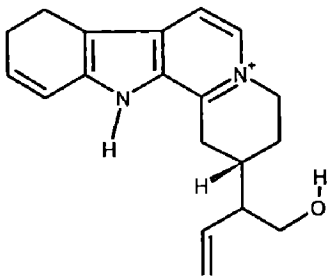
Gakunju et al. (1995) concluded Potent anti-malarial activity of the alkaloid nitidine (**20**) isolated from a Kenyan herbal remedy *toddalia asiatica* [29].

Oketch-Rabah et al., 1997 found two new antiprotozoal (**21, 22**) from *Vernonia brachycalyx* and (**23**) in 2000 one antiplasmodial coumarin from *Toddalia Asiatica* roots [30, 31].

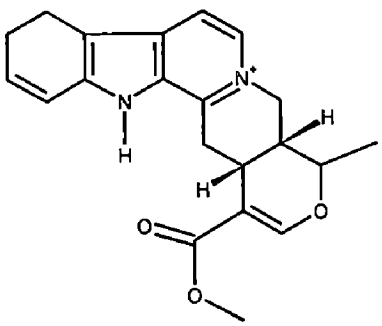
Cryptolepine (**24**), matadine (**25**), and serpentine (**26**) are three indoloquinoline alkaloids isolated from the roots of African plants; these alkaloids have been used in African folk medicine in the form of plant extracts for the treatment of multiple diseases, in particular as antimalarial drugs [32]. Cryptolepine is an indoloquinoline, extracted from the roots of the West African shrub *Cryptolepis sanguinolenta*, Metadine from *strychnos- gossweileri* and serpentine from *Rawolfia serpentina* showed *in vitro* antiplasmodial activity against *Plasmodium falciparum* [33, 34].



24. cryptolepine



25. Metadine



26. Serpentine

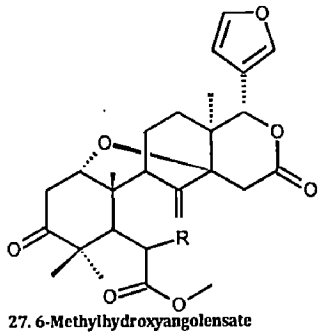
Bickii et al. (2000) concluded in vitro antimalarial activity of Limnoids (compounds **27, 28, 29, 30, 31, 32, 33, 34**) from *Khaya- gradifolia* (*maliaceae*) A considerable number of plant extracts and isolated compounds possess significant antimicrobial, anti-parasitic including antimalarial, anti-proliferative, anti-inflammatory, anti-diabetes, and antioxidant effects [35, 36].

Bickii et al. (2007) found new antiplasmodial agents (compounds **35, 36, 37**) from the stem bark of *Entandrophragma angolense* (Meliaceae) [37].

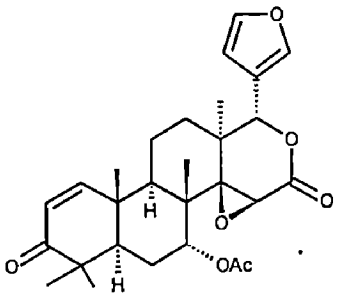
Kenmogne et al. (2006) extracted diterpenoids (compounds **38, 39, 40, 41**) from the seeds of *Aframomum zambesiacum* [38].

Tchuendem et al. (1999) extracted Anti-plasmodial sesquiterpenoids (compounds **42, 43, 44**) from the African *Reneilmia cincinnata* [39].

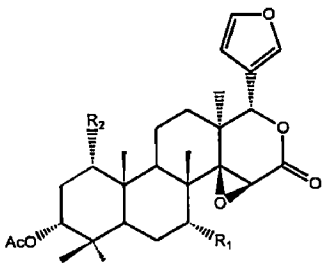
Ngouela et al. (2006) concluded Anti-plasmodial and antioxidant activities of constituents (**45, 46, 47**) of the seed shells of *Symphonia globulifera* [40].



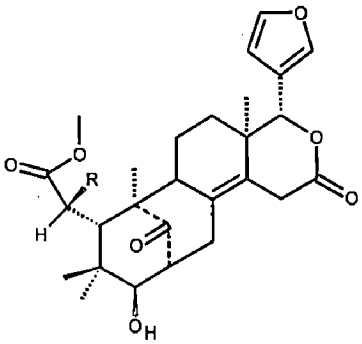
27. 6-Methylhydroxyangolensate
R = OH
28. Methylhydroxyangolensate; R = H



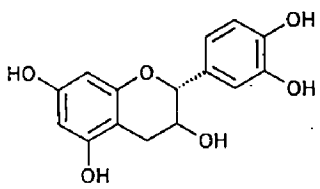
29. Gedunin



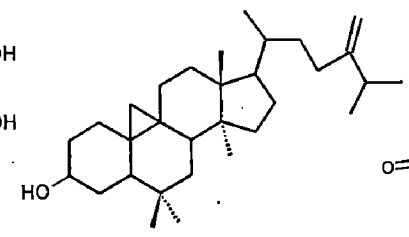
30. 7-deacetylkhivorin
31. 1-deacetylkhivorin



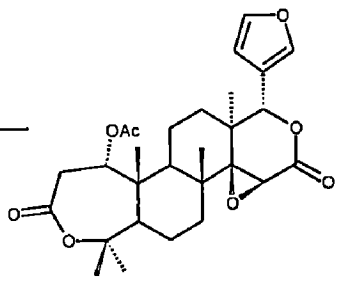
32. Swietenolide ; R = OH
33. 6-acetylswietenolide; R =OAc



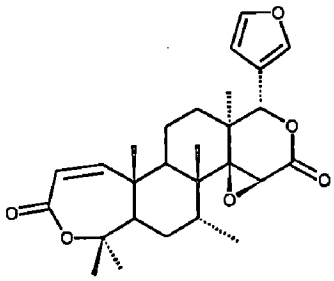
34. catechin



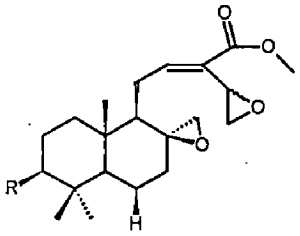
35. 24-methylene-cycloartenol



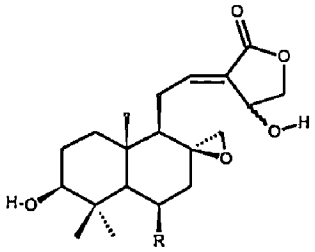
36. 7-alpha-Acetoxydihydronomilin



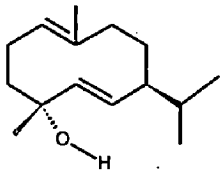
37. 7-alpha-Obacunnylacetate



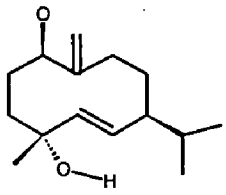
38. Aulocarpin-A; R = OH
39. 3-Deoxyaulocarpin-A; R = H



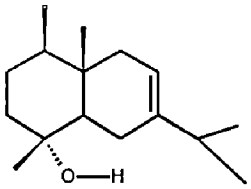
40. Zambesiacolactone-A; R = H
41. Zambesiacolactone-B; R = OH



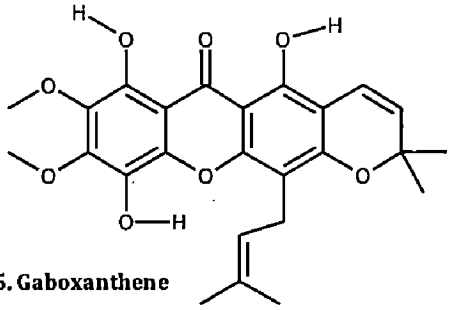
42. 1(10)E,5E-germacradien 4-beta-ol



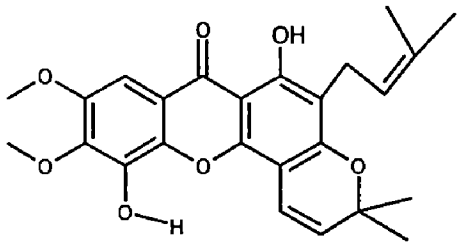
43. 5E,10(14)-germacraadien 1beta,4beta-diol



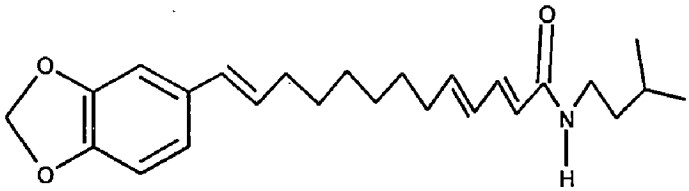
44. Oplodiol



45. Gaboxanthene



46. Symphonin

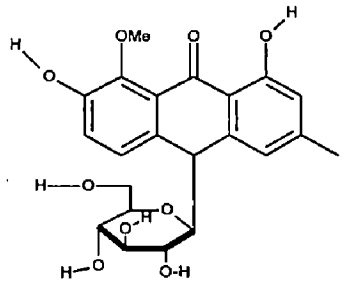


47. Pipyahyine

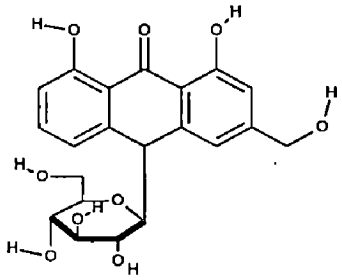
The genus *Aframomum* of the Zingiberaceae family includes 40 species and is most common in tropical and subtropical regions. Twenty species are found in Cameroon, where they are widely used in traditional medicine. The compounds isolated from plants of this genus include flavonoids, diaryl heptanoids , sesquiterpenes and labdane diterpenoids, specially in *Aframomum alboviolaceum* (Abreu and Noronha, 1997), *Aframomum aulacocarpos, Aframomum daniellii, Aframomum escapum, and Aframomum sceptrum*. A great deal of interest has been focused on the labdanes from *Aframomum* species, some of which exhibit antifungal, cytotoxic, and other biological activity [41].

Van Zyl et al. concluded in vitro activity of Aloe extracts (compounds **48, 49**) against *Plasmodium falciparum* [42].

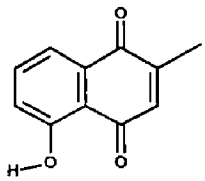
Likhitwitayawuid in 1998 found anti-malarial napthoquinones (compound **50**) from Nepenthes thorelii [43].



48. Homonataloin



49. Alion



50. Plumbagin

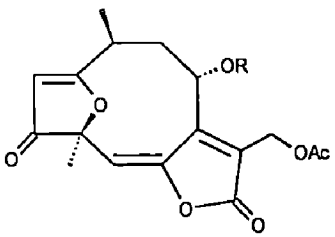
Pillay et al. (2007) extracted antiplasmodial hirsutinolides (compounds **51, 52**) from *Vernonia staehelinoides* and their utilisation towards a simplified pharmacophore [44].

Pillay et al. (2007) isolated antiplasmodial sesquiterpene (compounds **53, 54, 55, 56, 57, 58,**) lactones from *Oncosiphon piluliferum* [45, 46].

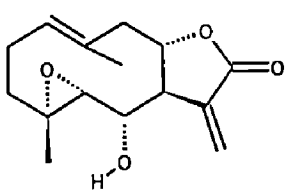
Kamatou et al. (2008) selected South African *Salvia species in search for* new antimalarial and anticancer active drugs and isolated compounds (**60 and 61**) from *Salvia redula* [47].

Clarkson in 2003 performed in vitro studies on abietane and totarane diterpenes isolated from *Harpagophytum procumbens* (Devil's Claw) and found antiplasmodial activity in the extracts (**62, 63, 64**) of plant *species* [48].

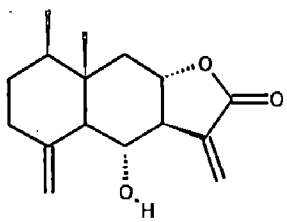
Campbell et al. found Bioactive Cytotoxic and antimalarial alkaloids (compounds **64, 65, 66, 67, 68**) from *Brunsvigia Littoralis* and *Brunsvigia radulosa* [49, 50].



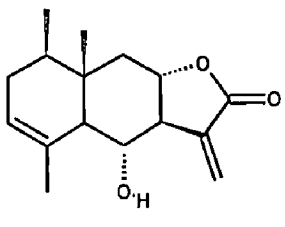
51. 8-alpha-5'-Acetoxysenecioglоxy-3-oxo -1-desoxy-1,2-dihydrohirstutinolide
52. 8-alpha-5'-Acetoxysenecioglоxy-3-oxo -1-desoxy-1,2-dihydrohirstutinolide



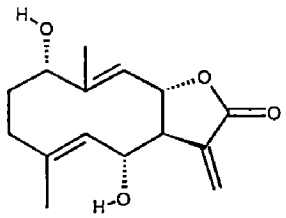
53. Germacranolide
54. Germacranolide



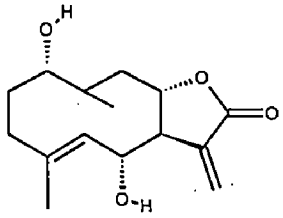
55. Desacetyl-beta-cyclo pyrethrosin



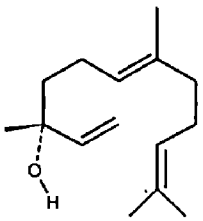
56. Sivasinolide



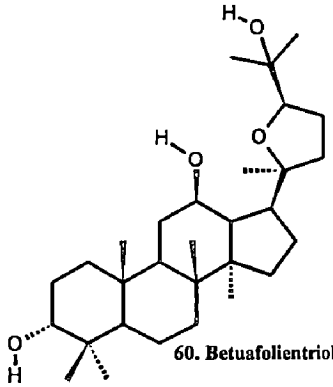
57. Tatridin-A



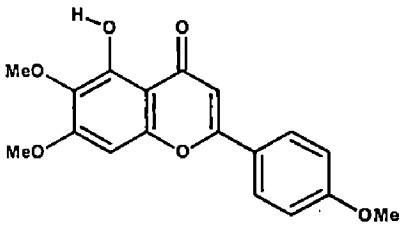
58. Taxachin



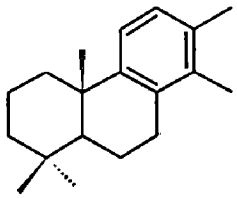
59.Nerolidol



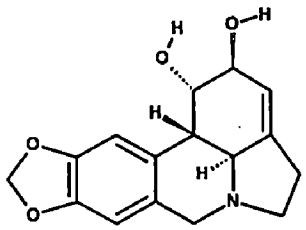
60. Betuafolientriol-oxide



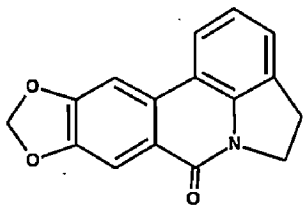
61. Salvigenin



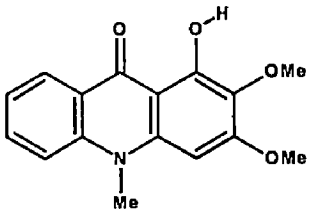
62. Totaratriene; R_1= OH, R_2= OH, R_3= -$CH(CH_3)_2$
63. Abietariene; R= OH, R_2=-$CH(CH_3)_2$, R_3= H
64. Totarol ; R= H, R_2= OH, R_3 =-$CH(CH_3)_2$



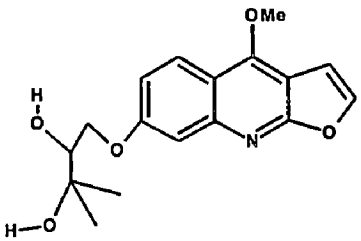
65. Lycorine



66. Anhydrolycorin-6-one

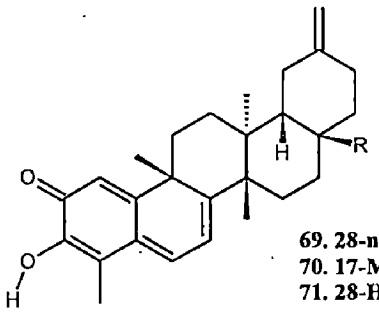


67.Arborinine

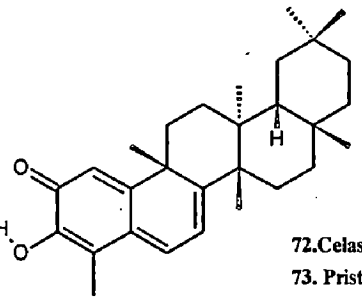


68. Evoxin

Compounds **69, 70, 71, 72, 73** and **74** were isolated from the roots of *Salacia kraussii* (Celastraceae) by bioassay-guided fractionation. The structures of the compounds were determined by DEPT and 2D NMR techniques. The isolates showed antimalarial activity in vitro. In vivo, 70 was found to be inactive against blood stages of *Plasmodium berghei* in mice after oral and parenteral administration, and the compound was toxic with increasing concentrations [51].



69. 28-nor-isoguesterin-17-carbaldehyde
70. 17-Methoxycarbonyl-28-norisoguesterin
71. 28-Hydroxyisoguesterin

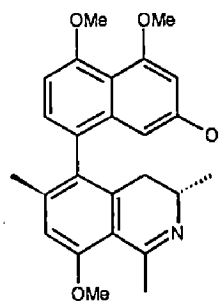


72.Celastrol; R1= Me, R_2=COOH
73. Pristimerin; R=Me, R_2=COOMe
74. Isiguesterol;R=H, R_2=CH_2OH

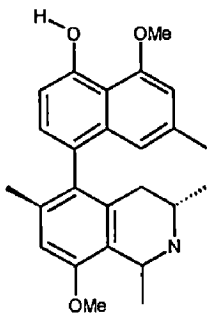
Alkaloids **74-93** have been assessed for activities against *Plasmodium falciparum* in vitro. Alkaloids allocrytopine, columbamine, dehydroocoteine, jatrorrhizine, norcorydine and ushinsunine shad values between 1 and 3 µg/ml. Compounds were also assessed for antiamoebic and cytotoxic activities, but none was significantly active which was moderately cytotoxic [52].

Bringmann et al. (2000) isolated two new naphthylisoquinoline alkaloids, ancistroealaines A (**75**) and B (**76**), from *Ancistrocladus ealaensis* and in vitro studies of these compounds showed their antiplasmodial activities [53].

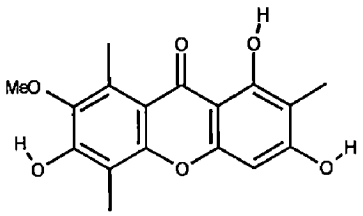
Xanthones (compounds **77, 78, 79, 80, 81, 82, 83, 84, 85 and 86**) were isolated from the stem bark of Pentadesma butyracea, were tested in vitro for antiplasmodial activity against a Plasmodium falciparum and nearly all of these xanthones exhibited good antiplasmodial activity [54].



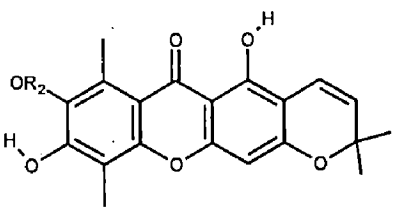
75.Ancistroealaines-A



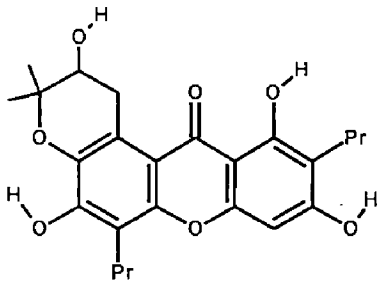
76. Ancistroealaines-B



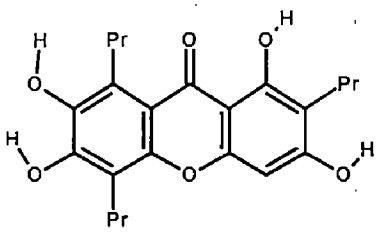
77. Xanthene-1; R_1= Ge, R_2=H, R_3=Pr
78. Xanthene-1; R_1=R_2=H, R_3=8'OH-Ge
79. Xanthene-1; R_1=R_2=Pr, R_3=H
80. Xanthene-1; R_1=R_2=H, R_3=Ge



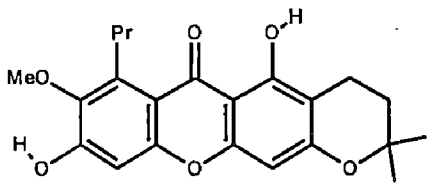
81. Xanthene-5; R_1= Pr, R_2= H, R_3=Pr
82. Xanthene-6; R_1= H, R_2= CH_3, R_3=Pr



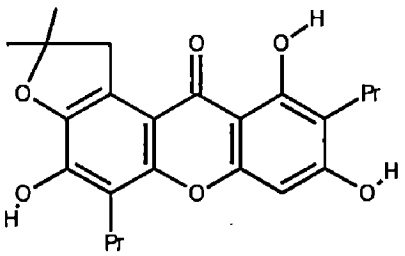
83. Xanthene-7



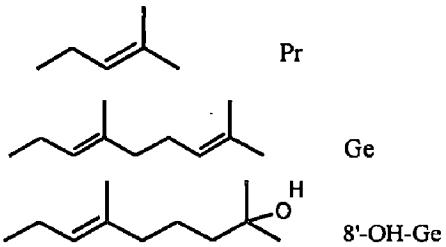
84. Xanthene-8



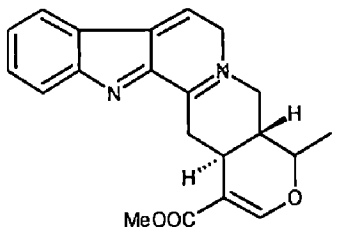
85. Xanthene-9



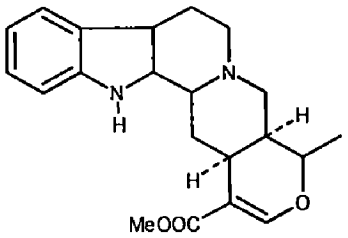
86. Xanthene-10



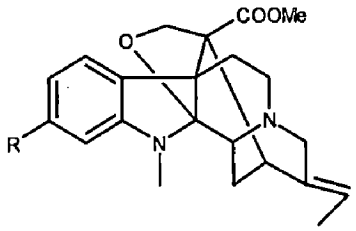
Okunji et al. (2005) found that *Picralima nitida* alkaloids (compounds **87, 88, 89, 90, 91, 92,** & **93**) possess in vitro antimalarial activity comparable to the clinical antimalarial chloroquine and quinine. The in vitro IC_{50} values for these alkaloids ranged from 0.01 - 0.9 µg/ml against *Plasmodium falciparum* [55].



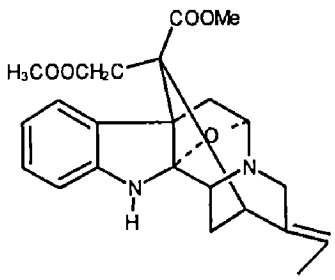
87. Alistonine



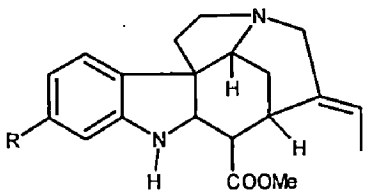
88.Akuammigine



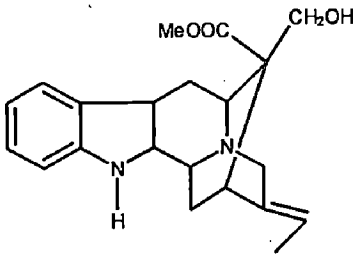
89. Akuammigine; R=H
90. Akuammine ; R=OH



91. Picraline



92. Akuammicine

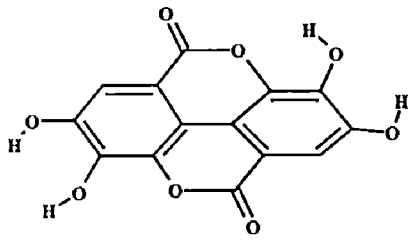


93. Akuammidine

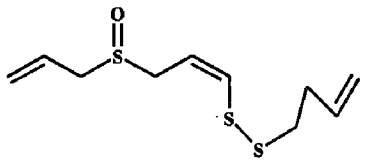
Adebayo et al (2011) concluded by his studies on medicinal plants from Nigeria that compounds 94-131 were found active against malaria parasites in vitro. The search for new

drugs based on plants is important due to the emergence and widespread of chloroquine-resistant and multiple drug-resistant malaria parasites, which require the development of new antimalarials, new phytotherapies that could be affordable to treat malaria, especially among the less privileged native people living in endemic areas of the tropics, mostly at risk of this disease [56].

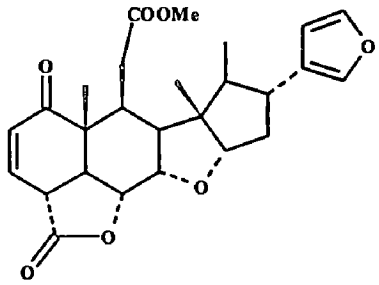
Banzouzi et al. studied antiplasmodial activities of Extracts (compounds **94-103**)of *Alchornea cordifolia* which exhibit in vitro activity against *Plasmodium falciparum* and found to be active constituent of the extract with IC_{50} in the range of 0.2 - 0.5 μM [57].



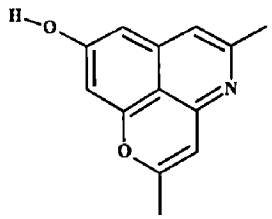
94. Ellagic acid



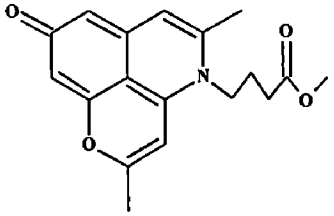
95. Ajoene



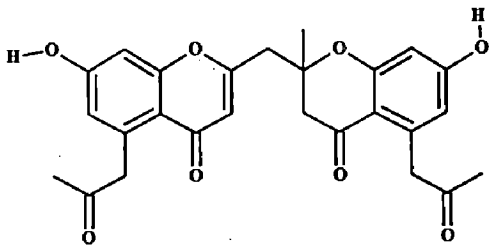
96. Nimbolide



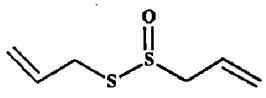
97. Cassiarin-A



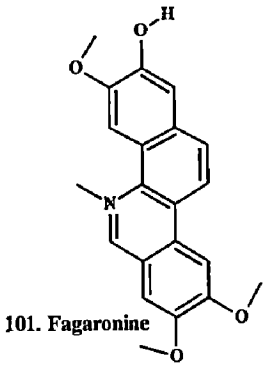
98. Cassiarin-A



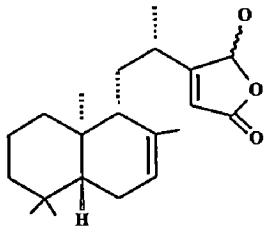
99. Bischromone-Chrobisiamine



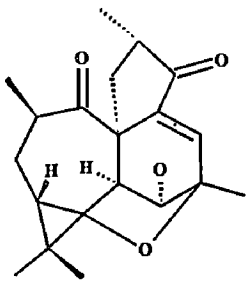
100. Allicin



101. Fagaronine



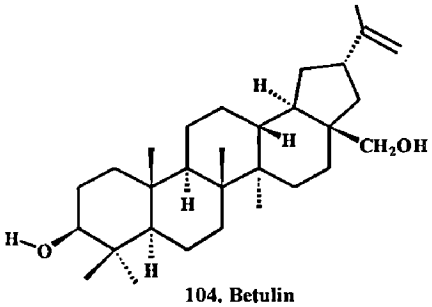
102. 12S,16R-Dihydroxy-ent-labda -7,13diene-15,16-olide



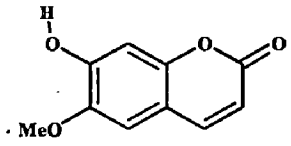
103. Steenkrotins-A

Mutai et al. (2008) isolated lupane triterpenes (compound **104**) from the stem bark of *Acacia mellifera* and his study showed the presence of bioactive agents in *Acacia mellifera* [58]

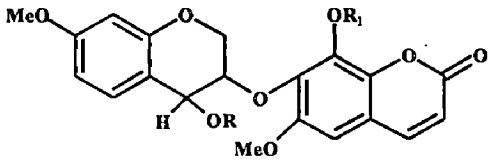
Adesanwo et al. (2004) extracted Eniotorin (compound 106) and Scopoletin (compound 105), anti-malarial Coumarins from the Root Bark of *Quassia undulate* and IC_{50} values are found to be 55 and 450 ng/ml respectively [59].



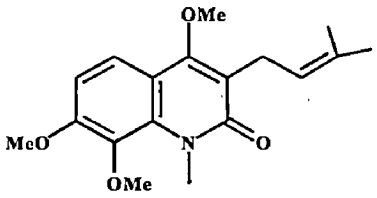
104. Betulin



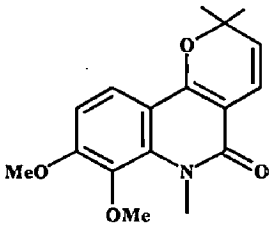
105. Scopoletin



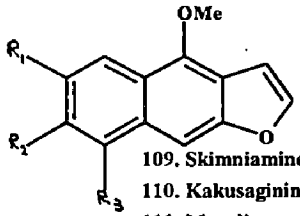
106. Eniotorin



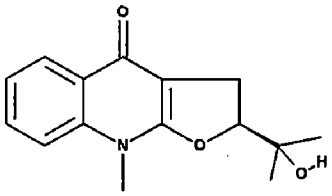
107. Methylpreskimmiamine



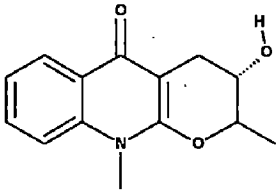
108. Veprisine



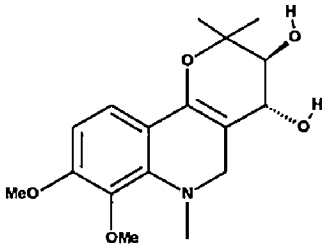
109. Skimniamine; R_2,R_3=OMe, R_1=H
110. Kakusaginine;R_1,R_2=OMe, R_3=H
111. Maculine ;R_1+R_2=OCH_2, R_3=H
112. Hindersiamine;R_1+R_2=OCH_2,R_3=OMe



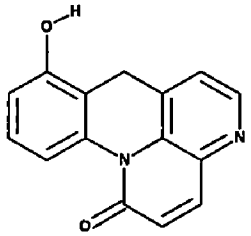
113. Platydesmine



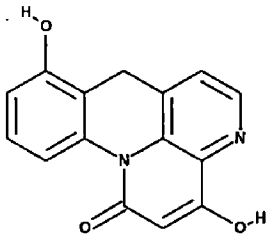
114. Ribalinine



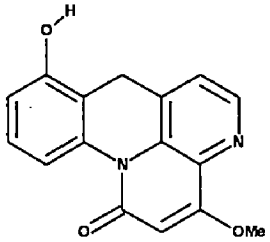
115. Araliposinine



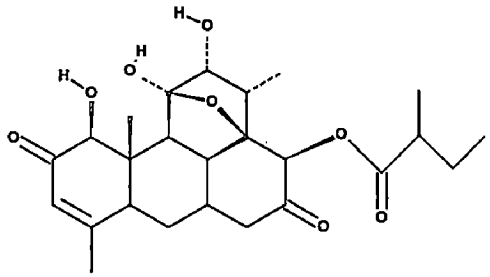
116. 11-Hydroxycanthin-6-one



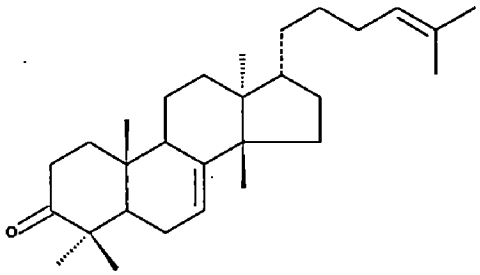
117. 4-hydroxycanthin-6-one



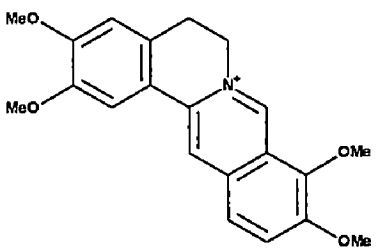
118. 4-Methoxycanthin-6-one



119. Allanthinone

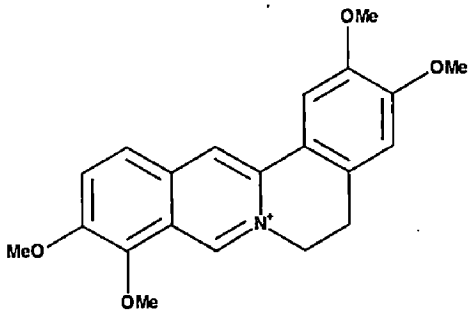


120. 3,21-Dioxotirucella-7-21-diene

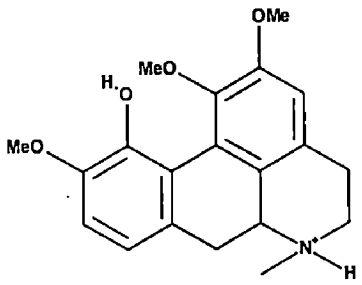


121. Palmatine

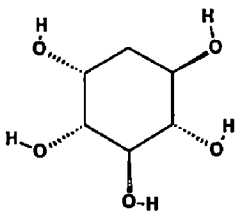
Compounds **107, 108, 109, 110, 111, 112, 113, 114 and 115** are extracts of *Araliopsis-tabuoensis,* compounds **116-120** from *Odyendyea gabonesis*, **121- 124** from Perianthus Longifolius and **125- 131** are taken from *Cordia globiferin.* All these compounds having IC_{50} value less than 5 µg/ml (except **131** IC_{50} >20 µg/ml).



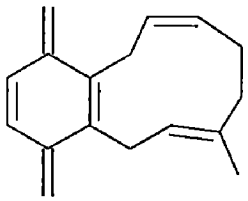
122. Jatrorrizine



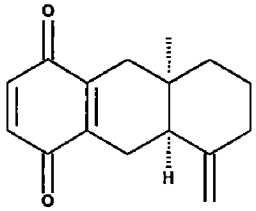
123. Isocoridinum-hydride



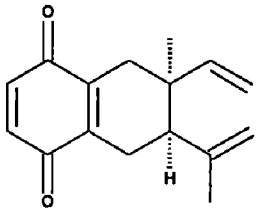
124.Viboquercitol



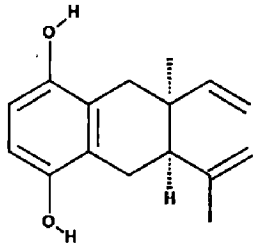
125. Globiferin



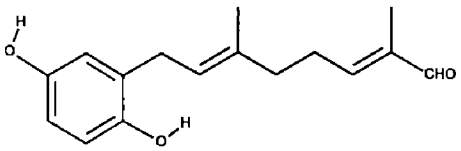
126. Cordiachrome-B



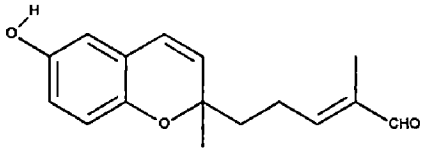
127. Cordiachrome-C



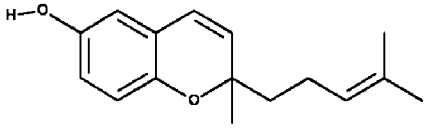
128. Cordiaquinol-C



129. Alliodorin



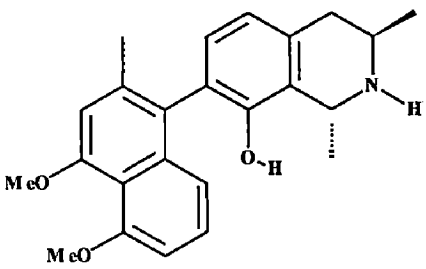
130. Elaeagin



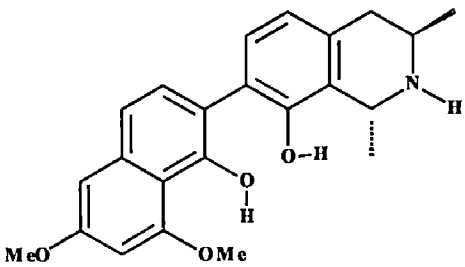
131. Cordiachromene

Francois et al (1997) shown antiparasitic activity of Naphthylisoquinoline alkaloid-containing extracts from species of the families Dioncophyllaceae (compounds **132, 133, 134, 135**) in Plasmo-dium berghei [60].

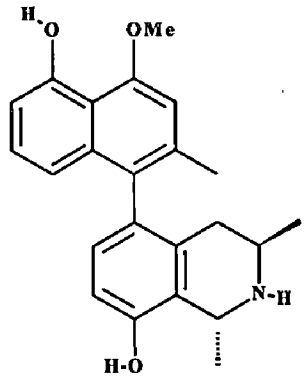
Benoit-Vical et al (2003) shown In vitro antimalarial activity of naphthyliso-quinoline alkaloids (compounds **136, 137, 138, 139, 140, 141**) isolated from extracts of the tropical liana Ancistrocladus korupensis and Ancistrocladus likoko and Structures were determined by spectroanalytical methods [61, 62, 63, 64, 65].



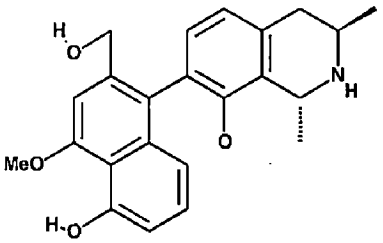
132. Dioncophylline-A



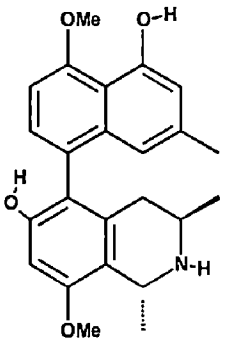
133. Dioncophylline-B



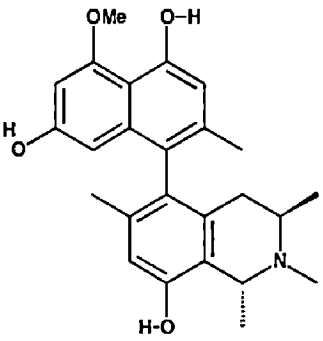
134. Dioncophylline-C



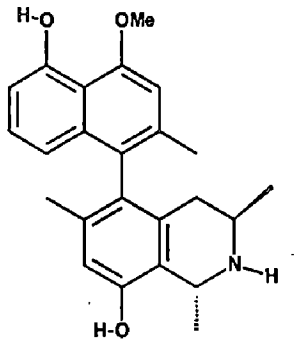
135. Dioncopelitine-E



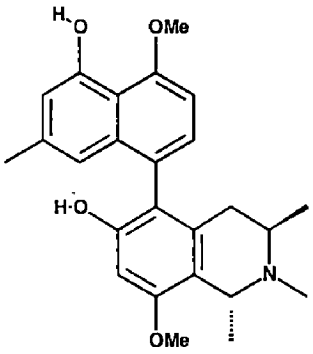
136. Korupensamine-E



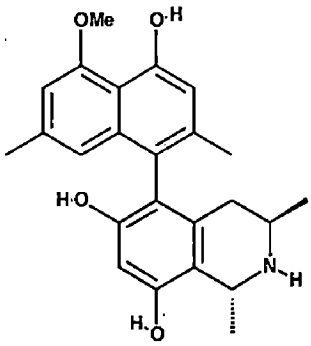
137. Ancistrolikokines-A



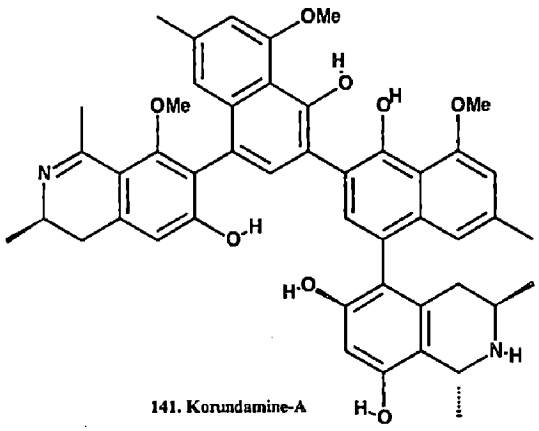
138. Ancistrolikokines-B



139. Ancistrolikokines-C



140. Korupensamine-A



141. Korundamine-A

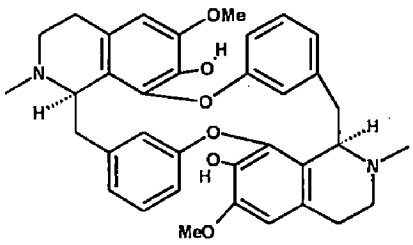
Angerhoffer et al. (1999) showed In vitro antiplasmodial activity of alkaloids isolates from *Cyclea barbata, Stephania pierrei, Stephania erecta, Pachygone dasycarpa, Cyclea atjehensis, Hernandia peltata, Curare candicans, Albertisia papuana, and Berberis valdiviana* (compounds **142, 143, 144, 145, 146, 147, 148, 149**) [66].

Roumy et al. (2006) showed in vitro antiplasmodial activity of azaanthuracene alkaloid (compound **150**) from *pseudoxandra Caspidata* [67].

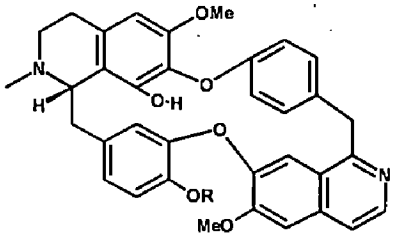
Simon et al.(2002) were found that alkaloids, budmunchiamines L4 (**151**) from leaves of *Albizia adinocephala* (Leguminosae) inhibit the malarial enzyme plasmepsin II [68].

Carraz et al (2008) showed that Tozapsine (**152, 153**) from *Strychnopsis thouarsii* is used in the Malagasy traditional medicine to combat malaria infection by targeting *Plasmodium* at its early liver stage. Structure of tozapsine was characterized by 2D NMR, MS, and CD spectral analysis [69].

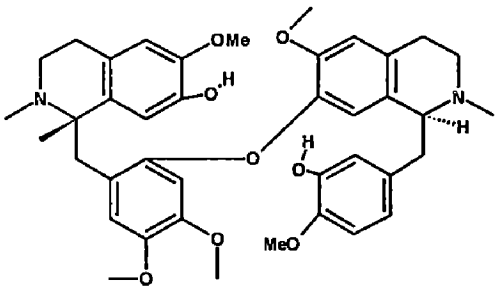
(Frédérich et al., 1999)A Reinvestigation of *Strychnos usambarensis* (**154**) and *Strychnos icaja* (**155**) resulted in the isolation of a tertiary phenolic bisindole alkaloid, 10'-hydroxyusambarensine, which was identified by detailed spectroscopic methods. And it was found that this Compound was moderately active against two strains of Plasmodium falciparum in vitro [70, 71].



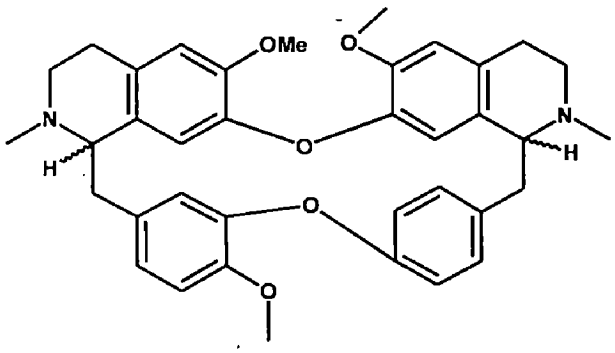
142.Cycleanine



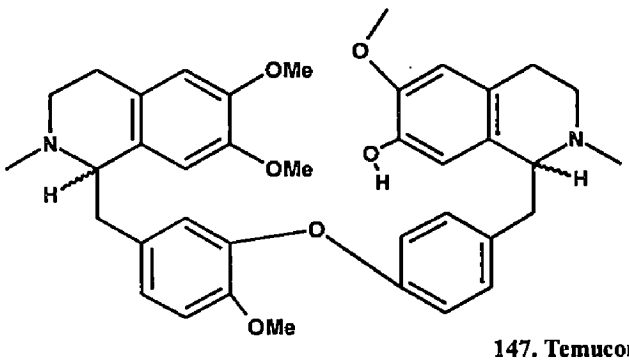
143. Cycleatjehine; R=H
144. Cycleathehemine; R=CH3



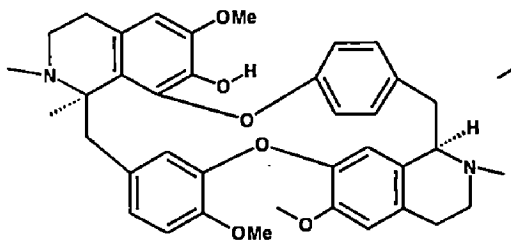
145.Makekulatine



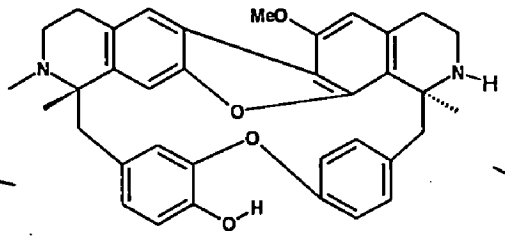
146. Repandine



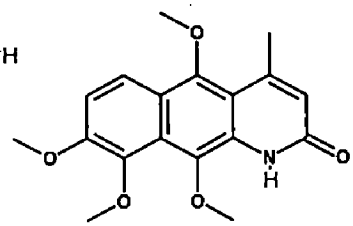
147. Temuconine



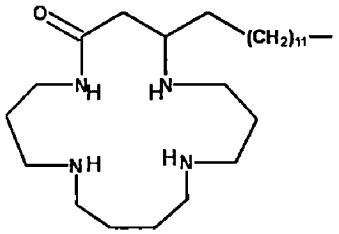
148. Curine



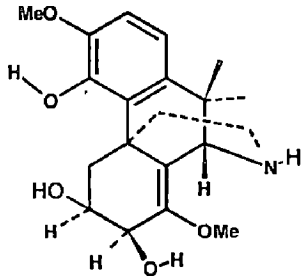
149. Norcosuline



150. Azanthuracene

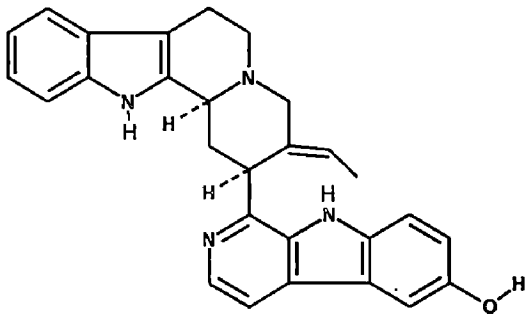


151. Budmunchiamine-L_4

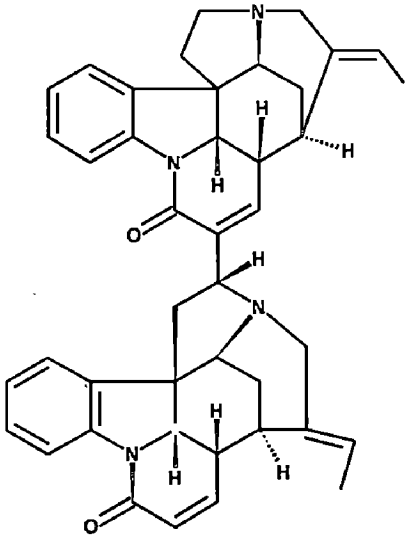


152. Tazopsine; R_1=OH, R_2= H

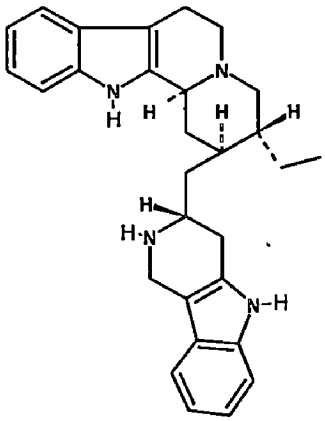
153. Sinucoculine; R_1= R_2= H



154. 10'Hydroxyusambarensis



155. Sungucine

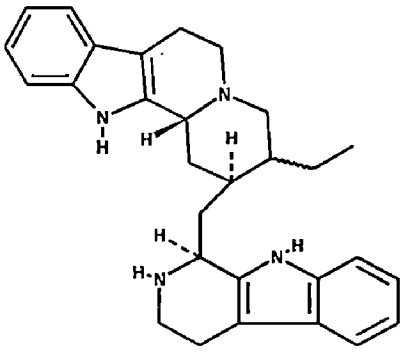


156. Ochralifuanine-A

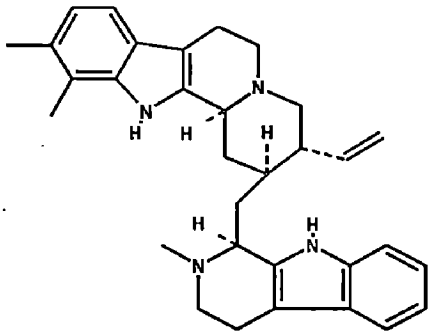
Frédérich et al (2002) showed in vitro antiplasmodial activities of alkaloids (compounds 156, 157, 158, 159) from various *Strychnos species* against Plasmodium falciparum. IC50 values for these alkaloids ranging from 80 nM to 190 nM [72].

Kuo et al (2003) identified new alkaloids (160, 161, 162) having in vitro cytotoxic and antimalarial activities isolated from the roots of *Eurycoma longifolia* [73].

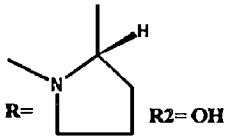
Topcu et al (2003) showed antimalarial activity of sesquiterpenes, compound **163,** extracted from *Laurencia obtuse*, with IC_{50} values of 2700 ngm/mL against *Plasmodium falciparum* [74].

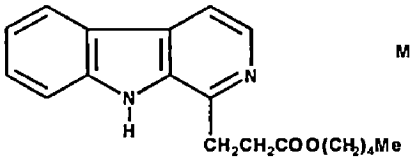


157. Ochralifuanine-F

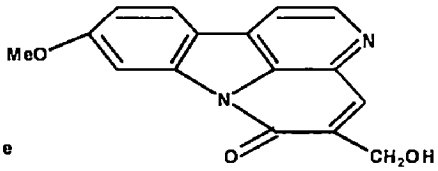


158. Usambarine; R1=R_2= H
159' Isostrychnopentamine;

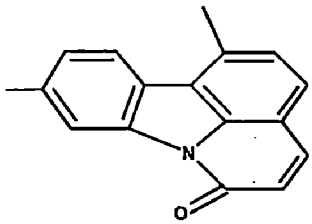




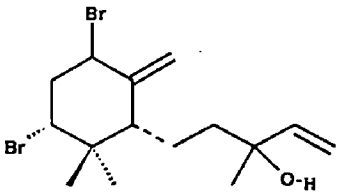
160. n-pentyl-beta-carboline-1-propionate



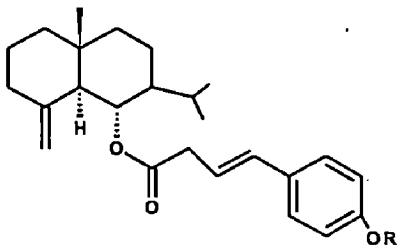
161. 5-Hydroxymethyl-9-methoxycanthin-6-one



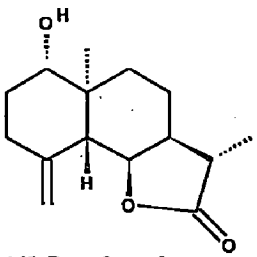
162. 1-Hydroxymethyl-9-methoxycanthin-6-one



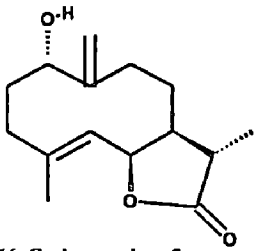
163. Bromo-Synderol



164. Eudesrn-4-ene



165. Benzofuran-2-one derivative

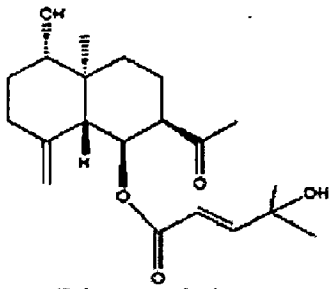


166. Cyclo-annulene-3-one derivative

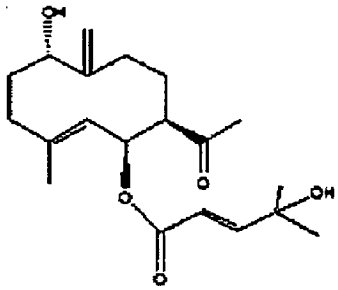
Prakash Chaturvedula et al (2004) and Garzo'n et al (2005) concluded Antiplasmodial Activity of Sesquiterpenoid Metabolites from *Melampodium camphoratum* (compound **164**) and a Caribbean Gorgonian Coral, *Eunicea sp* (compounds **165-169**) [75, 76].

Kraft et al (2003) studied *in vitro* antiplasmodial activities of extracts from *Vernonia colorata* (compounds **170, 171, 172**), and proved that *Vernonia colorata was* active against *Plasmodium falciparum* with IC_{50} values ranged from 4.3μg/mL to 12.6μg/mL [77].

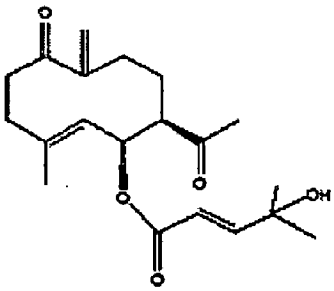
François et al (2003) and Karioti et al (2008) identified sesquiterpene lactones from *Amica montana* (**173, 174, 175**) and *Anthensis auriculata* (**176, 177, 178**) respectively which show *in vitro* activities against *Plasmodium falciparum.* Their IC50 values were situated in the range of 0.23 to 7.41 μM [78, 79].



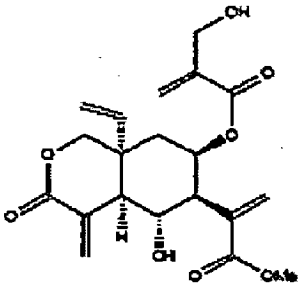
167. 2-enoate-derivative



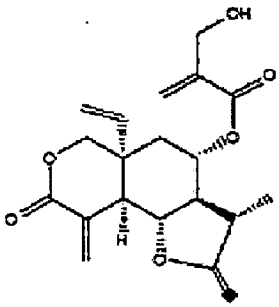
168. dimethylpent-2-enoate derivative



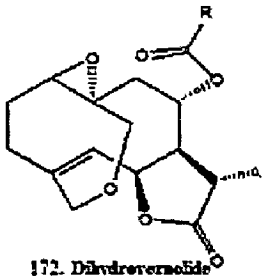
169. dimethylpent-2-enoate derivative



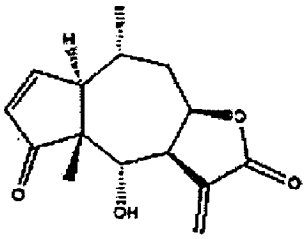
170. Vernodalol



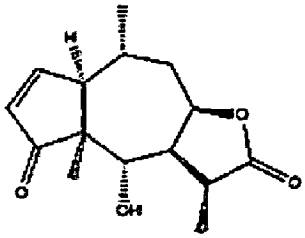
171. Dihydrovernidalin



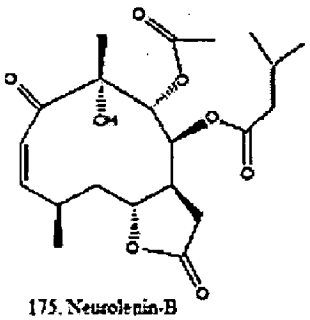
172. Dihydrovernolide



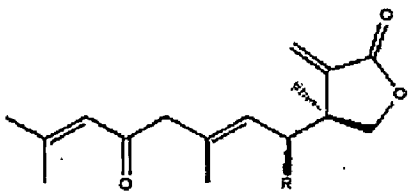
173. Helelin



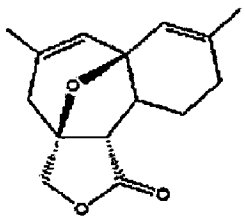
174. Dihydrohelelin



175. Neurolenin-B



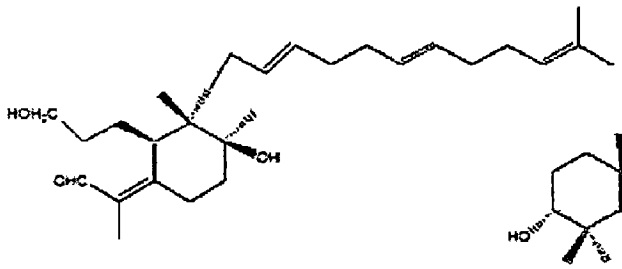
176. 4-Hydroxyanthecotulide; R=OH
177. 4-Acetoxyanthecotulide; R=OAc



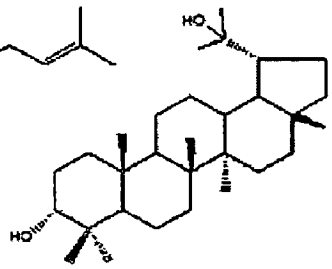
178. Anthecularin

Benoit-Vical et al (2003) showed in *vitro* activity of Iridal, a triterpenoidic compound extracted from *Iris-germanica*, on *Plasmodium falciparum* [80].

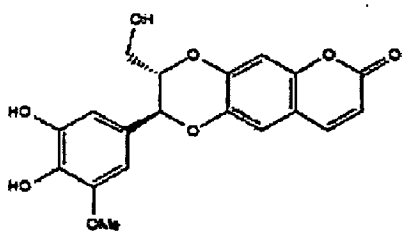
Cuiying Ma et al (2006) showed in vitro antimalarial activity of compounds (**180, 181, 182, 183, 184**) from Grewia bilamellata against *Plasmodium falciparum* [81].



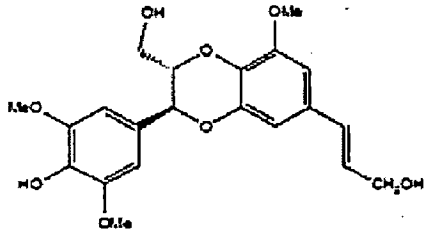
179. Iridal



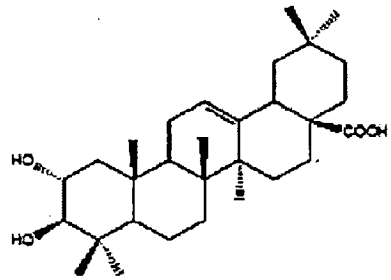
180. Lupandiol



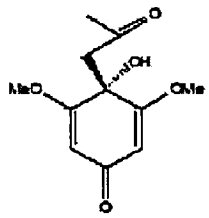
181. Grevain



182. Nordamin

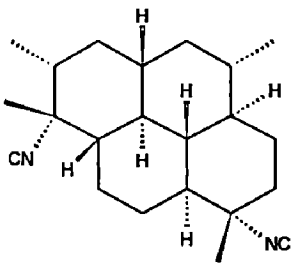


183. Dihydroxyolean

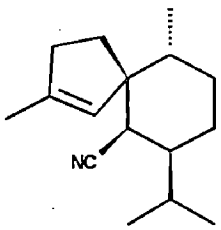


184. Dimethoxy-1-acetoxyquinol

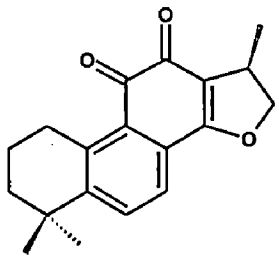
Limmatvapirat et al (2004) showed antiviral and cytotoxic activities of isoflavanquinone, abruquinone-B from the aerial parts of *Abrus precatorius* [82, 83, 84, 85, 86].



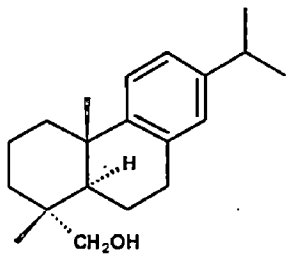
185. Diisocyanoadociane



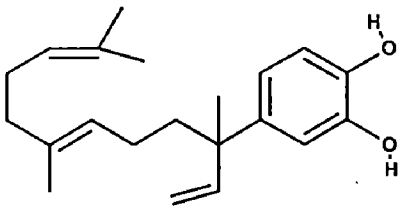
186. Axisonitrile-3



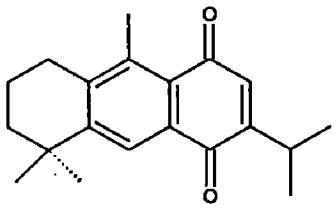
187. Cryptotanshinone



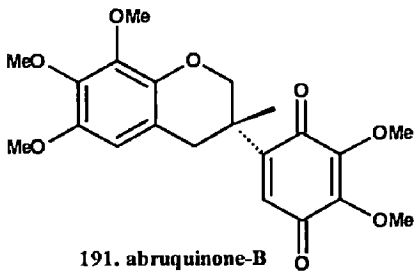
188. Dehydroabietinol



189. 4-nerolidykatechol



190.12,16-Dideoxyaegyptinone-B



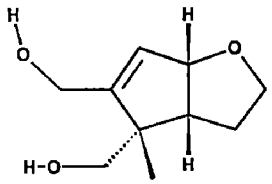
191. abruquinone-B

Tasdemir et al (2005) investigated anti-protozoal and inhibitory effect of *Scrophularia*

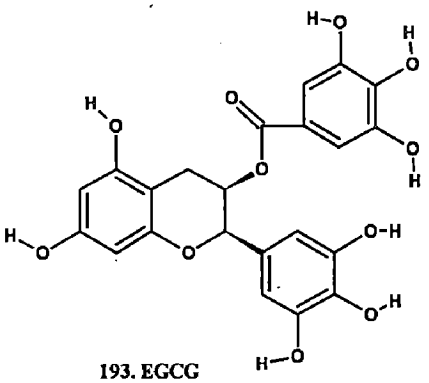
lepidota (compound **192**), an endemic plant of the Turkish flora, towards *Plasmodium falciparum* [87].

Sharma et al (2007), Oketch-Rabah et al (1997), George et al (2004) and Yenesew et al (2004) concluded *in vitro* anti-plasmodial activity of extracts of tea catechins(compound **193**), *Asparagus africanus* (compound **195**) (IC_{50} = 49 µM), *Cajanus cajan* (compound **196**) (IC_{50} <100 µgm/ml) *and Erythrina abyssinica* (compound **197**) (IC_{50} = 63 µgm/ml) against the *Plasmodium falciparum* [88, 89, 90, 91].

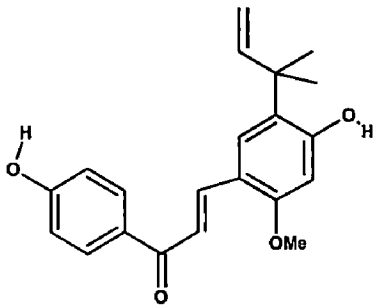
Likhitwitayawuid et al (1998) found xanthone (compounds 199, 200) from the bark of *Garcinia cowa*, possess in vitro antimalarial activity against *Plasmodium falciparum* with IC_{50} values ranging from 1.50 to 3.00µgm/ml [92].



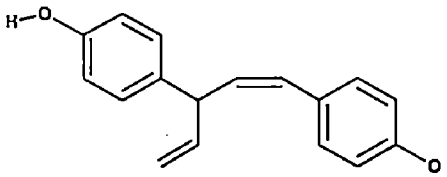
192. Iridoid



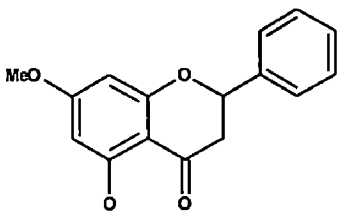
193. EGCG



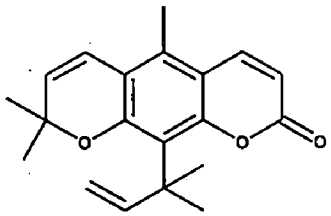
194. Licochalcone-A



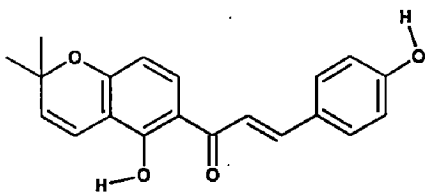
195. Nyasol



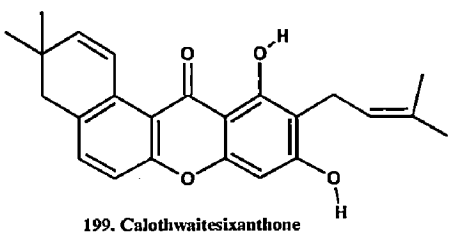
196. Pinostrobin



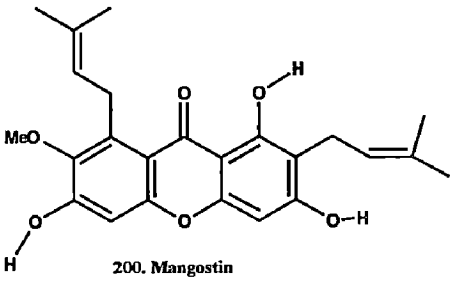
197. Dentatin



198. 4-Hydroxyonchocarpin



199. Calothwaitesixanthone

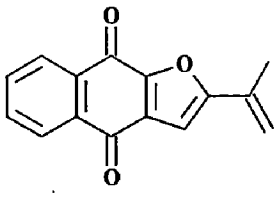


200. Mangostin

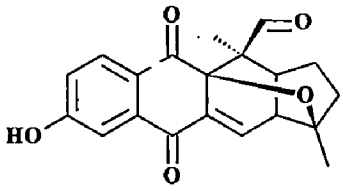
Weiss et al (2000) naphthoquinoids (compounds **201, 202**) from *Kigelia pinnata* rootbark possessed in vitro against *Plasmodium falciparum* strains with IC_{50} values 0.15 μM and 0.25 μM[93].

Tansuwan et al. (2007) identified in vitro activity of benzoquinone metabolites, 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (**203**) and xylariaquinone-A (**204**) from an endophytic fungus, *Xylaria sp.* against *Plasmodium falciparum*, IC_{50} values of 1.84 and 6.68 μM respectively [94].

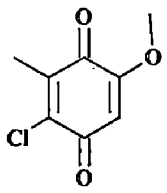
Laurent et al. (2006) found that Xestoquinone (compound **205**) from *Xestospongia* showed in vitro antiplasmodial activity against P. *falciparum* strain with an IC_{50} of 3 μM [95].



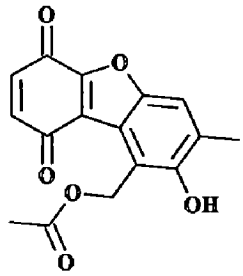
201. Naphthoquinoid



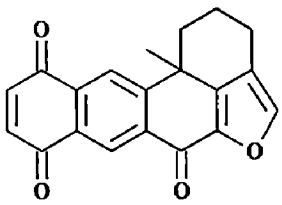
202. Isopinnatal



203. 2-chloro-5-methoxy -3-methyl-1,4-benzoquinone



204. Xylariaquinone-A



205.. Xestoquinone

CHAPTER 3

TOOLS AND TECHNIQUES

In the present work 205 natural products is taken for docking study from different plants species which are already found to be potent against malaria.

3.1. SKETCHING TOOLS

3.1.1. ChemDraw

ChemDraw is a molecular editor developed by the chem.-informatics company Cambridgesoft® andwas first used in 1986 in U.S. ChemdDraw is an outstanding package for chemical drawing. It creates stereochemically correct structures which can be saved as cdx files. It identifies stereochemistry using cahn-Ingold- prelog rules. It provide bond tools, ring tools, acyclic chain tools, automatic error check etc for sketching. It can automatically create structure from chemical name and vice versa. ChemDraw is based on Molecular mechanics for optimizing models. Moreover it also provides geometry optimization using MOPAC and Gaussian using AM1, PM3, MNDO and MINDO force field [96, 97].

3.1.2. Chem sketch

ACD/ChemSketch Freeware is one of the most accurate software from ACD/Labs developed to help chemists for drawing chemical structures, reactions, schematic diagrams and designing other chemistry related reports. It also includes features such as calculation of molecular properties (e.g., molecular weight, density, molar refractivity etc.), 2D and 3D structure cleaning and viewing, functionality for naming structures (fewer than 50 atoms and 3 rings), and prediction of log*P* [97, 98].

3.1.3. Accelrys Draw

Accelrys Draw enables scientists to draw and edit complex molecules, chemical reactions and biological sequences with ease, facilitating the collaborative searching, viewing, communicating, and archiving of scientific information [97, 99].

3.2. VISUALIZERS

3.2.1. Discovery studio visualizer

Molecular visualization is a key aspect of data analysis that can provide understanding about a molecular structure on certain interactions and biochemical reactions. DS Visualizer provides functionality for visualizing, analyzing and sharing biological and chemical data. It allows molecular data to be viewed from multiple perspectives by providing the options to view data through 3D structures [100].

3.2.2. Visual molecular dynamics (VMD)

VMD is a molecular modeling and visualization program for displaying, analyzing and displaying large biomolecules using 3-D graphics and analyze biopolymers such as proteins, nucleic acids, lipids, and membranes. It is written in C and C++ and has been developed by the theortical and computational biophysics group at University of University of Illinois and the Beckman Institute. Its original version was developed in 1992 by Mike Krogh, Bill Humphrey, and Rick Kufrin. The current developer of VMD is John E. Stone. VMD provides a wide range of molecular representations, coloring styles, and transparency and material properties (3). Molecules may be drawn as lines, bonds, CPK, licorice, VDW spheres, ribbons, tubes, surface, secondary structure cartoons, points, C-alpha traces, and surfaces. VMD is a general application for displaying molecules containing any number of atoms and is similar to other molecular visualization programs in its basic capabilities. VMD reads data files using an extensible plugin system, and supports Babel for conversion of other formats. User-defined atom selections can be displayed and exported to an image file [101].

3.2.3. Molegro Molecular Viewer (MMV)

Molegro Molecular Viewer (MMV) is an application for studying and analyzing how ligands interact with macromolecules. *Molegro Molecular Viewer* is developed by: Molegro ApS. Molegro Molecular Viewer is based on the notion of workspaces. The *workspace* is the central component and represents all the information available to the user in terms of molecules (proteins, ligands, cofactors, water molecules, and poses), user-defined constraints

(visualized as small spheres), cavities (visualized as a grid mesh), and various graphical objects (molecular surfaces, backbone visualizations, labels, etc) [102].

3.2.4. UCSF Chimera

UCSF Chimera is a highly extensible program for interactive visualization and analysis of molecular structures and related data, including sequence alignments, docking results, trajectories, and conformational ensembles. Chimera is developed by the Resource for Biocomputing, Visualization, and Informatics, funded by the National Institutes of Health National Center for Research Resources and National Institute of General Medical Sciences. It gives High-quality images [103].

3.2.5. Avogadro

Avogadro is an open source advanced molecule editor and visualizer designed for cross-platform use in computational chemistry, molecular modeling, bioinformatics, materials science, and related areas [104].

3.3. DOCKING TOOLS

205 natural plant products were docked using **AutoDock 4.2, MVD 5.0** and **iGEMDOCK 2.1** were used for docking simulation. For validation of data docking process has been repeated about 3-5 times for each compound.

3.3.1. AutoDock 4.2

The program AutoDock was developed to provide a procedure for predicting the interaction of small molecules with macromolecular targets. Autodock uses Lamarckian Genetic Algorithm (LGA), which is the hybrid of Genetic algorithm and local search algorithm for conformation searching. This algorithm first built a population of individuals (gene), each gene being a different random conformation of the docked compound [105, 106].

Method

Protein preparation- The crystal structure of wild type Plasmodium DHFR-TS complexed with WR99210, NADPH and dUMP was retrieved from Protein data bank (PDB code: 1J3I). Chain A was selected for the docking simulations. The inhibitor, cofactor NADPH and all water molecules were removed from the structure. Preparation of target protein with ADT (Autodock Tool) involved the addition of polar Hydrogen to the macromolecule, an essential step to correct calculation of partial charge. Finally Gasteiger charges were added for each atom of the macromolecule.

Ligands preparation- The database of 200 natural structures used in our molecular docking studies were derived from different medicinal plant extracts. WR99210 is used as reference molecule in our docking studies on DHFR. The three-dimensional models of all the molecules under investigation were built by ChemDraw software, ChemSketch and Symyx-Draw4.0. Resulting geometries were optimized by Avogadro and energy minimization was done by AM1 force field.

TABLE 3.1: SELECTION OF ALGORITHM AND SEARCH PARAMETERS

SEARCH PARAMETERS	SEARCH ALGORITHMS	RMSD VALUES
Genetic Algorithm	Lamarckian Genetic Algorithm	1.32
	Genetic Algorithm	1.89
	Simulated Annealing	2.03
	Local Search	2.11
Simulated Annaling	Lamarckian Genetic Algorithm	>2
	Genetic Algorithm	>2
	Simulated Annealing	>2
	Local Search	>2
Local Search Parameter	Lamarckian Genetic Algorithm	>2
	Genetic Algorithm	>2
	Simulated Annealing	>2
	Local Search	>2

Grid parameter setting and docking calculation- The docking area was assigned visually around the presumed active site. A grid of $76A^{\circ}\times76A^{\circ}\times76A^{\circ}$ with grid spacing of $0.375A^{\circ}$ was positioned around the active site with all ligand atom types using Autogrid, Additionally; an electrostatic map and a desolvation map were also calculated.

Each docking calculation consists of 25 million energy evaluation (ga_num_evals) using Lamarckian Genetic Algorithm Local Search method (GALS). All the parameters are set to defaults values except ga_run= **50**, maximum no. of generation (ga_num_generation)= 27000, mutation rate (ga_mutation _rate)= 0.02, crossover rate (ga_crossover_rate)= 0.8, Local search on an individual in the population (ls_search_frequency)= 0.06 and maximum no. of iterations per local search was set to 300. The docking results were clustered on the basis of root mean square deviation (rmsd) and were ranked on the basis of free energy of binding [107, 108, 109].

3.3.2. Molegro virtual docker

Molegro Virtual Docker (MVD) is an integrated environment for studying and predicting protein - ligand interactions. MVD handles all aspects of the docking process from preparation of the molecules to determination of the potential binding sites of the target protein, and prediction of the binding modes of the ligands. The identification of ligand binding modes is done by iteratively evaluating a number of candidate solutions (ligand conformations) and estimating the energy of their interactions with the macromolecule. MVD requires a 3-D structure of both protein and ligand. MVD performs flexible ligand docking, so the optimal geometry of the ligand will be determined during the docking. It has been found that Molegro Virtual Docker has higher docking accuracy than many others docking software.

Docking algorithms

a) **MolDock Optimizer:** The default search algorithm used in MVD is the MolDock Optimizer, which is based on an evolutionary algorithm. In MVD, selected parameters were used for the guided differential evolution algorithm: number of runs = 10 (by checking constrain poses to cavity option), population size = 50, maximum iterations = 2000, crossover rate = 0.90 and scaling factor = 0.50. A variance-based termination scheme was selected rather than root mean square deviation (RMSD). To ensure the most suitable binding mode in the binding cavity, pose clustering was employed, which led to multiple binding modes.

b) **MolDock SE**: MolDock SE (simplex evolution) is an alternative search heuristic which can be used together with either the *MolDock* or *MolDock [Grid]* scoring functions. Following parameters were set for pose generation, maximum iterations= 1500, population size= 50. The pose generator tests a number of different torsion angles, rotations and translations, evaluates the affected part of the molecule and chooses the value resulting in the lowest energy contribution. For poses generated, the energy threshold was set to 100.0. At each step, at least 10 min torsions were tested and the one giving the lowest energy was chosen. The 10 max tries number is lowered to the 10 quick try values. The Simplex

Evolution parameters were set at 300 maximum steps with neighbor distance factor of 1.0 [112, 113]

Scoring function

a) **MolDock Score:** The MolDock scoring function (MolDock Score) used by MVD is derived from the PLP scoring functions originally proposed by *Gehlhaar et al* and later extended by *Yang et al(2004)*. The MolDock scoring function further improves these scoring functions with a new hydrogen bonding term and new charge schemes. The docking scoring function, Escore, is defined by the following energy terms:

$$E_{score} = E_{inter} + E_{intra}$$

Where E_{inter} is the ligand-protein interaction energy

The ignore-distant-atoms option was used to ignore atoms far away from the binding site. Additionally, hydrogen bond directionality was set to check whether hydrogen bonding between potential donors and acceptors can occur. The binding site on the protein was defined as extending in X, Y and Z directions around the selected cavity with a radius of 15 Å.

b) **MolDock Score [GRID]:** The MolDock Score (Grid) is identical to the MolDock Score except that hydrogen bond directionality is not taken into account. The grid-based scoring function provides a 4-5 times speed-up by pre-calculating potential-energy values on an evenly spaced cubic grid. The rest of the terms in the MolDock Score (Grid) version (i.e., internal ligand energy contributions and constraint penalties) are identical to the standard version of the scoring function. A grid resolution of 0.80 Å was set to initiate the docking process.

c) **Plant Score**: The PLANTS scoring function (PLANTS Score) used by MVD is derived from the PLANTS scoring function originally proposed by Korb et al..

Validation of Docking Protocol

In order to develop the docking methodology, we first try to demonstrate that bound conformations could be reproduced in silico. For this purpose, WR99210 from the complexes 1J3I was re-docked in MVD. The fitness evaluation of each re-dcoked pose was evaluated by considering the RMSDs values, docking scores and position of pose. The selected re-docked pose was further evaluated by its interactions and energetic analysis to investigate the efficiency of the docking search algorithm and scoring function by comparing its values with the bound conformation [114, 115, 116].

Method:

Protein preparation

3-D X-ray crystallized structure of wild type Pf-DHFR (PDB: 1J3I) was downloaded from the Protein Data Bank. The downloaded protein has two chains A, B, C and D with 233, 231, 426 and 427 residues respectively together with NADPH, DUMP and WR99210 as ligands. It also contains 787 water molecules of crystallization. The co-crystallized ligands and water molecules were removed using Discovery studio3.1 and protein structure was prepared by using MVD. The prepared protein was then taken as receptor protein. Binding sites of WR99210 has been selected for docking simulation [114].

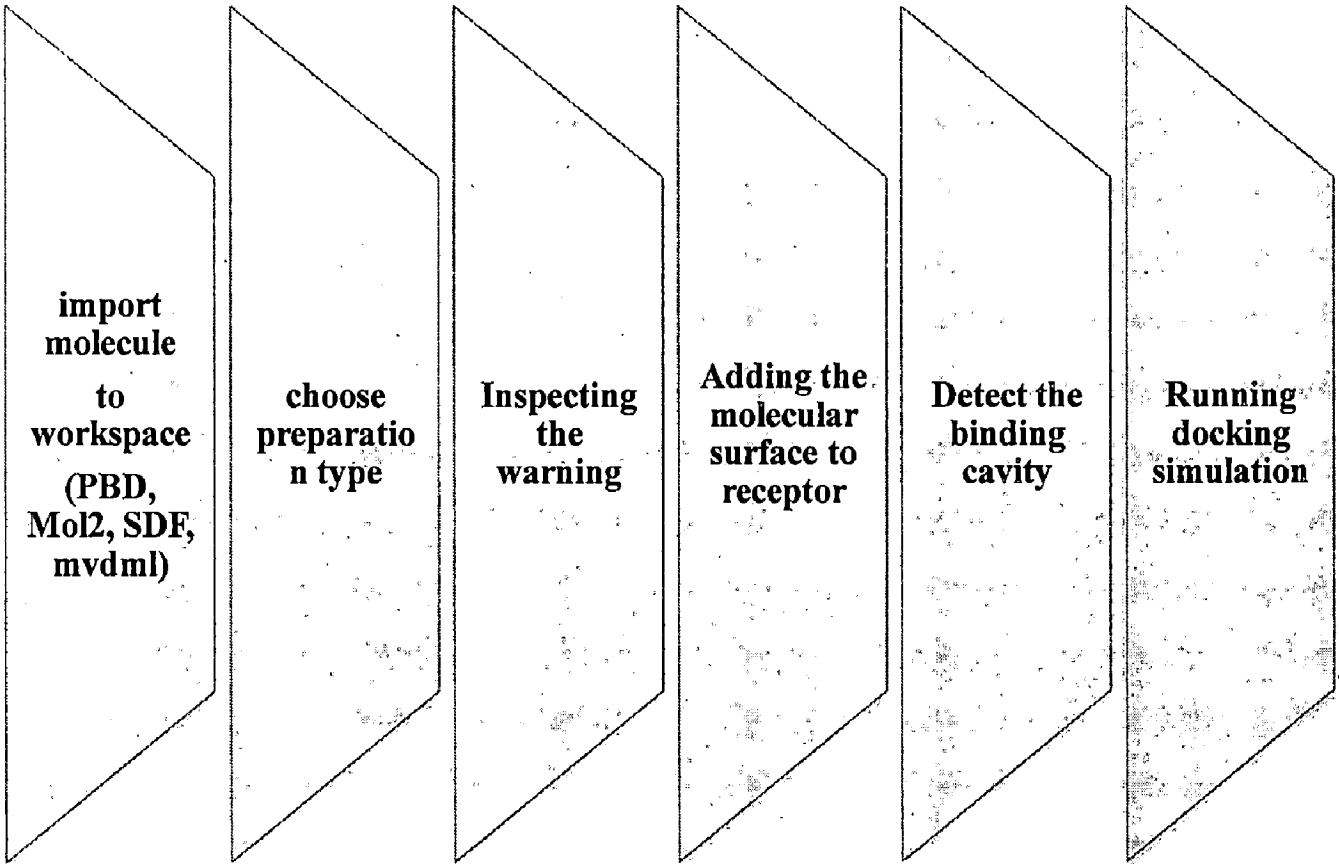
Ligands preparation

All the selected ligands were prepared by Chemdraw 8.2, energy is minimized usind AM1 force field and geometry is optimized by Avogadro software.

Docking study with MVD

It is automated docking software. The preparation of protein and ligands were done using default parameters, which automatically adds the missing hydrogen atoms. The software has module to create surface over receptor molecule and to give possible binding site for its activity. On average, 10 docking runs were made to obtain high docking accuracy. The active site region of the receptor protein for WR99210 was chosen for docking, which is already

known from literature. It gives ten conformations for each ligand and returns five outputs with MolDockScore and Rerank score and other thermodynamically calculated values. The MolDockScore is an anonymous value on which we have to suggest the best docked ligand with its conformation. It also shows hydrogen bond information, which suggests the formation of stable complex between ligand and receptor molecule.



Working scheme of Molegro Virtual Docker (MVD)

The validity of docking protocol mainly focuses on the similarity of re-docked poses to the crystallographically identified bound orientations. Each docking protocol returned multiple docking poses and a symmetry corrected RMSD was computed for all poses. Of these, MolDock SE combined with MolDock Score (Grid) gave the lowest *RMSD* values for co-crystallized ligands, that is only ***0.42 Å*** deviations between the top-ranked poses and the experimental structures of WR99210 (Figure1). The RMSD value demonstrates that MVD is accurate in reproducing the experimental binding mode [114].

TABLE 3.3.2.1: RMSD Values of Re-docked WR99210 with wild type Pf-DHFR(1J3I)

SCORING FUNCTION	SEARCH ALGORITHMS	RMSD VALUES
Moldock score	Moldock SE	0.57
	Moldock Optimizer	1.89
	Iterated Simplex	0.94
MolDock score[grid]	Moldock SE	0.42
	Moldock Optimizer	0.61
	Iterated Simplex	0.82
Plants Score	Moldock SE	0.69
	Moldock Optimizer	0.95
	Iterated Simplex	1.23
Plants Score[Grid]	Moldock SE	0.65
	Moldock Optimizer	0.74
	Iterated Simplex	0.83

Selection of algorithm and scoring function

As search algorithm MolDcok SE in combination with the scoring function MolDock Score (Grid) gives the lowest RMSD values of re-docked poses with reference to the bound crystal conformations. Also In 1J3I, the total pose energy of the bound WR99210 was found to be -118.34 kcal/mol (-129.705 kcal/mol for re-docked pose). Therefore, we decided to apply this docking protocol for Docking.

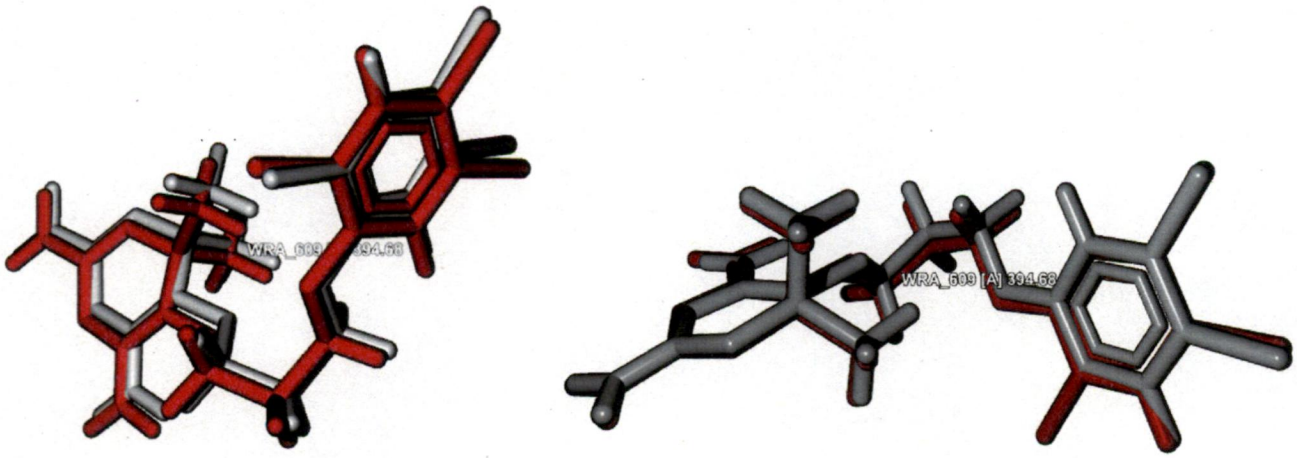


Figure 3.3.2.1: Crystal structure and re-docked pose of RW99210

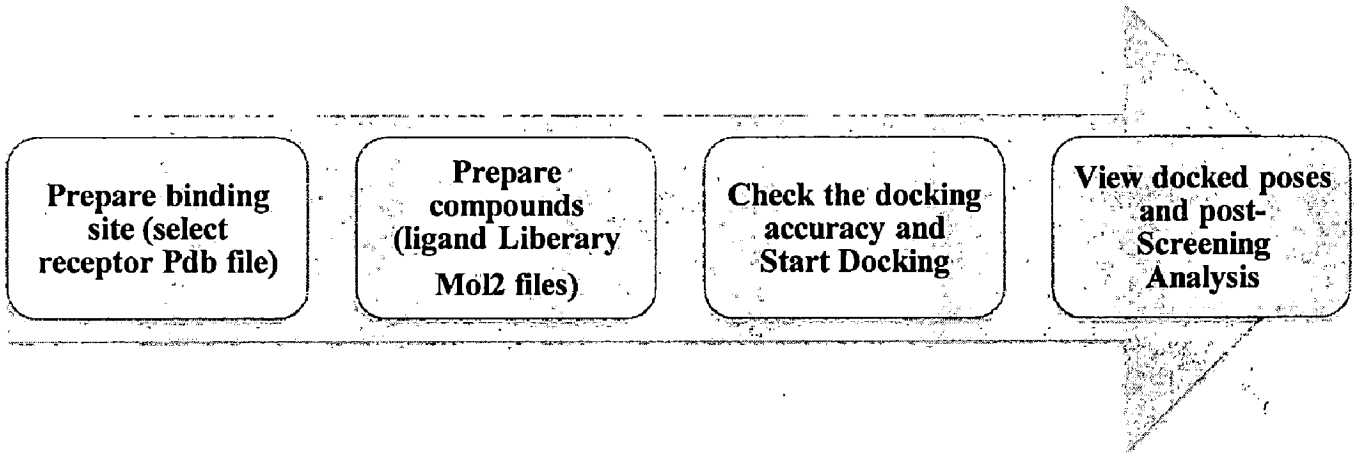
3.3.3. iGemdock

Method

iGemDock is another docking software which is developed by the University of National Chiao Tung, Taiwan. iGEMDOCK is a graphical-automatic drug discovery system, for integrating docking, screening, post-analysis, and visualization. An empirical scoring function and evolutionary approaches are adopted in iGEMDOCK. iGEMDOCK computes a ligand conformation and orientation relative to the binding site of target receptor by using a generic evolutionary method (GEM). After the ligands and protein were prepared, we set the size of binding site (8 Å) and the parameters such as initial step sizes ($\sigma = 0.8$, $\Psi = 0.2$ (in radius)), family competition length ($L = 2$), population size ($n = 300$), recombination probability ($pc = 0.3$), generations (80) and number of solutions ($n = 10$) to values typically employed in a standard docking run [117, 118, 119].

Ligand preparation:

Before Docking we need to generate 3-D ligand files. iGEMDOCK can accepts the Mol, Sybyl Mol2 and Pdb file format for ligand files. But we generally take the ligand files in mol2 format as the input of iGEMDOCK. Thus we prepared a ligand library in MOL2 format for virtual screening.



Virtual screening protocol of iGemdock

3.4. OTHER TOOLS

3.4.1. Open-Babel

OpenBabel is an open chemical software toolbox mainly used for converting chemical file format. It can convert over 100 file format. In addition to convert file format it offers a complete programming liberary for developing chemistry software. The liberery is written in C++ language [120].

3.4.2. PharmaGist

Prediction of molecular interactions is a major goal in rational drug design. PharmaGist is a freely available web server for detecting pharmacophores or the spatial arrangement of features that enables a molecule to interact with the target receptor. The employed method is ligand based and does not require the structure of the receptor. Instead, the input is a set of structures of drug-like molecules that are known to bind to the receptor. The method is highly efficient, we can take up to 32 drug-like molecules in a single mol2 format file and it takes few minutes on a stardard PC. Another important characteristic of the method is the capability of detecting pharmacophores shared by different subsets of input molecules. This capability is a key advantage when the ligands belong to different binding modes [121].

CHAPTER 4

RESULT AND DISCUSSION

Docking analysis

The most important parts of any screening study are the analysis of the docking results. We have used three docking/Screening engines for our study.

Strategies used:

(1) First we have re-docked the crystal structure third generation inhibitor WR99210, a known active inhibitor against target protein, and the result were used to define as a baseline energy value for the selection of screening result.

(2) The rank of each compound was determined by the binding free energy of the lowest energy cluster. We found that mostly the most populated cluster coincided with the lowest energy cluster, but in some cases we have violated this convention. For example in the case of "4-hydroxy canthin-6-one" we got two clusters. Lowest energy cluster has energy of -8.05Kcal/mol with only single conformation, while most populated cluster has remaining 96% conformation with average binding energy of -7.80Kcal/mol. In this case we have ranked this compound by considering its free energy as -7.80Kcal/mol, instead of -8.05 Kcal/mol. This is done because only ranking by lowest docked energy can sometime favors unreliable single member cluster that is prone to disappear in the repeated docking or when docking parameters are modified. Further largest cluster are less sensitive to change in docking parameters, which result in more stable ranking. Several studies have shown that in docking calculation the most populated cluster of the docked ligand conformations are better predictor of the native state than the usual approach of selecting the lowest energy cluster.

(3) Furthermore Autodock have a typical error of ±2 Kcal/mol in the prediction of free energy of binding, so the estimated free energy of binding should not be used as a sole criterion for selection of ligand ranking. Visual inspection of the docked pose and interaction can greatly help to increase the success rate. In the visual inspection we are focused on following three things

(A) Is a ligand bound inside a pocket or pit in the receptor?

(B) Are non-polar atoms in the ligand docked near non-polar atom of the receptor? Are polar atoms in the ligands docked near polar atom in the receptor?

(C) Hydrogen bond interactions and hydrophobic interactions with the binding site residues of *Pf*- DHFR.

Results of Docked compounds with their Autodock binding energies and no. of Hydrogen bonds, MVD moldock score and iGEMDOCK fitness score are given in Table 4.1 (Annexure).

Top ranked compounds- On the basis of extensive docking following compounds are selected as top scorer-

Ochrolifuanine-A which was first extracted from *Strychnos* species, seems to be the most potent hit with Autodock binding energy -12.07kcal/mol (Figure 4.1) and GEMDOCK fitness score -114.7Kcal/mol and MVD Moldock Score is found as -176.166kcal/mol (Figure 4.2). The docked conformation found by AutoDock and MVD are comparable (Figure 4.3). This compound binds tightly to binding site by hydrogen bond interaction with Ser108 and Tyr 170. One side of binding site is completely hydrophobic with residues like Cys15, Ala16, Leu40, Val 45, Leu46, Ile 164 and Val195 while in just opposite site lipophilic residues are concentrate in a very small space, including residues Thr107, Ser108, Ser111 and 117, Gly165, Gly166 and Tyr170. Interestingly one Pyrrole ring of ligand also shows σ-π interaction with Gly166 and π-π interaction with Phe58 (Figure 4.4).

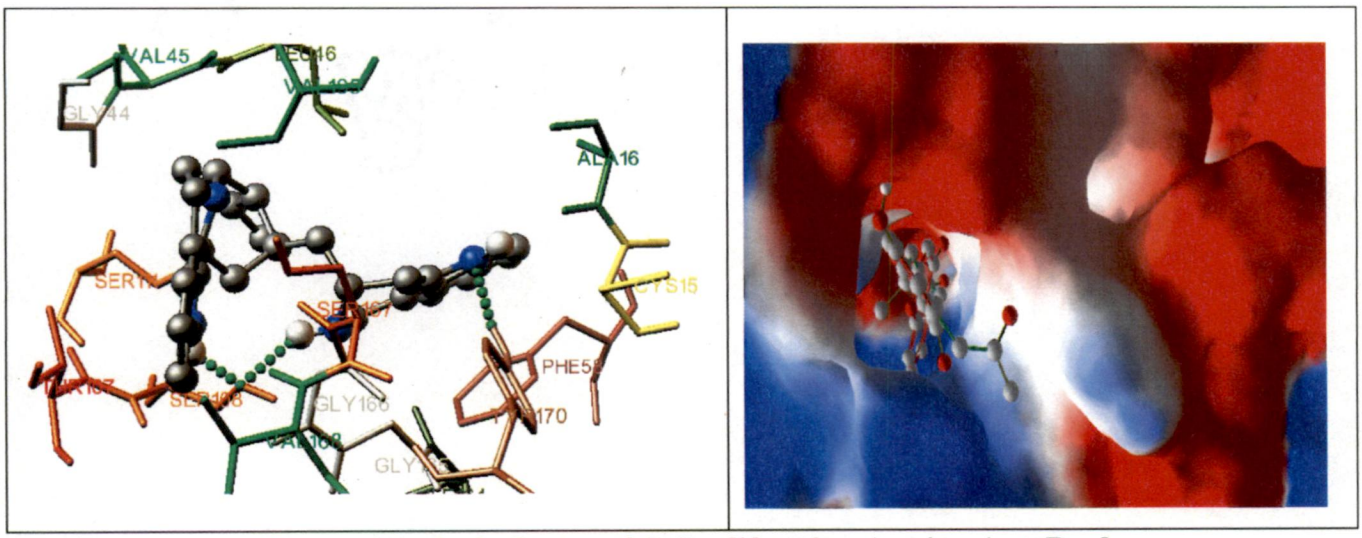


Figure 4.1: docked pose of Ochralifuanine-A using AutoDock

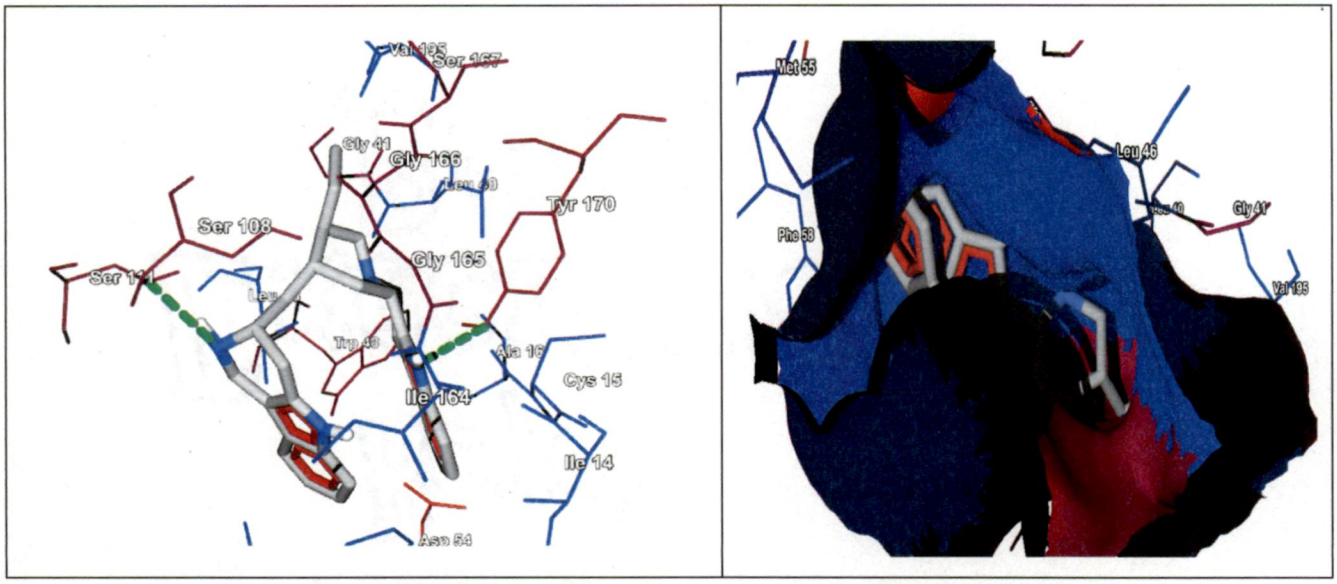


Figure 4.2: Docked pose of Ochralifuanine-A (energy= -176.67 kcal/mol) using MVD

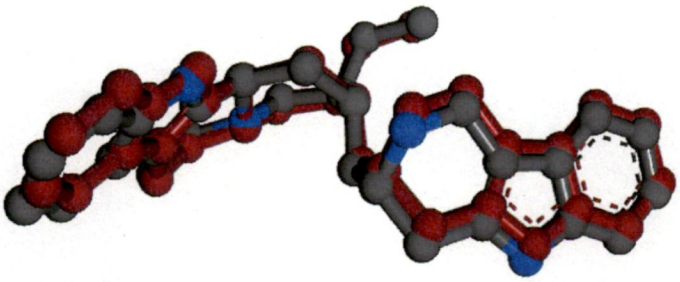


Figure 4.3: comparable (RMSD= 0.61 A°) docked conformation of Ochralifuanine-A using AutoDock4.2 (gray) and MVD5.0 (red)

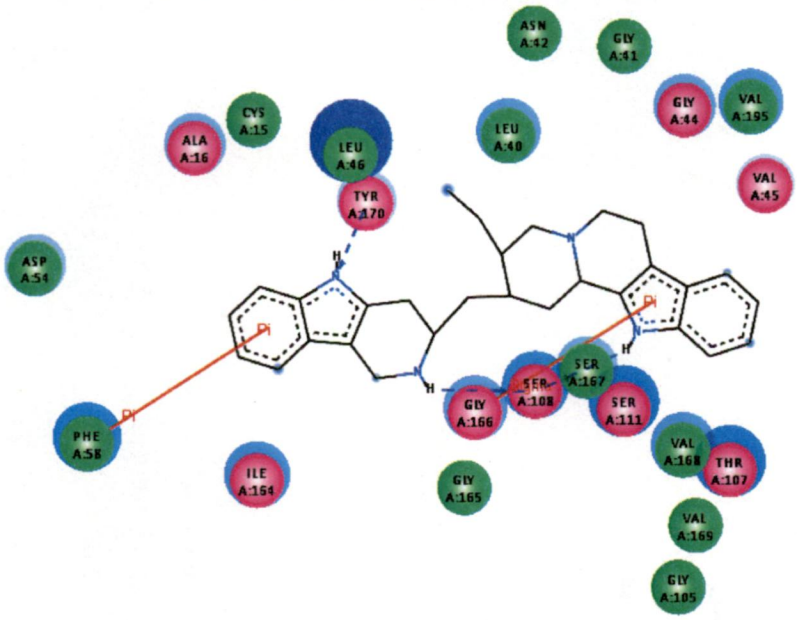


Figure 4.4: 2-D diagram of Ochralifuanine-A showing pi-pi and sigma-pi interaction

Bischromone-chrobisiamine is placed among the top ranked compound with Binding energy value -11.73kcal/mol, K_i in nanomolar range (2.51 nM) and its minimum energy conformation is shown in Figure 4.5. The chromenone moiety occupies a hydrophobic pocket of Ile14, Cys15, Ala16, Phe58, and Ile164 with only one hydrogen bond between Ala 16 with cyclic carbonyl oxygen while the remaining half is buried inside a region rich with Amino acid having low hydropathy index (Asn42, Lys43, Gly44, Thr107, Ser108 and Ser111) with two Hydrogen bonds with Gly44 and Asp194. Similar result can be shown with the other Docking engine MVD which gives high value of MolDock Score -167.247 for Bischromone and similar residues were involved in hydrogen bond interaction (Figure 4.6). iGemdock Score were also found to be good (-133.6). A 2-D diagram for Bischromone has been shown in the Figure 4.7.

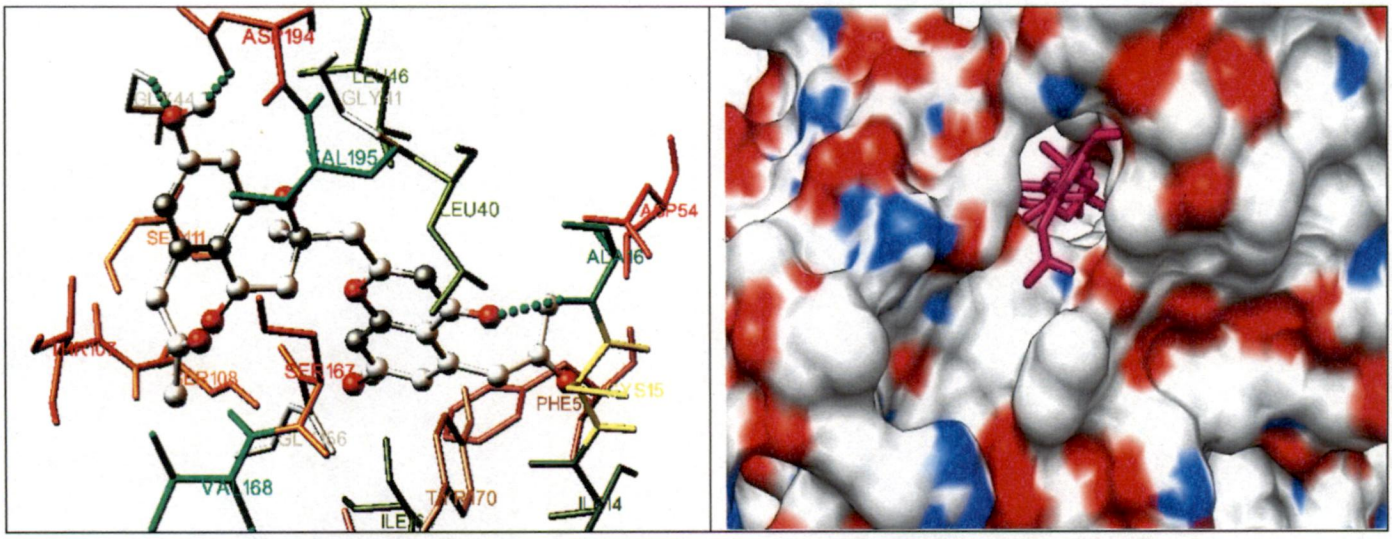


Figure 4.5: Docked pose of Bischromone-Chrobisiamine using AutoDock

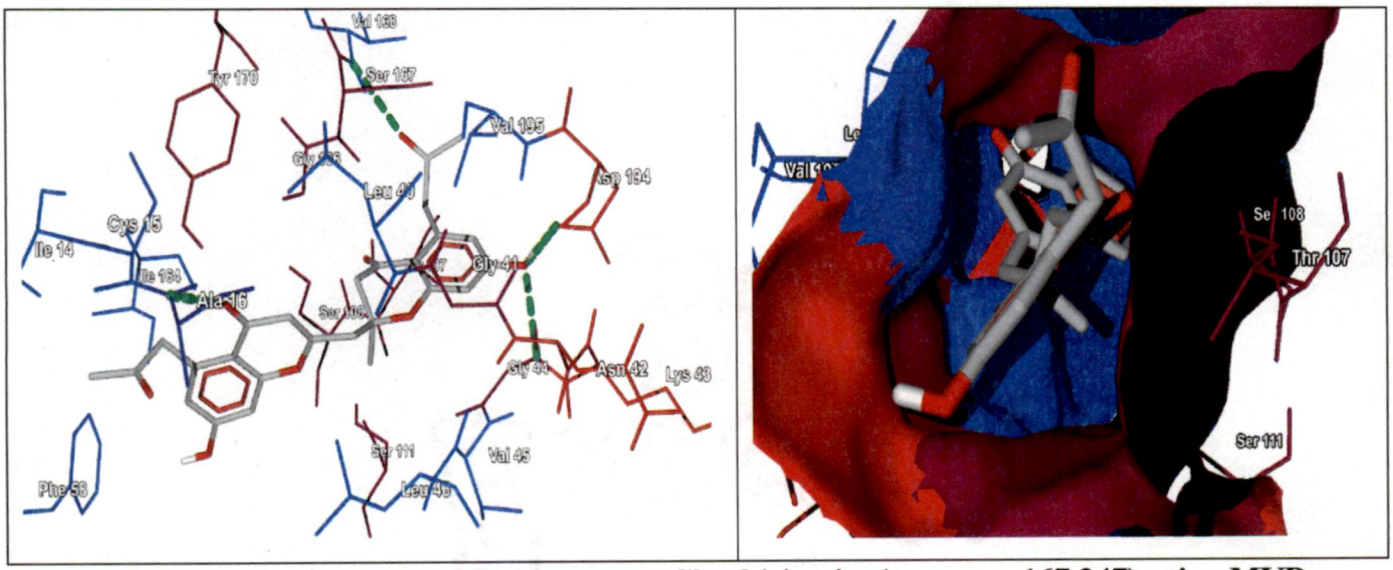


Figure 4.6: Docked pose of Bischromone-Chrobisiamine(energy= -167.247) using MVD

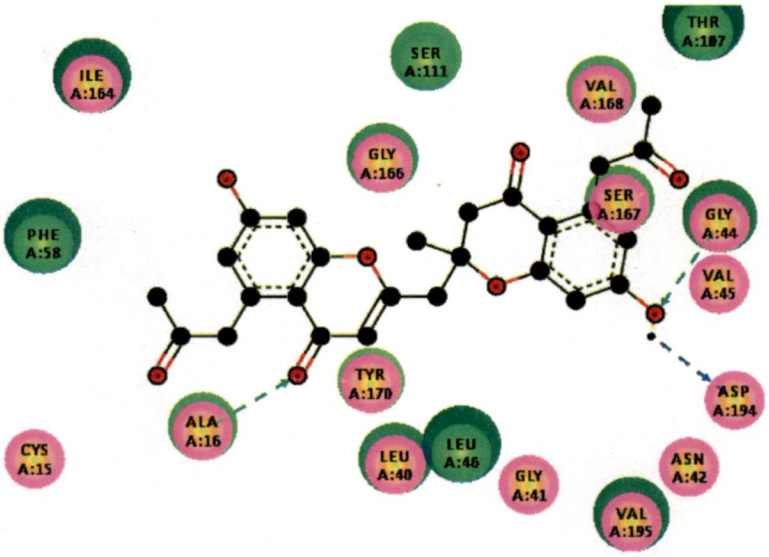


Figure 4.7: 2-D diagram showing interaction for Bischromone-chrobisiamine

Xanthene group of compounds- All these compounds are the active constituents of *Pentadesma butyracea* plant extract and possess same docked pose in the binding space. This group of compounds (77, 78, 79, 80, 81, 82, 83, 84, 85, 86) gave high docking score in all the docking engines (AutoDock with energies -8.96, -10.13, -8.98, -8.16, -9.14, -9.22, -9.47, -9.30, -8.98, -9.79 and 2, 4, 3, 4, 3, 5, 3, 4, 1, 4 hydrogen bonds respectively; MVD MolDock score -143.873, -153.919, -145.90, -139.496, -125.805, -139.734, -140.105, -138.353, -125.788, and -147.407; iGemDock gave fitness score from -112.6, -156.2, -125.1, -120.7, -114.6, -132.1, -128.0, -154.7, 106.5 and -117.8). These Compounds occupied similar hydrophobic binding pocket lined with Lys43, Asn44, Gly66, Thr107, Ser108 and Asp 194. The most promising compound among these xanthene compounds shows four hydrogen bonds with Gly44, Ser108, Gly166 and Asp194. Interestingly, two hydrogen bond interactions (with Asp194 and Gly44) were conserved in all the Xanthene derivatives.

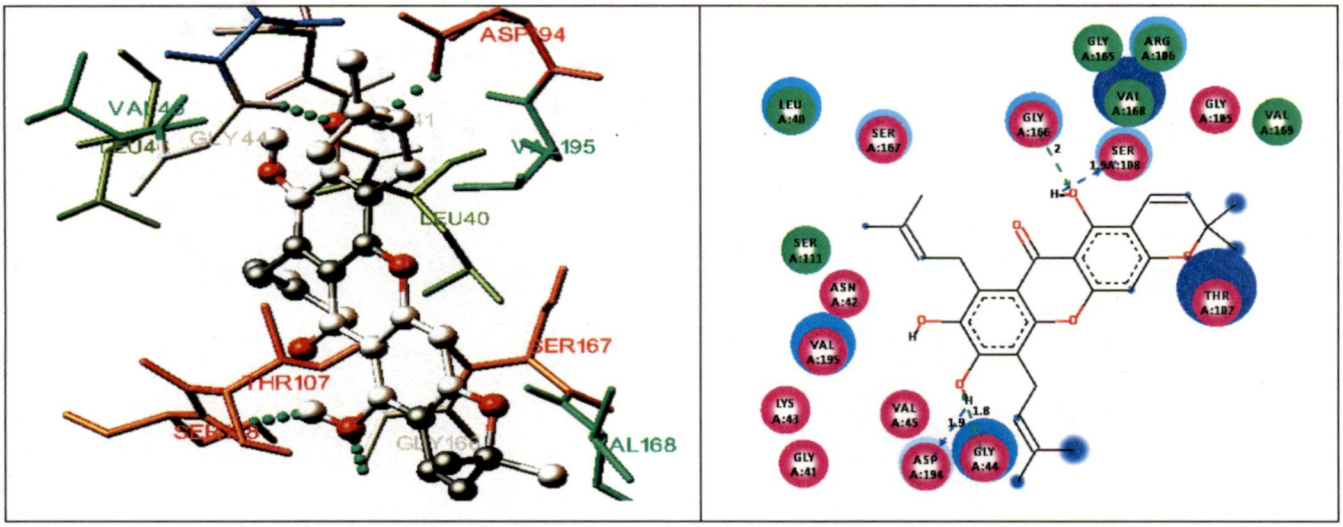


Figure 4.8: Docked pose of compound 78 using AutoDock

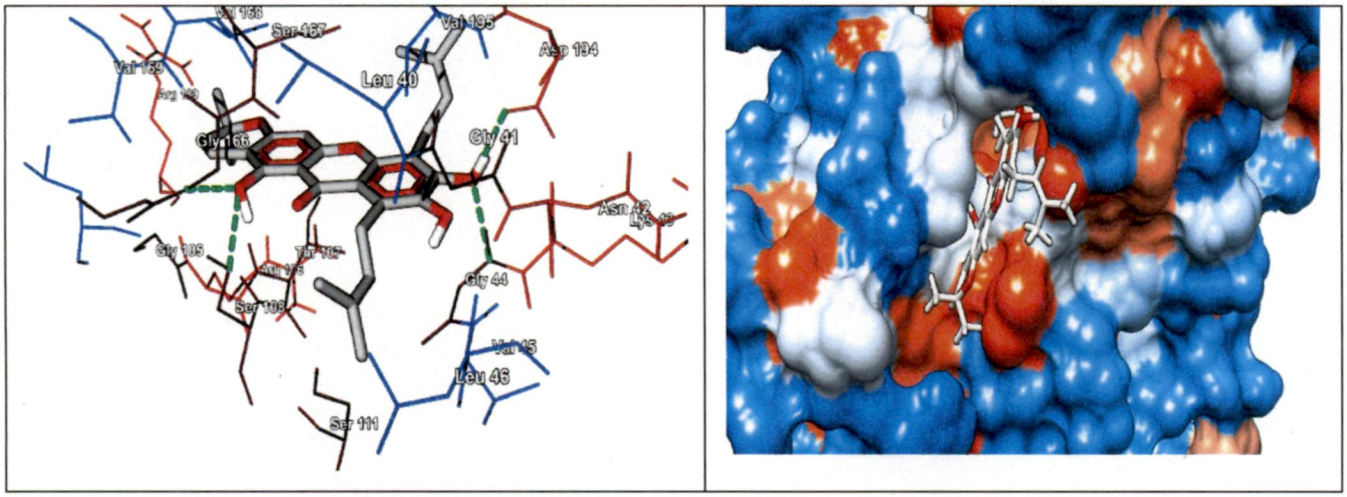


Figure 4.9: Docked pose of compound 78 (energy= -153.918) using MVD

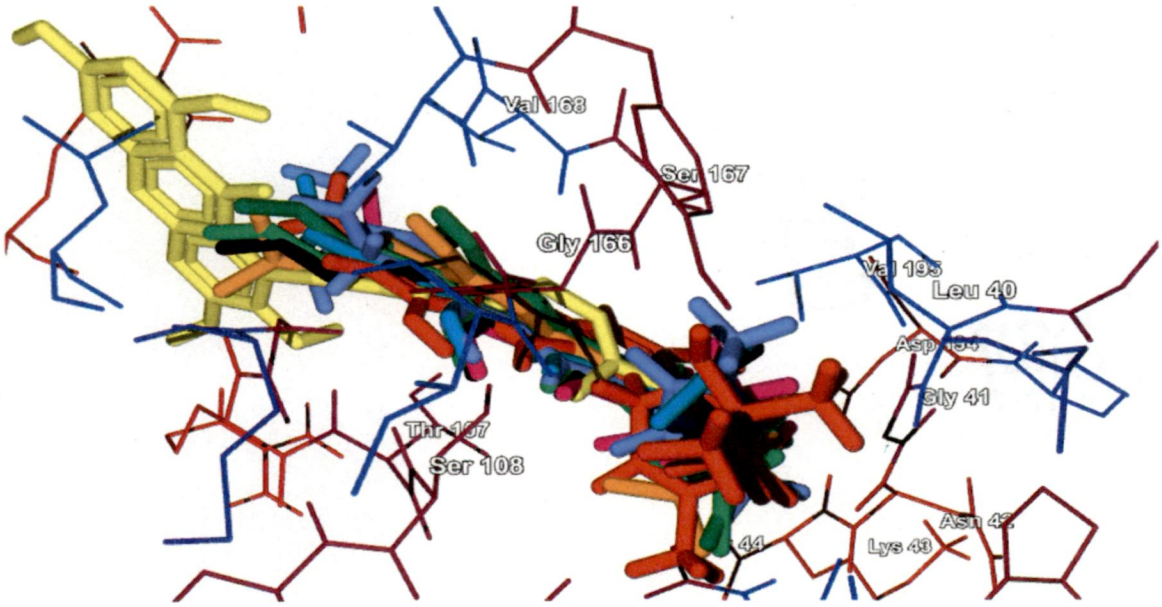


Figure 4.10: Docked poses of all Xanthene compounds.

Korupensamine and Ancistrolikokines- The Korupensamine and Ancistrolikokines have been isolated from a sample of *Ancistrocladus korupensis*, both are isoquinoline derivatives. This isoquinoline moiety is involved in some hydrophobic interaction with Ala16, Phe58, and Ile 164 along with hydrogen bond with Ala16 and Asp 54. This hydrophobic pocket is quite similar to one observed in the case of bischromone but the position of two ligands inside the binding pocket is slightly different, probably because of the lesser flexibility of these isoquinoline. AutoDock scores for Korupensamine and Ancistrolikokines are -10.21 and -9,89 kcal/mol respectively, Moldock scores are -137.83 and -136.82 kcal/mol respectively and iGemDock fitness scores are -125.9 and -122.2 kcal/mol respectively. Docked poses of Korupensamine and Ancistrolikokines has been shown in the Figures 4.11, 4.12, 4.13.

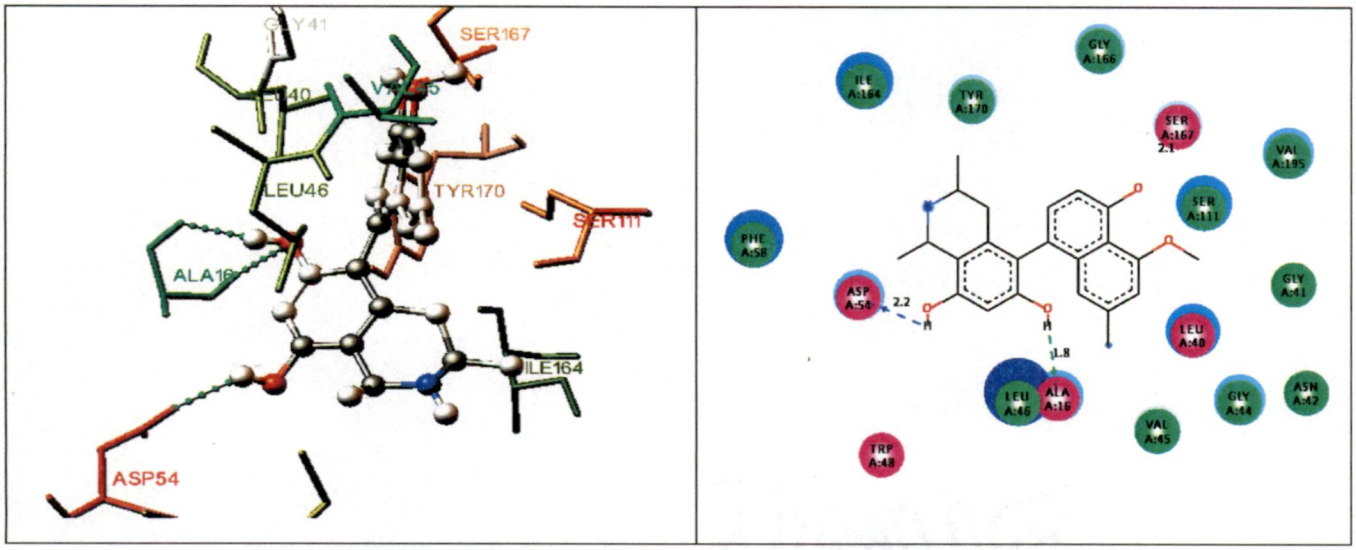


Figure 4.11: Docked pose of Korupensamine-A using AutoDock

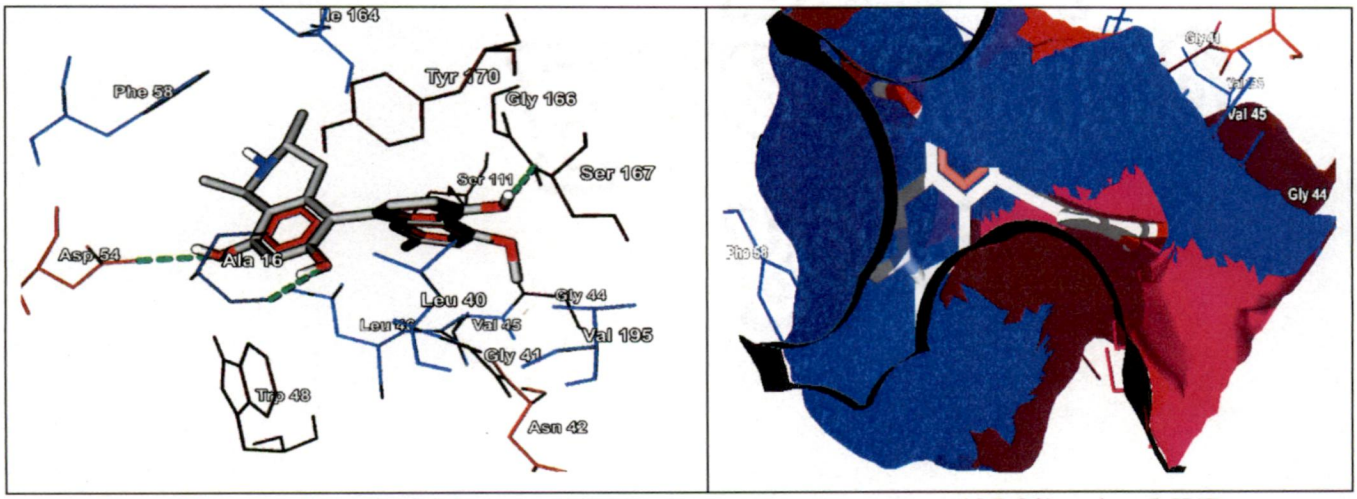


Figure 4.12: Docked pose of Korupensamine (energy= -137.83) using MVD

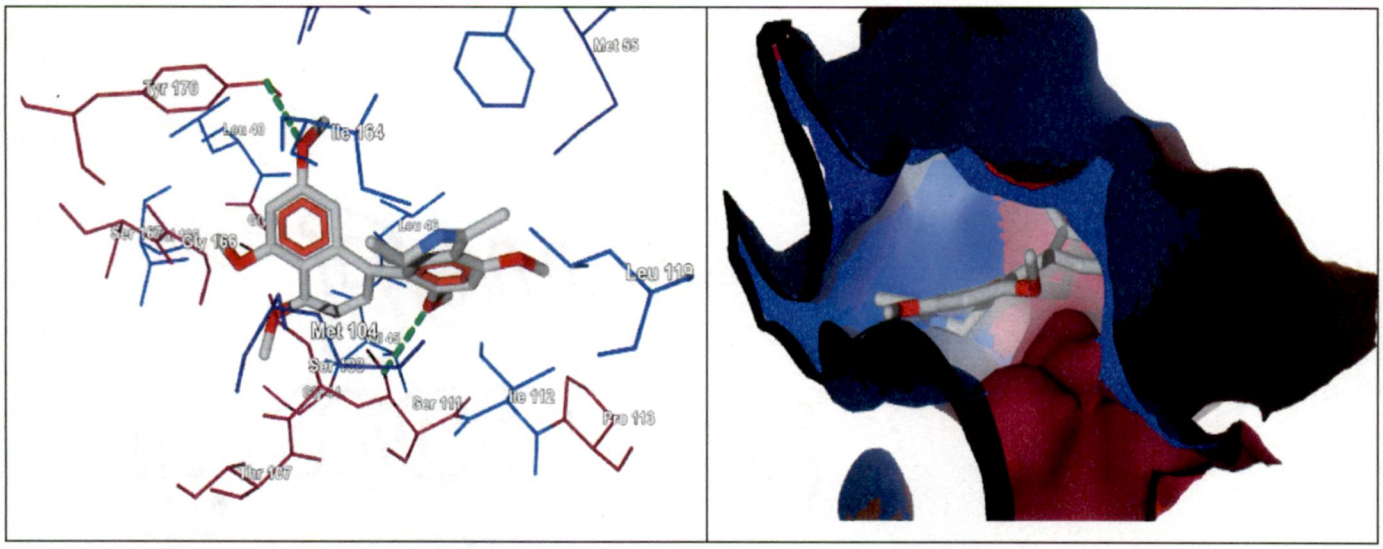


Figure 4.13: Docked pose of Ancistrolikokines (energy= -136.82) using MVD

7-Deacetylkhivorin is a limonoid and first obtained from the bark and seed extract of *Khaya grandifoliola. This compound* occupy a surface pit, rich with hydrophilic and Polar amino acid Gly44, Arg106, Thr107, Ser108, Gly166 and Ser167 with Four hydrogen bond interaction with Arg106, Thr107, Thr130 and Val 169.

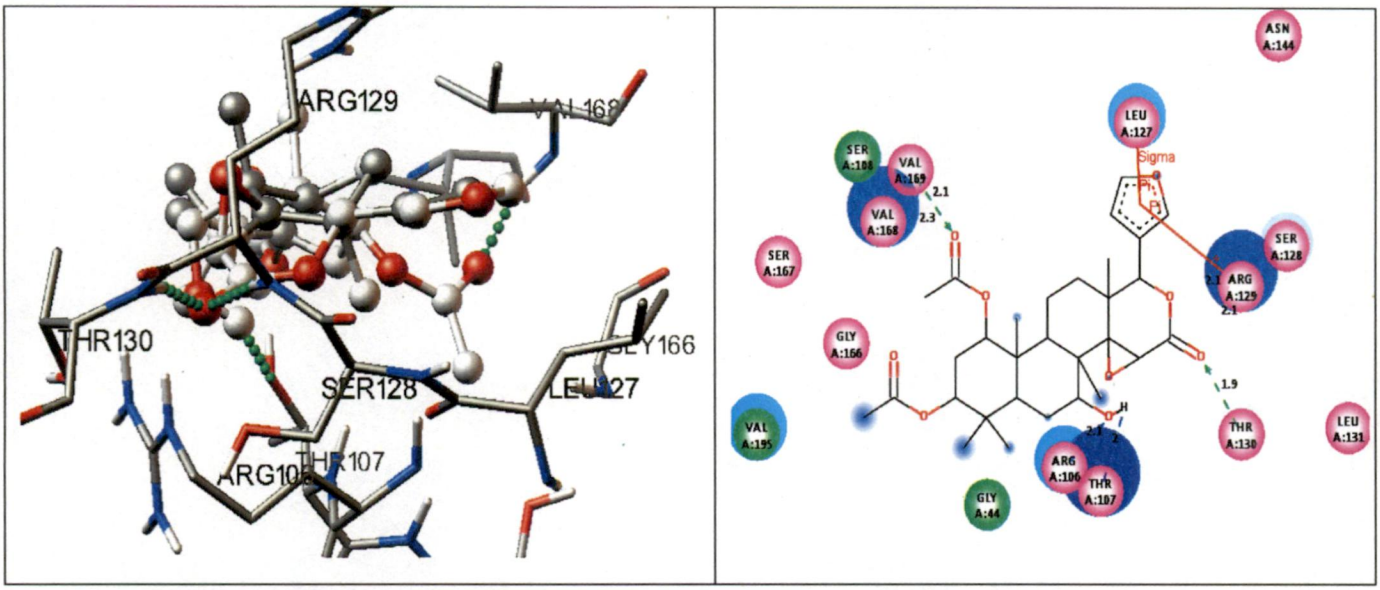


Figure 4.14: Docked pose of 7-deacetylkhivorin (energy= -9.10 kcal/mol) using AutoDock

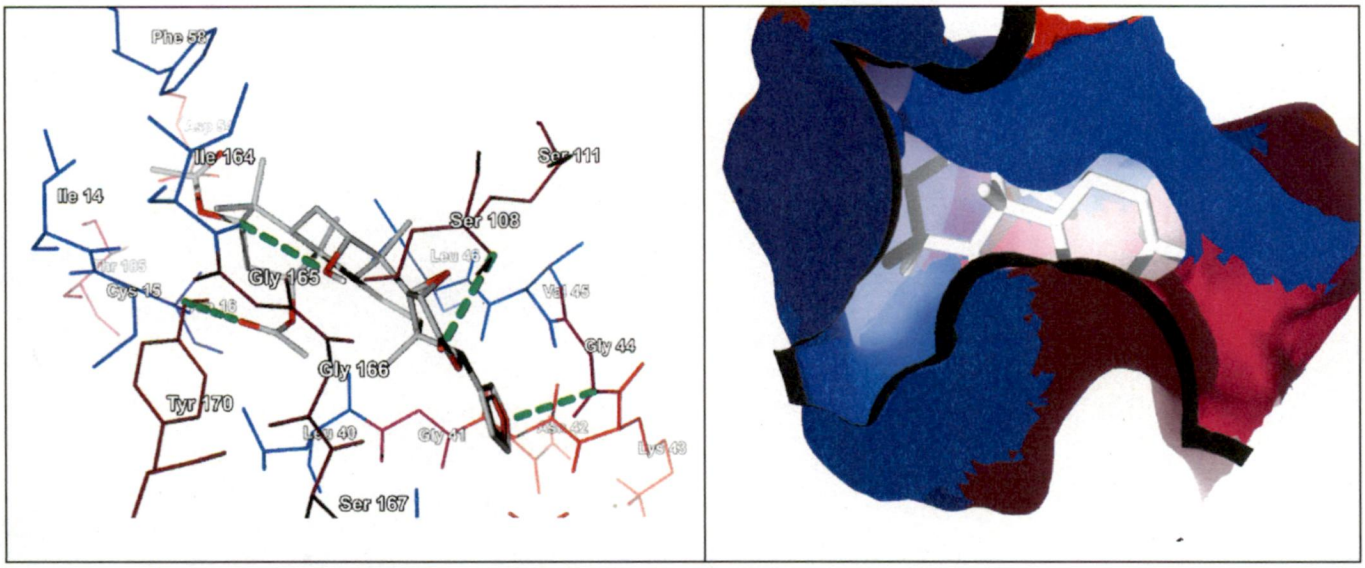


Figure 4.15: Docked pose of 7-deacetyl-khivorin (energy= -144.634) using MVD

Alianthione is a rigid pentacyclic compound and first extracted from *Odyendyea gabonesis*. This compound completely fit and buried into hydrophobic surface of Cys15, Ala16, Met55, Phe58 and Leu119. In addition Ser108, ALA16 and Tyr170 are involved in Hydrogen bonding. Notably, an σ-π interaction with Phe58 is also observed. Docked poses are shown in the figures 4.16 and 4.17.

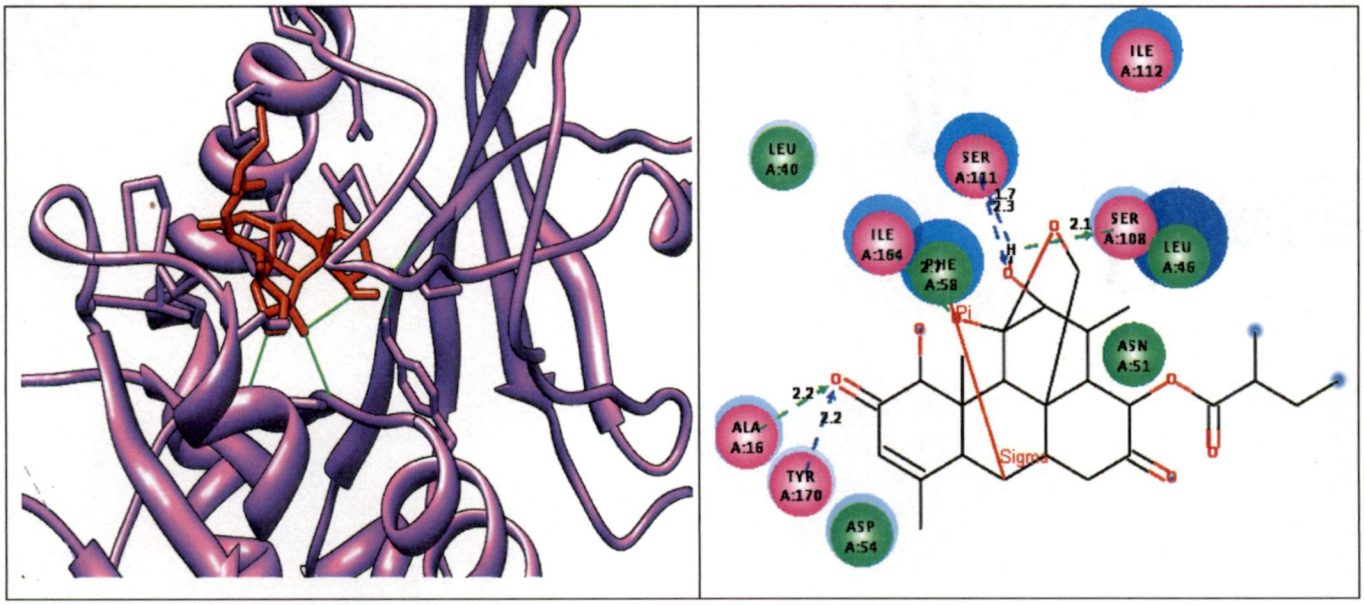


Figure 4.16: Docked pose of Alianthione (energy = -10.28 kcal/mol) using AutoDock

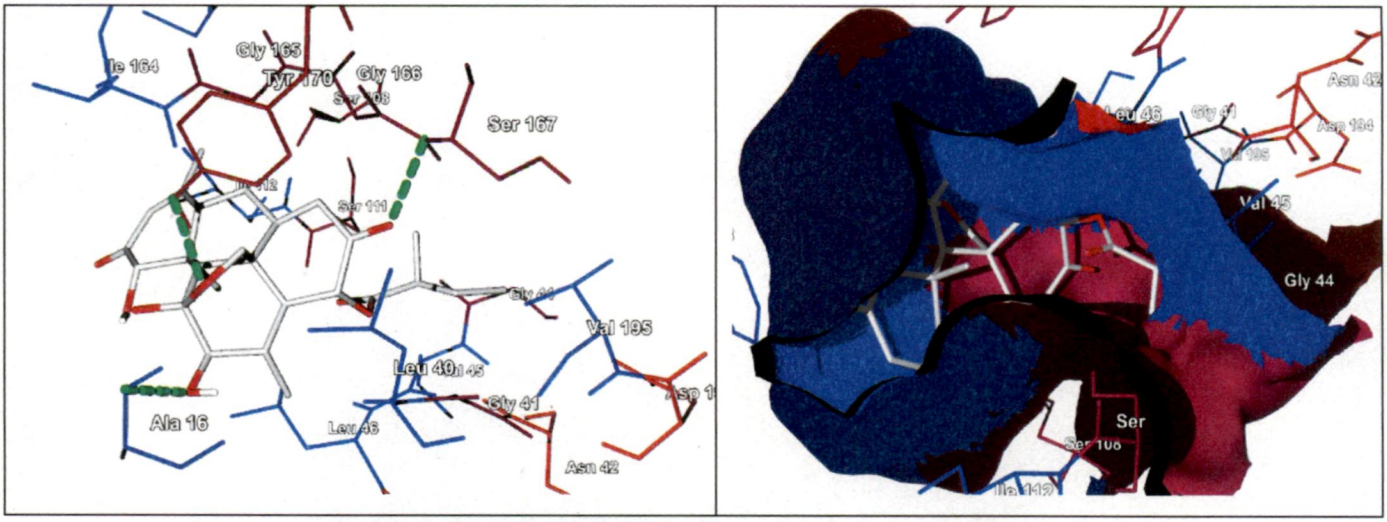


Figure 4.17: Docked pose of Alianthione (energy= -144.432) using MVD

Most of the top scorers have shown one or more common hydrogen bonding interaction with Ala 16, Gly 44, Ser 108 and Asp 194.Similarly, some pocket residues with high hydropathy index like Ile14, Cys 15, Ala 16, Phe 58 and Ile 164 were also found to be highly conserved, indicating the significant role of these residues in binding. Next we focused on the structural and spatial characteristics of the ligands. It seems that Chromanone skeleton and fused heterocyclic rings play an important role in protein-ligand interactions [122].

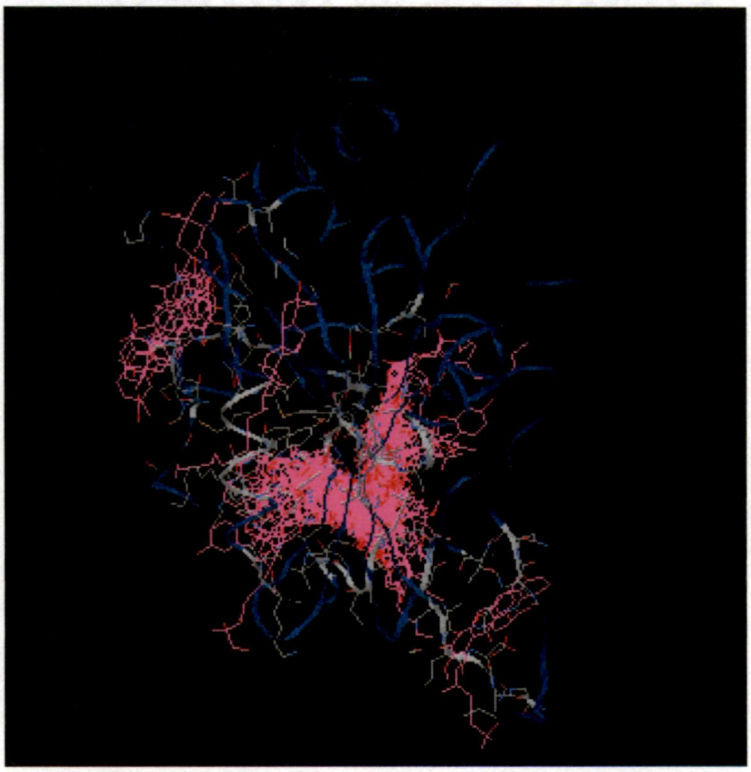


Figure 4.18: Virtual screening using iGemDock

Seven compounds, **Ochrolifuanine-A, Bischromone-chrobisiamine, Alianthione, Korupensamine, Xanthene, Ancistrolikokines, and 7-Deacetylkhivorin** inhibited P. falciparum DHFR domain with Ki values of 1.42, 2.51 29.09, 30.81, 37.71, 56.10, 220 nM respectively. Kinetic analysis showed that these compounds competitively inhibited the enzyme with respect to the substrate dihydrofolate. These compounds serve as leads for developing new DHFR inhibitors, since their skeletal structures are different from other DHFR inhibitors AutoDock4.2, iGemdock and MVD runs resulted in binding energy scores from -4.6 to -12.07 kcal/mol, -63 to -156.2 and -64.347 to -176.166 kcal/mol. Among the 210 inhibitors selected for docking studies, an excellent correlation was observed in some cases and some of these experimentally reported molecules showed a high dock score than the crystal structure inhibitor.

Therefore, molecular docking using AutoDock4.2 suggests the importance of evaluating the prediction accuracy of various molecules as evidenced by a correlation coefficient of 0.68 between experimental activities (IC_{50}) and AutoDock binding energies (Figure 4.19), which is an acceptable value in such types of docking practices. This result suggests that Autodock have performed well in predicting the binding energies and also rationalized the mechanism by which these inhibitors work.

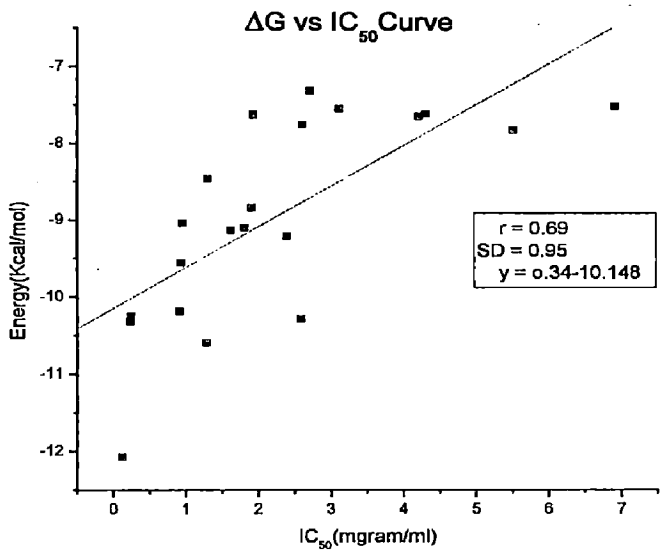


Figure 4.19: Energy (AutoDock) vs IC_{50} Curve

CONCLUSION

In the Dissertation we have presented a structure based screening approach to search for novel lead compounds for the inhibition of Pf-DHFR enzyme which play important role in the life cycle of plasmodium species and require for growth. For this purpose Crystal structure of wild type Pf-DHFR (PDB Id: 1J3I) with known active sites is taken as target for docking studies.

Docking and screening method were carried out to find novel lead compounds showing better result compare to known inhibitor WR99210 (AutoDock energy= -8.35 kcal/mol, MolDock score= -127.598 kcal/mol) using Autodock4.2, MVD and iGemdock. We have identified 7 new Pf- DHFR inhibitors whose structures were completely unrelated to those of the classical antifolates. We found that the binding energies of top ranked molecules in Kcal/mol with autodock4.2, MVD and iGEMDOCK having better energy score compare to 3rd generation inhibitor WR99210. **Ochrolifuanine-A, Bischromone-chrobisiamine** and **Xanthene group** are found to be most promising hits among the top scored ligands. Two factors seems to be especially important in binding are that residues Ala16, Gly44, Ser108, Ile164 and Asp194 are common in hydrogen bonding interactions in most of ligand-protein complexes and residues Ile14, Cys15, Ala16, Phe58, and Ile164 shown in Hydrophobic pocket. These new compounds may act as potent inhibitors for Pf-DHFR which can be further proved by experimental methods.

References

1. Delfino, R. T.; Santos-Filho, O. A.; Figueroa-Villar, J. D. *Biophys. Chem.* **2002**, *98*, 287–300.
2. *World Malaria Report* **2011**, WHO, Fact Sheet.
3. Donald, J.A. Eds. *Burger's Medicinal Chemistry and Drug Discovery, 6th ed.*; John Wiley & Sons: U.K, 2003.
4. Hansch, C.; Sammes, S.; Taylor, J. B. *Comprenhensive Medicinal Chemistry*: Quantative Drug Design, *5th ed.;* Elsevier: Landon, **2005;** Chapter 3.
5. Hansch, C.; Sammes, S.; Taylor, J. B. *Comprenhensive Medicinal Chemistry*, Quantative Drug Design, *5th ed.;* Elsevier: Landon, **2005;** Chapter 4.
6. http://www.nlm.nih.gov/medlineplus/ency/article/000621.htm
7. Fairhurst, R. M.; Wellems, T. E. Plasmodium species (Malaria). In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases*,7th ed. Philadelphia, Pa: Elsevier Churchill Livingstone; **2009**; chap 275
8. Wells, T. N. C. *Wells Malaria Journal* **2011**, *10*, 1–12.
9. Das, N.; Bhadoria, P. B. S. Traditional Knowledge and Indigenous uses of Tribal Medicinal resources in India. In: Govil, G. N.; Singh, V. K.; Arunachalam, C. eds. *Recent Progress in Medicinal Plants*; Stadium Press LLC, U.S.A. **2006**, *PP 1-48* Eds.
10. Saad, B.; Azaizeh, H.; Said, O. *eCAM* **2005**, *2*, 475– 479.Tipsuwan, W.; Srichairatanakool, S.; Kamchonwongpaisan, S.; Yuthavong, Y.; Uthaipibull, Y. *Maejo Int. J. Sci. Technol.* **2011**, *5*, 401–412
11. Kompis, I. M.; Islam, K.; Then, R. *Chem. Rev.* **2005**, 105, 593–620.
12. Yuthavong, Y. *Microbes Infect.* **2002**, *4*, 175-182.
13. Tawari, N. R.; Bag, S; Degani, M. S. *Curr. Pharm. Des.* **2011**, *17*, 712–51
14. Shoichet, B. K. *J. Med. Chem.* **2003**, *46*, 2304–2312.
15. Rastelli, G.; Pacchioni, S.; Sirawaraporn, W.; Sirawaraporn, R.; Parenti, M. D.; Ferrari, A. M. *J. Med Chem.* **2003**, *46*, 2834–2845.
16. Ridley, R. G. *Nature* **2002**, *415*, 686–693.
17. Schweitzer, B. I.; Dicker, A. P.; Bertino, J. R. *FASEB J.* **1990**, *4*, 2441– 2452.
18. Prakash, N.; Patel, S.; Faldu, N. J.; Ranjan, R.; Sudheer, D. V. N. *JCSB* **2010**, *3*, 70–73.
19. Gahtori, P.; Ghosh, S.K.; Parida, P.; Prakash, A.; Gogoi, K.; Bhat, H. R.; Singh, U. P. *Exp. Parasitol* **2012**, *130*, 292–299.
20. Bhat, H. R.; Ghosh, S. K.; Prakash, A.; Gogoi, K.; Singh, U. P. *Lett. Appl. Microbial.* **2012**, *54*, 483–486.
21. Muthaura, C. N.; Keriko, J. M.; Derese, S.; Yenesewc, A.; Rukunga, G. M. *Exp. Parasitol.* **2011**, *127*, 609–626.

22. Kraft, C.; Jenett-Siems, K.; Siems, K.; Jakupovic, J.; Mavi, S.; Bienzle, U.; Eich, E. *Phytother. Res.* **2003,** *17*, 123–128.
23. Yenesew, A.; Induli, M.; Derese, S.; Midiwo, J.O.; Heydenreich, M.; Peter, M.G.; Akala, H.; Wangui, J.; Liyala, P.; Waters, N.C. *Phytochem.* **2004**, *65*, 3029–3032.
24. Kuria, K.A.M.; Chepkwony, H.; Govaerts, C.; Roets, E.; Busson, R.; DeWitte, P.; Zupko, I.; Hoornaert, G.; Quirynen, L.; Maes, L.; Janssens, L.; Hoogmartens, J.; Laekeman, G. *J. Nat. Prod.* **2002,** *65*, 785–793
25. Murata, T.; Miyase, T.; Muregi, F. W.; Naoshima-Ishibashi, Y.; Umehara, K.; Warashina, T.; Kanou, S.; Mkoji, G. M.; Terada, M.; Ishih, A. *J. Nat Prod.* **2008,** *71*, 167–174.
26. Rukunga, G. M.; Muregi, F. W.; Omar, S. A.; Gathirwa, J. W.; Muthaura, C. N.; Peter, M. G.; Heydenreich, M.; Mungai, G. M. *Fitoterapia* **2008**, *79*, 188–190.
27. Rukunga, G. M.; Muregi, F. W.; Tolo, F. M.; Omar, S. A.; Mwitari, P.; Muthaura, C. N.; Omlin, F.; Lwande, W.; Hassanali, A.; Githure, J.; Iraqi, F. W.; Mungai, G. M.; Kraus, W.; Kofi-Tsekpo, W. M. *Fitoterapia* **2007**, *78*, 455–459.
28. Gakunju, D. M. N.; Mberu, E. K.; Dossaji, S. F., Gray, A. I., Waigh, R. D.; Waterman, P. G.; Watkins, W. M. *Chemotherapy* **1995**, *39*, 2606–2609.
29. Oketch-Rabah, H. A.; Lemmich, E.; Dossaji, S. F.; Theander, T. G.; Olsen, C. E.; Cornett, C.; Kharazmi, A.; Christensen, S. B. *J. Nat. Prod.* **1997**, *60*, 458–461.
30. Oketch-Rabah, H. A.; Mwangi, J. W.; Lisgarten, J.; Mberu, E. K. *Fitoterapia* **2000**, *71*, 636–640.
31. Dassonneville, L.; Bonjean, K.; Pauw-Gillet, M. C. De.; Colson, P.; Houssier, C.; Quetin-Leclercq, J.; Angenot, L.; Bailly, C. *Biochem.* **1999,** *38*, 7719–7726.
32. Lectercq, J. Q.; Coucke, P.; Delaude, C.; Warin, R.; Bassleer, R.; Angenot, L. *Phytochem.* **1991**, *30*, 1697–1700.
33. Wright, C. W.; Allen, D.; Phillipson, J. D.; Kirby, G. C.; Warhust, D. C.; Massiot, G.; Olivier, L. M. L. *J. Ethnopharmacol.*, **1993**, *40*, 41–45.
34. Kuete V.; Efferth *T. Front. pharmacol.*, **2010,** *1*, 1-17.
35. Bickii, J.; Njifutie, N.; Foyere, J. A.; Basco, L. K.; Ringwald, P. *J. Ethnopharmacol.* **2000,** *69*, 27–33.
36. Bickii, J.; Feuya Tchouya, G. R.; Tchouankeu, J. C.; Tsamo, E. *Afr. J. Trad. CAM* **2007**, *4*, 135–139.
37. Kenmogne, M.; Prost, E.; Harakat, D.; Jacquier, M. J.; Frédérich, M.; Sondengam, L. B.; Zèches, M.; Waffo-Téguo, P. *Phytochem.* **2006,** *67*, 433–438.
38. Tchuendem, M. H. K.; Mbah, J. A.; Tsopmo, A.; Ayafor, J. F.; Sterner, O.; Okunjic, C. C.; Iwu, M. M.; Schuster, B. M. *Phytochem.* **1999**, *52*, 1095–1099.
39. Ngouela, S.; Lenta, B. N.; Tchamo Noungoue, D.; Ngoupayo, J.; Boyom, F.; Tsamo, E.; Gut, J.; Rosenthal, P. J.; Connolly, J. D. *Phytochem.* **2006**, *67*, 302–306.
40. Pillay, P.; Mahara,j V. J.; Smith, P. J. *J. Ethnopharmacol.* **2008**, *119*, 438–454.

63. Bringmann, G.; Messer, K.; Brun, R.; Mudogo, V. *J. Nat. Prod.* **2002**, *65*, 1096-1101.
64. Bringmann, G.; Dreyer, M.; Faber, J. H.; Dalsgaard, P. W.; Staerk, D.; Jaroszewski, J. W.; Ndangalasi, H.; Mbago, F.; Brun, R.; Christensen, S. B. *J. Nat. Prod.* **2004**, *67*, 743-751.
65. Hallock, Y. F.; Cardellina, J. H.; Schäffer, M.; Bringmann, G.; Francois, G.; Boyd, M. R. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1729- 1734.
66. Angerhofer, C. K.; Guinaudeau, H.; Wongpanich, V.; Pezzuto, J. M.; Cordell, G. A. *J. Nat. Prod.* **1999**, *62*, 59- 66.
67. Roumy, V.; Fabre, N.; Souard, F.; Massou, S.; Bourdy, G.; Maurel, S.; Valentin, A.; Moulis, C. *Planta Med.* **2006**, *72*, 894-902.
68. Ovenden, S. P. B.; Cao, S.; Leong, S.; Flotow, H.; Gupta, M. P.; Buss, A. D.; Butler, M. S. *Phytochemistry*, **2002**, *60*, 175–177.
69. Carraz, M.; Jossang, A.; Rasoanaivo, P.; Mazier, D.; Frappier, F. *Bioorg. Med. Chem.* **2008**, *16*, 6186-6192.
70. Frédérich, M.; Tits, M.; Hayette, M. P.; Brandt, V.; Penelle, J.; DeMol, P.; Llabrès, G.; Angenot, L. *J. Nat. Prod.* **1999**, *62*, 619-622.
71. Frédérich, M.; De Pauw, M. C.; Prosperi, C.; Tits, M.; Brandt, V.; Penelle, J.; Hayette, M. P.; DeMol, P.; Angenot, L. *J. Nat. Prod.* **2001**, *64*, 12-16.
72. Frédérich, M.; Jacquier, M. J.; Thépenier, P.; De, M. P.; Tits, M.; Philippe, G.; Delaude, C.; Angenot, L.; Zèches-Hanrot, M. *J. Nat. Prod.* **2002**, *65*, 1381-1386.
73. Kuo, P. C.; Shri, L.S.; Damu, A. G.; Su, C. R.; Huang, C. H.; Ke, C. H.; Wu, J. B.; Lin, A. J.; Bastow, K. F.; Lee, K. H.; Wu, T. S. *J. Nat. Prod.* **2003**, *66*, 1324-1327.
74. Topcu, G.; Aydogmus, Z.; Imre, S.; Gören, A. C.; Pezzuto, J. M.; Clement, J. A.; Kingston, D. G. *J. Nat. Prod.* **2003**, *66*, 1505- 1508.
75. Prakash Chaturvedula, V. S.; Farooq, A.; Schilling, J. K.; Malone, S.; Derveld, I.; Werkhoven, M. C. M.; Wisse, J. H.; Ratsimbason, M.; Kingston, D. G. I. *J. Nat. Prod.* **2004**, *67*, 2053-2057.
76. Garzo´n, S. P.; Abimael D. Rodrı´guez, A. D.; Sa´ nchez, J. A.; Ortega-Barria, E. *J. Nat. Prod.* **2005**, *68*, 1354-1359.
77. Kraft, C.; Jenett-Siems , K.; Siems, K.; Jakupovic, J.; Mavi, S.; Bienzle, U.; Eich, E. *Phytother. Res.* **2003**, *17*, 123–128.
78. François, G.; Passreiter, C. M. *Phytother. Res.* **2004**, *18*, 184–186.
79. Karioti, A.; Skaltsa, H.; Zhang, X.; Tonge, P. J.; Perozzo, R.; Kaiser, M.; Franzblau, S. G.; Tasdemir, D. *Phytomedicine* **2008**, *15*, 1125-1129.
80. Benoit-Vical, F.; Imbert, C.; Bonfils, J. P.; Sauvaire, Y. *Phytochemistry* **2003**, *62*, 747- 751.
81. Ma, C.; Zhang, H. J.; Tan, G. T.; Hung, N. V.; Cuong, N. M.; Soejarto, D. D.; Fong, H. H. S. *J. Nat. Prod.* **2006**, *69*, 346-350.

82. Wright, A. D.; Wang, H.; Gurrath, M.; König, G. M.; Kocak, G.; Neumann, G.; Loria, P.; Foley, M.; Tilley, L. *J. Med. Chem.* **2001**, *44*, 873-885.
83. Sairafianpour, M.; Christensen, J.; Stærk, D.; Budnik, B. A.; Kharazmi, A.; Bagherzadeh, K.; Jaroszewski J. W. *J. Nat. Prod.* **2001**, *64*, 1398-1403.
84. Ziegler, H. L.; Jensen, T. H.; Christensen, J.; Staerk, D.; Hägerstrand, H.; Sittie, A. A.; Olsen, C. E.; Staalsø, T.; Ekpe, P.; Jaroszewski, J. W. *Planta Med.* **2002**, *68*, 547-549.
85. Moein, M. R.; Pawar, R. S.; Khan, S. I.; Tekwani, B. L.; Khan, I. A. *Phytother. Res.* **2008**, *22*, 283-285.
86. Limmatvapirat, C.; Sirisopanaporn, S.; Kittakoop, P. *Planta Med.* **2004**, *70*, 276-278.
87. Tasdemir, D.; Güner, N. D.; Perozzo, R.; Brun, R.; Dönmez, A. A.; Calis, I.; Rüedi, P. *Phytochem.*, **2005**, *66*, 355-359.
88. Sharma, S. K.; Parasuraman, P.; Kumar, G.; Surolia, N.; Surolia, A. *J. Med. Chem.* **2007**, *50*, 765-775.
89. Oketch-Rabah, H. A.; Dossaji, S. F.; Christensen, S. B.; Frydenvang, K.; Lemmich, E.; Cornett, C.; Olsen, C. E.; Chen, M.; Kharazmi, A.; Theander, T. *J. Nat. Prod.* **1997**, *60*, 1017-1022.
90. Brøgger Christensen, S. *Phytother. Res.* **2004**, *18*, 128-130.
91. Yenesew, A.; Induli, M.; Derese, S.; Midiwo, J. O.; Heydenreich, M.; Peter, M. G.; Akala, H.; Wangui, J.; Liyala, P.; Waters, N. C. *Phytochem.*, **2004**, *65*, 3029-3032.
92. Likhitwitayawuid, K.; Phadungcharoen, T.; Krungkrai, *J. Planta Med.* **1998**, *64*, 70-71.
93. Weiss, C. R.; Moideen, S. V. K.; Croft, S. L.; Houghton, P. J. *J. Nat. Prod.* **2000**, *63*, 1306-1309.
94. Tansuwan, S.; Pornpakakul, S.; Roengsumran, S.; Petsom, A.; Muangsin, N.; Sihanonta, P.; Chaichit, N. *J. Nat. Prod.* **2007**, *70*, 1620-1622.
95. Laurent, D.; Jullian, V.; Parenty, A.; Knibiehler, M.; Dorin, D.; Schmitt, S.; Lozach, O.; Lebouvier, N.; Frostin, M.; Alby, F.; Maurel, S.; Doerig, C.; Meijer, L.; Sauvain, M. *Bioorg. Med. Chem.* **2006**, *14*, 4477-4482.
96. www.cambridgesoft.com
97. Li, Z.; Wan, H.; Shi, Y.; Ouyang, P. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 1886-1890.
98. ACD/ChemSketch Freeware, version 10.00, Advanced Chemistry Development, Inc.,Toronto, ON, Canada, www.acdlabs.com, **2012**.
99. http://accelrys.com/products/informatics/cheminformatics/draw/index.html
100. Matsuzawa N., Seto J., DixonD. A., J. Phys. Chem. A, **1997**, *101*, 9391.
101. Humphrey, W., Dalke, A. and Schulten, K., ``VMD - Visual Molecular Dynamics" *J Molec. Graphics* **1996**, *14.1*, 33-38.
102. www.molegro.com/MMV_Manual.

103.Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. *J Comput Chem.* **2004**, *25*, 1605-1612.
104.http://avogadro.openmolecules.net
105.Morris, G. M.; Goodsell, D. S.; Huey, R.; Olson, A. J. *J. Computer-Aided Molecular Design* , **1996**, *10*, 293-304.
106.Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comp. Chem.* **1998**, *19*, 1639-1662.
107.Homsen, R. *BioSystems* **2003**, *72*, 57-73.
108.Wei, G.; Yan-Ling, Z.; Lu, A.; Yan-Jiang, Q. *Chin. J. Nat. Med.* **2010**, *8*, 51–56.
109.Morris, G. M.; Goodsell,D. S.; Pique, M. E.; Lindstrom, Huey, R.; Forli, S.; Hart, W. E.; Halliday, S.; Belew, R.; Olson, A. J. "AutoDock 4.2" *User Guide* **2010** www.scripps.edu.
110.Choowongkomon, K.; Theppabutr, S.; Songtawee, N.; Day, N. P. J.; White, N. J., Woodrow, C. J., Imwong, *M. Malaria Journal* **2010**, *9*, 1-10
111.Ali, H. I.; Tomita, K.; Akaho, E.; Kambara, H.; Miura, S.; Hayakawa, H.; Ashida, N.; Kawashima, Y.; Yamagishi, T.; Ikeya, H.; Yoneda, F.; Tomohisa Nagamatsu, T. *Bioorg. Med. Chem.* **2007**, *15*, 242–256.
112.Vianna, C. P.; de Azevedo Jr., W. F. *J. Mol. Model.* **2012**, *18*, 755–764.
113.Jayashree, B. S.; Thomas, S.; Nayak, Y. *Med. Chem. Res.* **2010** *19*, 193–209.
114."Molegro Virtual Docker" *User Manual* **2011**, *http://www.molegro.com*
115.Zaheer-ul-Haq; Khan, W. *J. Comput. Aided Mol. Des.* **2011** *25*, 81–101.
116.Fogel, G. B.; Cheung, M.; Pittman, E.; Hecht, D. *J Comput Aided Mol Des.* **2008**, *22*, 29–38.
117.Drug Design and Systems Biology Laboratory of National Chiao Tung University, Taiwan "iGemDock 2.1" *User manual* **2008** *http://gemdock.life.nctu.edu.tw/dock*
118.Yang, Y. M.; Chen, C. C. **2004**, *55*, 288-304.
119.Hsu, K. C.; Chen, Y. F.; Lin, S. R.; Yang, J. M. Hsu et al. BMC Bioinformatics **2011**, *12*, 1-11.
120.O'Boyle, N. M.; Banck, M.; James, C. A.; Morley, C.; Vandermeersch, T.; Hutchison, G. R. *J Cheminf* **2011**, *3*, 1-14.
121.Kozakov, D.; Clodfelter, K.H.; Vajda, S.; Camacho, C. J. *J. Med. Chem.* **2004**, *89*, 867-875.
122.Rukunga, G. M.; Muregi, F. W.; Omar, S. A.; Gathirwa, J. W.; Muthaura, C. N.; Peter, M. G.; Heydenreich, M.; Mungai, G. M. *Fitoterapia* **2008** *7*,188–190.

ANNEXURE

TABLE 4.1: Docking Results using AutoDock, iGemDock and MVD

Ligands	AutoDock Result		iGemdock fitness score	MVD moldock score
	binding Energy	No of H.B.		
1	-8.16	4	-90.3	-121.583
2	-7.82	4	-90.5	-115.567
3	-8.2	3	-99.3	-116.02
4	-8.86	4	107.2	-138.003
5	-8.87	2	-104.9	-142.773
6	-7.79	0	-91.5	-89..321
7	-9.02	2	-112.1	-146.988
8	-9.06	4	-106.3	-151.521
9	-6.1	4	-100.1	-145.779
10	-7.2	5	-93.5	-103.121
11	-11.09	1	-85.3	-125.505
12	-7.98	1	-77.3	-91.670
13	-7.62	1	-78.5	-90.017
14	-10.19	0	-114.6	-99.624
15	-10	3	-90.2	-142.329
16	-9.56	1	-104.7	-128.632
17	-7.9	2	-100.6	-107.362
18	-7.96	1	-112.1	-107.911
19	-7.49	3	-115.6	-102.311

20	-8.88	1	-103.6	-121.254
21	-8.76	3	-121.1	-120.123
22	-6.63	2	-101.4	-89.912
23	-7.71	1	-84.5	-93.325
24	-7.08	1	-99.3	-90.278
25	-9.42	0	-110.2	-89.328
26	**-8.91**	**2**	**-123.2**	**-139.911**
27	-7.53	2	-124.5	-111.785
28	-10.35	0	-110.7	-125.643
29	**-9.1**	**4**	**-114.28**	**-144.634**
30	-6.72	2	-117.6	-78.873
31	-8.49	1	-118.2	-131.127
32	-8.26	0	-97.6	-123.542
33	-11.08	0	-79.5	-118.269
34	-7.32	1	-114.1	-116.218
35	-11.37	0	-96.6	-118.980
36	-8.52	1	-113.6	-132.210
37	-7.21	3	-98.3	-118.325
38	-9.12	3	-92.2	-137.564
39	-8.22	2	-95.3	-124.673
40	-7.47	2	-113.8	-118.729
41	-7.47	2	-117.6	-119.098
42	-7.33	2	-81.5	-117.825
43	-5.22	0	-99.1	-65.378

44	-8.46	1	-105.3	-119.234
45	-8.22	2	-125.5	-117.256
46	-6.01	?	-105.2	-79.786
47	-9.37	3	-105.2	-132.267
48	-9.49	7	-106.7	-132.879
49	-6.21	2	-106.8	-100.648
50	-5.93	2	-80.2	-69.107
51	-9.94	4	-108.7	-131.254
52	-8.16	2	-103.5	-120.759
53	-6.67	3	-94.8	-79.899
54	-7.55	2	-89.3	-88.994
55	-7.75	2	-102.7	-90.238
		2	-81.7	-105.672
		3	-76.2	-111.658
		2	-86	-71.387
	.1	1	-88.7	-119.103
	.3	1	-103	-99.897
	8.42	1	-105.4	-119.785
	-9.04	2	-103	-119.731
	-8.47	1	-91.5	-112.849
	-7.74	3	-82.2	-78.975
	-7.61	1	-102.1	-77.879
	-6.75	2	-107.7	-68.839
67	-7.21	5	-85.6	-75.482

68	-9.08	0	-95.1	-111.478
69	-9.9	2	-82.4	-126.937
70	-8.87	2	-86.7	-112.679
71	-9.2	0	-91.5	-118.275
72	-8.51	1	-85.4	-113.896
73	-10.09	1	-78.6	-127.001
74	-8.76	1	-89.9	-112.879
75	-8.43	2	-94.4	-110.982
76	-8.56	3	-115.6	-111.982
77	**-8.96**	**4**	**-112.6**	**-143.873**
78	**-10.13**	**4**	**-156.2**	**-153.919**
79	**-8.98**	**4**	**-125.1**	**-145.90**
80	**-8.16**	**4**	**-120.7**	**-139.496**
81	**-9.14**	**5**	**-114.6**	**-125.805**
82	**-9.22**	**3**	**-132.1**	**-139.734**
83	**-9.47**	**4**	**-128**	**-140.105**
84	**-9.30**	**1**	**-154.7**	**-138.353**
85	**-8.89**	**4**	**-106.5**	**-125.788**
86	**-9.79**	**1**	**-117.8**	**-147.407**
87	-9.02	0	-105.1	-117.382
88	-9.13	0	-102.9	-118.843
89	-7.57	1	-116.6	-106.574
90	-7.32	2	-92.2	-109.823
92	-8.25	1	-110.9	-123.982

92	-7.84	2	-90.5	-114.985
93	-8.14	5	-91.4	-125.876
94	-7.71	2	-100.3	-100.345
95	-5.16	1	-72.2	-69.995
96	-10.5	2	-91.2	-121.674
97	-7.33	1	-108.3	-102.743
98	-7.51	3	-120.3	-108.920
99	**-11.73**	**2**	**-133.6**	**-167.247**
100	-4.26	1	-131.1	-64.347
101	-8.24	1	-128.7	-120.289
102	-9.21	1	-95.8	-125.749
103	-9.6	2	-79.6	-112.897
104	-11.22	2	-87.8	-139.027
105	-5.87	4	-88.3	-74.321
106	-8.95	1	-103	-118.721
107	-7.66	1	-117.6	-102.229
108	-7.33	0	-87.7	-85.583
109	-6.56	2	-84.6	-78.754
110	-6.56	1	-84.7	-74.943
111	-6.87	1	-83.4	-75.843
112	-6.67	3	-93.6	-74.998
113	-7.21	2	-94.9	-98.234
114	-7.39	2	-81.8	-99.995
115	-6.64	1	-83.9	-78.996

116	-7.56	0	-81.7	-99.673
117	-7.8	1	-96.4	-101.234
118	-7.66	3	-103.6	-100.723
119	**-10.28**	**1**	**-113.3**	**-134.276**
120	-11.5	0	-121.7	-121.324
121	-7.44	2	-96.7	-99.657
122	-8.17	0	-108.9	-102.273
123	-4.62	7	-117.4	-66.537
124	-5.6	2	-72.8	-71.891
125	-8.08	3	-78.9	-108.832
126	-8.01	2	-89.4	-106.345
127	-7.3	2	-98.8	-98.875
128	-6.85	4	-84.9	-87.885
129	-7.7	3	-104.9	-99.668
130	-8.1	2	-88.9	-113.375
131	-7.99	2	-79.1	-96.452
132	-9.36	2	-141.3	-119.849
133	-9.1	1	-114.3	-108.934
134	-8.32	4	-119.4	-107.621
135	-9.44	1	-122.2	-111.723
136	-7.76	1	-88.9	-95.874
137	-8.27	3	-117.9	-104.873
138	-9.89	2	-122.2	-126.928
139	-8.3	2	-126	-112.228

140	**-10.21**	**4**	**-125.9**	**-129.606**
141	-9.5	2	-120	-113.119
142	-7.53	2	-120.2	-101.112
143	-7.02	1	-99.5	-88.833
144	-6.66	1	-78.6	-79.872
145	-6.53	3	-110.6	-87.374
146	-7.55	1	-95.7	-96.456
147	-10.32	2	-90.2	-115.236
148	-7.04	1	-88.5	-88.992
149	-7.16	1	-107	-94.487
150	-6.88	2	-99.9	-81.238
151	-7.96	3	-99	-102.743
152	-7.54	2	-99.5	-101.897
153	-7.69	1	-89.9	-97.028
154	-10.65	0	-107.6	-98.873
155	-7.84	3	-109.6	-107.897
156	**-12.07**	**1**	**-106**	**-176.166**
157	-6.42	0	-104.3	-66.643
158	-10.86	1	-118	-113.743
159	-5.58	0	-103.9	-67.843
160	-6.44	3	-99.1	-79.433
161	-7.2	3	-94.1	-98.876
162	-7.12	2	-75.5	-96.774
163	-7.16	3	-68.6	-96.998

164	-10.68	1	-85.1	-113.673
165	-7.62	2	-88.3	-101.976
166	-7.34	3	-91.8	-103.446
167	-8.59	3	-85.1	-123.859
168	-8.23	3	-85.8	-119.233
169	-8.29	3	-108.7	-121.732
170	-7.7	2	-95.3	-94.232
171	-7.69	1	-97.1	-98.654
172	-8.25	3	-97.7	-111.211
173	-8.2	3	-88.5	-110.832
174	-8.17	0	-86.1	-107.833
175	-8.98	2	-92.3	-109.722
176	-7.72	2	-102.2	-100.82
177	-7.91	0	-78	-79.728
178	-9.02	1	-85.3	-99.289
179	-8.15	1	-90.3	-97.899
180	-11.19	2	-100.4	-127.029
181	8.09	4	-106.1	-125.973
182	-8.16	3	-96.1	-115.383
183	-9.04	2	-96.3	-124.526
184	-5.94	0	-89	-78.976
185	-9.04	0	-88.7	-91.373
186	-7.99	2	-69	-100.843
187	-8.78	2	-98.4	-109.283

188	-9.13	2	-96.5	-119.474
189	-7.84	1	-86.6	-102.473
190	-8.79	3	-87.7	-122.844
191	-8.34	3	-82.3	-119.234
192	-5.77	2	-71.1	-68.978
193	-6.87	2	-77.9	-89.995
194	-8.92	4	-95.9	-125.786
195	-6.83	3	-91.3	-99.832
196	-8.08	5	-96.2	-110.656
197	-9	3	-122.4	-129.986
198	-9.5	1	-87.6	-109.864
199	-9.87	1	-123.3	-117.897
200	-7.92	1	-117.7	-98.987
201	-7.06	1	-79.6	-88.863
202	-8.37	3	-104.6	-120.287
203	-5.37	3	-67.0	-69.985
204	-6.78	2	-101.3	79.647
205	-9.34	2	-107	-119.4