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CHLOROQUINE IN MALARIA MANAGEMENT; A COMPARATIVE STUDY WITH 4-AMINOQUNOLONE

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ABSTRACT :

Malaria is a mosquito-borne disease caused by Plasmodium species, with P. falciparum being the most deadly. Diagnosis involves blood smears, RDTs, and PCR. Chloroquine, a 4-aminoquinoline, was once the first-line treatment due to its efficacy and low cost. Its mechanism involves inhibition of heme detoxification in the parasite's food vacuole. However, resistance has limited its use.Chloroquine's physicochemical properties include good solubility, a log P of ~4.5, and a melting point of 87–94°C. It is assayed using UV spectrophotometry or HPLC. Structure-activity relationship (SAR) highlights the importance of the quinoline ring and basic side chain for activity.Compared to other 4-aminoquinolines like amodiaquine, piperaquine, and nivaquine, chloroquine has limited market use today. Amodiaquine and piperaquine are preferred in ACTs due to better resistance profiles. Evaluation is done via in vitro assays (IC50) and in vivo models (P. berghei in mice).Despite resistance, chloroquine remains important in understanding antimalarial drug action, with newer 4-aminoquinolines offering improved efficacy and availability.

INTRODUCTION

One of the most deadly infectious diseases that affect people is malaria. Due to its prevalence in less developed nations and areas, it poses clinical and economic challenges and significantly impedes socioeconomic advancement. The unicellular protozoan parasites that cause malaria are members of the genus Plasmodium. In addition to humans, these parasites also infect other species, such as birds, mammals, and reptiles. Each of the more than 200 Plasmodium species that have been officially described so far infects a particular range of hosts. There are just five species of Plasmodium that normally infect people and cause malaria in vast parts of the world: P. falciparum, P. vivax, P. malariae, P. ovale, and P. knowlesi. While P. knowlesi is naturally maintained in macaque monkeys, the first four are unique to humans.

TYPES OF MALARIA

The severity of malaria transmission will depend on the kinds (species) of Anopheles that are prevalent in a region at a certain moment.

- **Plasmodium falciparum.** As it progresses through its 48-hour life cycle, P falciparum exhibits adherence properties that lead to the sequestration of the parasite in tiny postcapillary vessels. This species is responsible for the most malignant form of malaria; it can infect RBCs of all ages, resulting in high levels of parasitemia.
- **Plasmodium vivax.** When such an infection is not treated, it typically lasts 2 to 3 months, with decreasing frequency and severity of paroxysms; 50% of patients with P vivax infection have a relapse within weeks to 5 years after the initial illness P vivax infects only young RBCs and therefore results in little parasitaemia.
- **Plasmodium ovale**. These infections are similar to P vivax infections, although generally more mild; P ovale infection commonly resolves without treatment; like P vivax, P ovale infects only immature RBCs, and parasitemia is usually lower than that seen with P falciparum.
- **Plasmodium malariae**. Individuals infected with this species of Plasmodium remain asymptomatic several times longer than those infected by P vivax or itspecies of Those with infected this species of Plasmodium for a much longer period compared to patients infected with P vivax or a itspecies.
- **Plasmodium knowlesi.** Autochthonous cases have been reported from Malaysian Borneo, Thailand, Myanmar, Singapore, the Philippines and other countries in the region; human cases of simian malaria likely also occur in Central and South America; patients with this, or any other simian species, should be treated as aggressively as for falciparum malaria, because P knowlesi may cause fatal disease.

INCUBATION PERIOD

MALARIA PARASITE NAME	INCUBATION PERIOD
Plasmodium Falciparum	9 To 14 Days
Plasmodium Vivax	8 To 17 Days

Plasmodium Ovale	16 To 18 Days
Plasmodium Malariae	18 To 40 Days

CAUSE OF MALARIA

- Of the species of the genus Plasmodium that infect humans, four are able to produce the disease:
- IN HUMANS;
- Plasmodium vivax
- Plasmodium ovale
- Plasmodium malariae
- Plasmodium knowlesi
- Plasmodium falciparum
- PATHOPHYSIOLOGY
- Two key phases of the parasite lifecycle are,
- Asexual cycle—Asexual cycle takes place in infected host.
- Sexual cycle - Sexual cycle takes place in mosquito.
- Asexual cycle

•Human disease transmission initiates when plasmodial "sporozoites" are inoculated from the salivary gland into humans through a bite and subsequent blood extraction.

These slender motile forms of the parasite are swiftly disseminated via the bloodstream to the liver, where they infiltrate hepatic parenchymal cells and commence a phase of asexual reproduction.

Through this amplification mechanism, a single sporozoite can produce between 10,000 and 30,000 offspring merozoites.

The engorged infected liver cell ultimately ruptures, releasing motile merozoites into the bloodstream. These subsequently penetrate the red blood cells and proliferate 60 to 20-fold every 48 to 72 hours.

When the parasite concentration attains 50 per microliter of blood, the symptomatic phase of infection commences.

Merozoites swiftly infiltrate erythrocytes upon entering the bloodstream and transform into "trophozoites." Attachment is facilitated through particular erythrocyte surface receptors.

During the initial phase of the intra-erythrocytic cycle, tiny "ring forms" of the four parasite species emerge, exhibiting similarities under light microscopy.

As the trophozoites enlarge, species-specific traits become apparent, pigmentation becomes discernible, and the parasite adopts a "irregular shape."

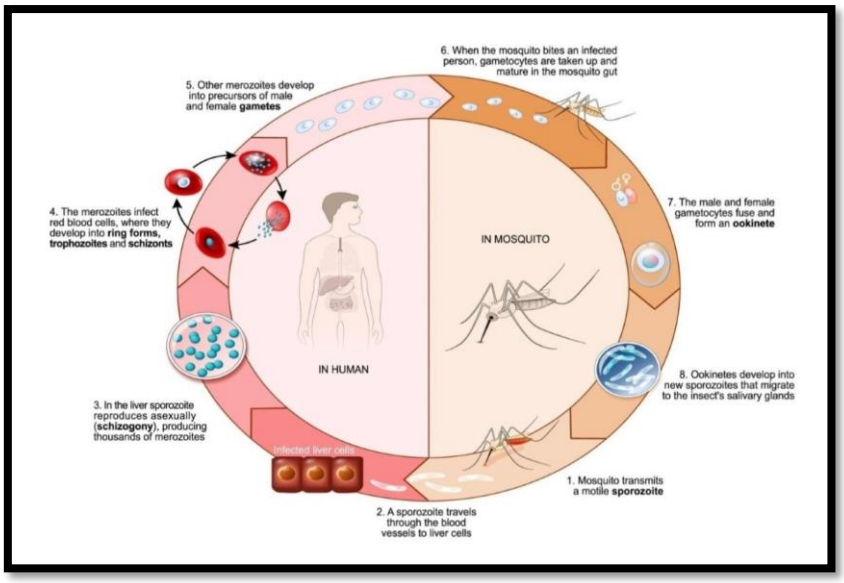
By the conclusion of the 48-hour intraerythrocytic cycle, the parasite has ingested virtually all the hemoglobin and expanded to fill the majority of the red blood cells. It is presently referred to as "shizont".

Multiple nuclear divisions have occurred, resulting in the rupture of red blood cells to release 6-30 daughter merozoites.

Following a succession of asexual cycles or immediately upon release from the liver, certain parasites differentiate into morphologically separate, longer-lived sexual forms (gametocytes) capable of transmitting malaria.

Sexual cycle

- After being ingested in the blood meal of a biting female anopheles' mosquito, the male and female gametocyte form a "zygote", matures in to ookinete, which penetrates and encysts in the mosquito gut wall.
- The resulting oocysts expands by asexual division until it bursts to liberate motile sporozoites which then migrate in to the hemolymph to the salivary gland of mosquito to await inoculation in to another human at the next feeding.



ETIOLOGY

There are four common Plasmodium species that primarily affect humans, with a fifth species in Southeast Asia, known as *Plasmodium knowlesi*, which can rarely infect humans and lead to severe disease. However, this species generally infects monkeys. The majority malaria cases worldwide are caused by *P falciparum*, followed by *P vivax*.

SIGNS AND SYMPTOMS OF MALARIA

- Fever
- Chills
- General feeling of discomfort
- Headache
- Nausea and vomiting
- Diarrhea
- Abdominal pain
- Muscle or joint pain
- Fatigue
- Rapid breathing
- Rapid heart rate
- Cough

DIAGNOSIS

1. Blood Smear Test
2. Antigen Based Rapid Diagnostic Test
3. Clinical Diagnosis
4. Polymerase Chain Reaction

BLOOD SMEAR TEST

Microscopy remains the definitive method for diagnosing malaria infection. The methods of slide preparation, staining, and examination are established and standardized, as is the assessment of parasite density, which is a significant advantage of microscopy and can be readily evaluated on a thick film. Rapid diagnostic tests (RDTs) are increasingly supplanting microscopy in primary healthcare facilities across endemic nations. A multitude of tests is available on the market, and their accuracy is being carefully assessed by the W.H.O. in collaboration with other organizations. Numerous rapid diagnostic tests (RDTs), utilizing both HRP-2 and p-LDH, have about 100% sensitivity at a relatively low parasite density of 200 parasites/μL, and demonstrate good specificity for P. falciparum malaria infection.

A clinical diagnosis of malaria is customary among physicians. This method is the most economical and commonly employed. Clinical diagnosis relies on the patient's signs, symptoms, and physical examination findings. The initial symptoms of malaria are generic and diverse, encompassing fever, headache, weakness, myalgia, chills, disorientation, stomach discomfort, diarrhea, nausea, vomiting, anorexia, and pruritus. A clinical diagnosis of malaria remains difficult due to the non-specific characteristics of the signs and symptoms, which significantly overlap with those of other prevalent and potentially fatal diseases, such as frequent viral or bacterial infections and other febrile disorders. The symptomatology of malaria overlaps with those of other tropical diseases, undermining diagnostic precision, which may lead to the indiscriminate administration of antimalarials and jeopardize the quality of care for patients with non-malarial fevers in endemic regions. The Integrated Management of Childhood Illness (IMCI) has established clinical algorithms for the management and diagnosis of prevalent pediatric ailments by inadequately trained healthcare providers in underdeveloped countries with insufficient laboratory diagnostic equipment. A commonly employed clinical method for malaria diagnosis, when compared to a fully qualified pediatrician with laboratory support, demonstrated extremely low specificity (0-9%) yet exhibited 100% sensitivity in African contexts. The absence of specificity highlights the dangers of differentiating malaria from other febrile conditions in children based solely on clinical assessment. A recent study indicated that the application of the IMCI clinical algorithm led to a 30% over-diagnosis of malaria. Consequently, the precision of malaria diagnosis can be significantly improved by integrating clinical and parasitological findings.

ANTIGEN TESTS

Antigen-based rapid diagnostic tests (RDTs) are often more accurate than blood smears at predicting the presence of malaria parasites. For areas where microscopy is not available, or where laboratory staff are not experienced at malaria diagnosis, there are RDTs that require only a drop of blood. Immunochromatographic tests have been developed, distributed and field tested. These tests use finger-stick or venous blood, the completed test takes a total of 15–20 minutes, and the results are read visually as the presence or absence of colored stripes on the dipstick, so they are suitable for use in the field. One disadvantage is that dipstick tests are qualitative but not quantitative – they can determine if parasites are present in the blood, but not how many.

TREATMENT

Treatment of malaria depends on the following factors:

1. Type of infection.

2. Severity of infection.
3. Status of the host.
4. Associated conditions/ diseases.

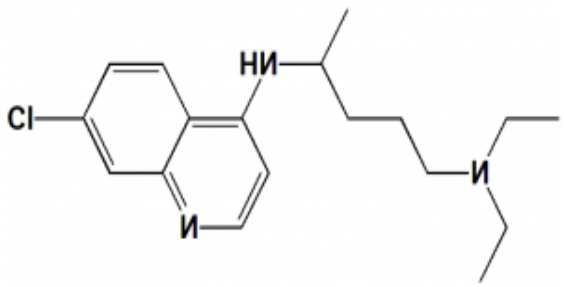
CLASSIFICATION OF ANTIMALARIAL DRUGS

SL NO	CLASSES	DRUGS
1	CINCHONA ALKALOIDS	Quinine, quinidine
2	4-AMINO QUINOLINES	Chloroquine, amodiaquine Piperaquine
3	8-AMINOQUINOLINES	Primaquine, tafenoquine
4	BIGUANIDES	Proguanil (chloroguanide)
5	DIAMINOPYRIMIDINES	Pyrimethamine
6	NAPHTHYRIDINE	Pyronaridine
7	SESQUITERPINE LACTONE	Artesunate, artemether, arteether, arterolane
8	SULFONAMIDES AND SULFONES	Sulfadoxine, sulfamethopyrazine, dapson
9	NAPHTOQUINONE	Atovaquone
10	ANTIBIOTICS	Tetracycline, Doxycycline, Clindamycin

The treatment of malaria depends on several factors, including the species of the infecting parasite, the severity of the disease, the patient's age, pregnancy status, and any underlying health conditions. Here's an overview of the common approaches to treating malaria:

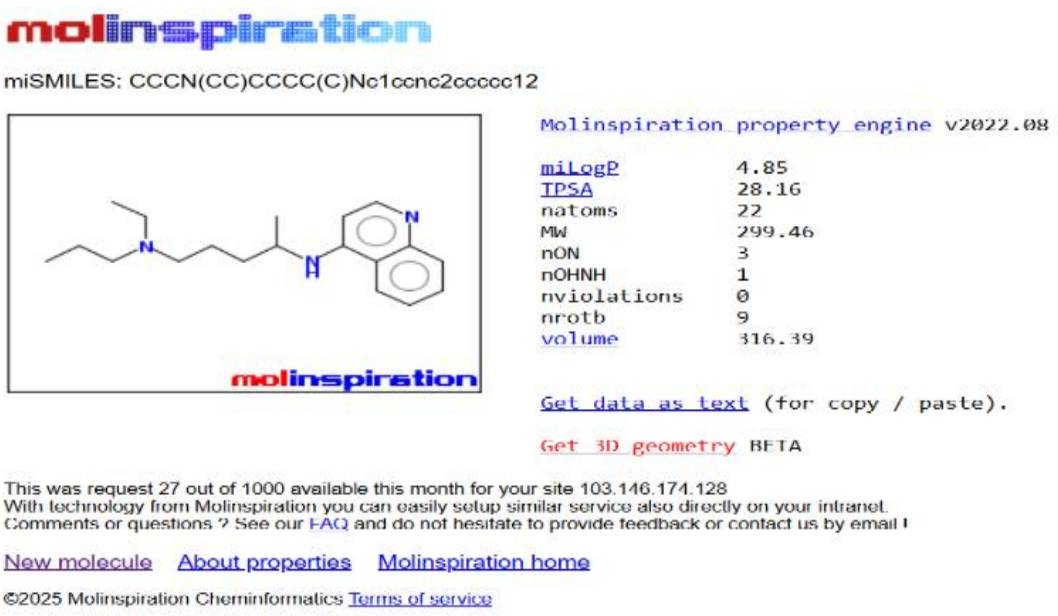
1. **Antimalarial Drugs**: Antimalarial medications are the cornerstone of malaria treatment. The choice of drug depends on the species of the infecting parasite and the drug resistance patterns in the region where the infection was acquired.
2. **Supportive Care:** In addition to antimalarial drugs, supportive care is essential, especially for severe malaria cases. This may include interventions to manage complications such as fluid replacement therapy for dehydration, blood transfusions for severe anemia, and treatment of other concurrent infections.
3. **Prevention of Complications:** Prompt and effective treatment of malaria helps prevent complications such as cerebral malaria, renal failure, severe anemia, and respiratory distress, which can be life-threatening.
4. **Vector Control:** Preventing mosquito bites through the use of insecticide-treated bed nets, indoor residual spraying, and other vector control measures is crucial for preventing malaria transmission and reducing the risk of infection.
5. **Intermittent Preventive Treatment (IPT):** IPT involves the administration of antimalarial drugs to vulnerable populations, such as pregnant women and infants, at
6. regular intervals, regardless of whether they are infected. This strategy helps prevent malaria-related morbidity and mortality in high-transmission areas.

CHLOROQUINE



PHYSICOCHEMICAL PROPERTIES MOLINSPIRATION-

Molinspiration offers broad range of cheminformatics software tools supporting molecule manipulation and processing, including SMILES and SD file conversion, normalization of molecules, generation of tautomers, molecule fragmentation, calculation of various molecular properties needed in QSAR, molecular modelling and drug design, high quality molecule depiction,molecular database tools supporting substructures and similarity searches.



The physicochemical properties of chloquine studied using molinspiration was found to be as-

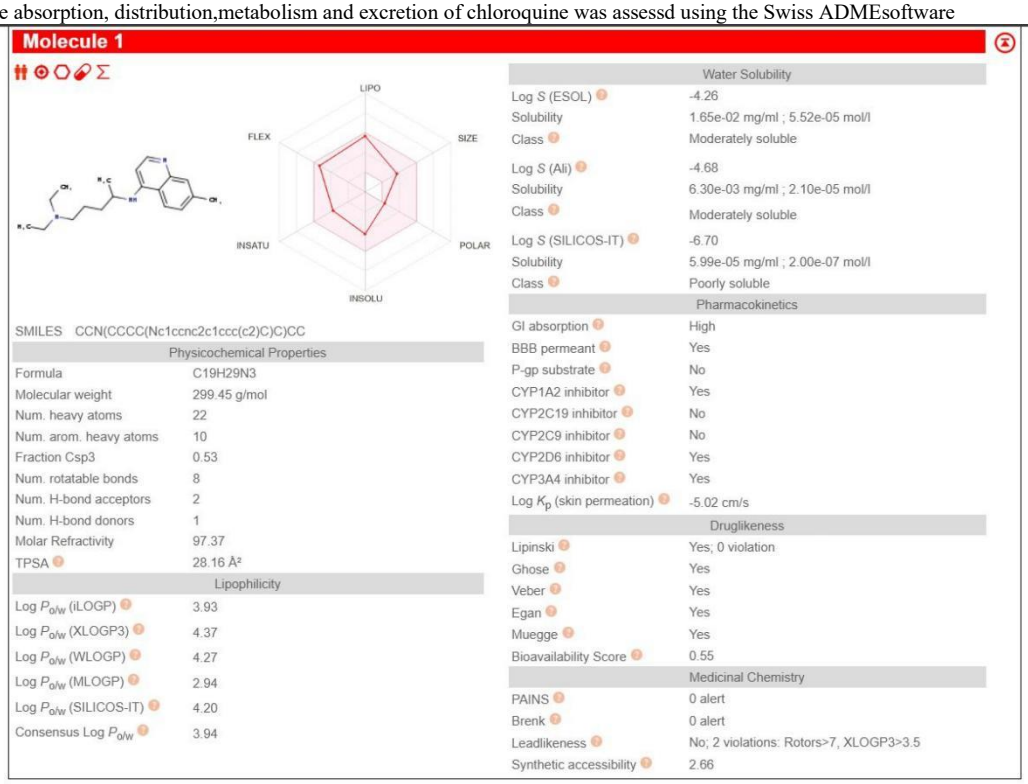
PHYSICOCHEMICAL PROPERTY	**VALUE**
Mi LogP	4.85
Number of Atoms	22
Molecular weight	299.46
Number of Hydrogen Atom Donors	3
Number of Hydrogen Atom Acceptors	1

LIPINSKI'S RULE OF FIVE

Lipinski's rule of five, also known as Pfizer's rule of five or simply the rule of five (RO5), is a rule of thumb to evaluate druglikeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would likely make it an orally active drug in humans. The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). However, the rule does not predict if a compound is pharmacologically active. Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria; • No more than 5 hydrogen bond donors (the total number of nitrogen hydrogen and oxygen–hydrogen bonds) • No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms) • A molecular mass less than 500 daltons • A calculated octanol-water partition coefficient (log P) that does not exceed 5 Since all the above conditions are satisfied by chloroquine, it is considered to comply with Lipinski's Rule of Five.

ADMET Prediction:

Swiss ADME allows to compute physicochemical descriptors as well as predict pharmacokinetics properties and druglike nature of one or multiple small molecules. The absorption, distribution,metabolism and excretion of chloroquine was assessd using the Swiss ADMEsoftware



Bioavailability Radar

Bioavailability Radar is displayed for a rapid appraisal of drug-likeness. Six physicochemical properties are taken into account: lipophilicity, size, polarity, solubility, flexibility and saturation. A physicochemical range on each axis was defined by descriptors and depicted as a pink area.

Physicochemical property

The physicochemical property of Isoniazid was analysed using SwissADME and was found to be;

PHYSICOCHEMICAL PROPERTY	VALUE
Molecular weight	319.87 g/mol
Number of heavy atoms	22
Number of aromatic heavy atoms	12
Number of hydrogen bond donors	1
Number of hydrogen bond acceptors	3

Lipophilicity

The partition coefficient between n-octanol and water (log Po/w) is the classical descriptor for Lipophilicity. It has a dedicated section in Swiss ADME due to the critical importance of this physicochemical property for pharmacokinetics drug discovery. Swiss ADME give

access to five freely available predictable models; which are XLOGP3, WLOGP, MLOGP, SILICOS-IT, iLOGP.

The lipophilicity of Isoniazid was studied using Swiss ADME which determines the solubility, the ability to penetrate through cell barriers, and transport across the membrane.

LIPOPHILICITY	VALUE
LogPo/w(ILOGP)	4.34
LogPo/w(XLOGP3)	4.63
LogPo/w(WLOGP)	4.70
LogPo/w(MLOGP)	3.67
LogPo/w(SILICOS-IT)	3.95
ConsenusLogPo/w	4.26

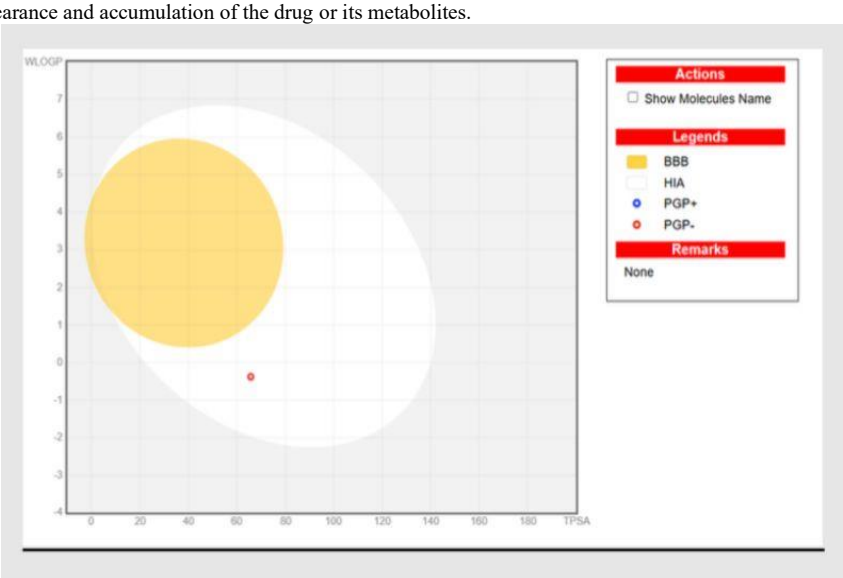
Water solubility

soluble molecule greatly facilitates many drug development activities, primarily the ease of handling and formulation. Moreover, for discovery projects targeting oral administration, solubility is one major property influencing absorption. As well, a drug meant for parenteral usage has to be highly soluble in water to deliver a sufficient quantity of active ingredient in the small volume of such pharmaceutical dosage. Two topological methods to predict Water Solubility are included in Swiss ADME. The first one is an implementation of the ESOL model36 and the second one is adapted from Ali et al.

The water solubility of Isoniazid was studied using Swiss ADME.

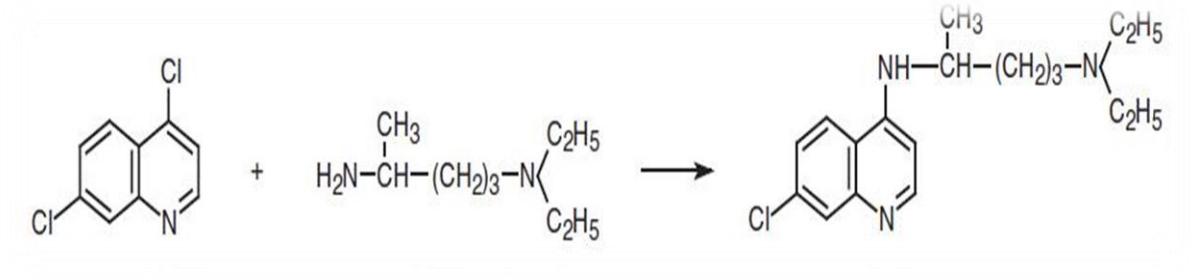
Pharmacokinetics

Pharmacokinetics is essential for the knowledge about interaction of molecules with cytochromes P450 (CYP). This superfamily of isoenzymes is a key player in drug elimination through metabolic biotransformation. The pharmacokinetics of 5-fluorouracil was studied using Swiss ADME. study shows that Isoniazid is not an inhibitor of CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4. Hence the drug do not possess any pharmacokinetics-related drug-drug interactions leading to toxic or other unwanted adverse effects due to the lower clearance and accumulation of the drug or its metabolites.



The boiled-egg allows for intuitive evaluation of passive gastrointestinal absorption (HIA) and brain penetration (BBB) in function of the position of the molecules in the WLOGP- versus-TPSA referential. In addition the points are coloured in blue if predicted as actively effluxed by P-gp (PGP+) and in red if predicted as non-substrate of P-gp (PGP−). In case Isoniazid is located in white region of boiled egg which shows that Isoniazid has high probability of passive absorption by the gastrointestinal tract. Isoniazid is not subject to active efflux by P-gp. (PGP+(red dot)

SYNTHESIS



4,7Dichlroquinoline 4-Amino-1-diethylamino pentane Chloroquine

Chloroquine is synthesized in a multi-step process starting with the formation of 4,7-dichloroquinoline, typically from aniline derivatives via cyclization and chlorination. This intermediate undergoes nucleophilic substitution with diethylamine at the 4-position to form 7-chloro-4-diethylaminoquinoline. In the final step, it is alkylated with 4,4-diaminopentane, introducing the side chain and yielding chloroquine base. The base can then be converted to its pharmaceutical salt form, such as chloroquine phosphate, by reaction with phosphoric acid.

ASSAY

- Grind the tablets into a fine powder to homogenize.
- Weigh a portion of the powder containing 0.5 g of chloroquine phosphate or sulphate.
- Add 20 ml of 1M sodium hydroxide to the weighed powder.
- Weigh 20 tablets from each coded sample.
- Extract the mixture with 4 × 25 ml portions of chloroform.
- Pool the four chloroform extracts.
- Evaporate the combined extract to approximately 10 ml.
- Add 40 ml of anhydrous glacial acetic acid to the concentrated chloroform extract.
- Titrate the mixture with 0.1M perchloric acid using non-aqueous potentiometric titration.

COMPARITIVE STUDY OF MALARIAL DRUGS

Mechanisms of Action and Efficacy

Chloroquine	Inhibits heme polymerization in the parasite's food vacuole. Accumulated toxic ferriprotoporphyrin IX (free heme) kills the Plasmodium parasite. Highly effective in the past, now limited by resistance; still used for *P. vivax* and autoimmune disorders.
Amodiaquine	Same as chloroquine: inhibits heme detoxification. Also believed to have immune-modulatory effects. Similar to chloroquine but more effective in resistant malaria; hepatotoxicity risk exists.
Piperaquine	Same MoA as chloroquine but with stronger binding to heme, better retention in food vacuole. Long-acting due to high lipophilicity. Highly potent against resistant *P. falciparum*, especially when combined with artemisinin derivatives; long half-life offers post-treatment prophylaxis.

Saftey Profiles

Chloroquine is generally well tolerated at standard antimalarial doses, but its long-term use, particularly in autoimmune diseases like lupus or rheumatoid arthritis, is associated with retinal toxicity. This can lead to irreversible retinopathy, especially with cumulative dosing, hence regular ophthalmologic monitoring is recommended. Additionally, chloroquine can cause cardiac side effects, particularly QT interval prolongation, which may predispose to serious arrhythmias in susceptible individuals. Other rare adverse effects include neuropsychiatric symptoms, such as irritability, confusion, or hallucinations. Despite these risks, chloroquine is considered safe in pregnancy and is still used where Plasmodium strains remain sensitive.

Amodiaquine has a safety profile that limits its use to short-course antimalarial therapy, especially in combination with artesunate. The most serious risks associated with amodiaquine are hepatotoxicity and hematologic toxicities, including agranulocytosis and neutropenia, which can be life-threatening. These effects are more commonly seen with repeated or prolonged use, making it unsuitable for long-term prophylaxis. Due to its Mannich base side chain, it may also increase the risk of liver enzyme elevation and liver damage. Patients receiving amodiaquine should be monitored for liver function and complete blood counts, especially during repeated dosing.

Piperaquine is generally well tolerated but has one notable safety concern: prolongation of the QT interval. This effect is dose-dependent and more pronounced when used in combination therapies (e.g., with dihydroartemisinin), necessitating caution in patients with existing cardiac conditions or those taking other QT-prolonging drugs. Unlike amodiaquine, piperaquine does not typically cause hepatotoxicity or bone marrow suppression, making it safer in terms of liver and hematologic side effects. However, due to its long half-life and cardiac effects, ECG monitoring is recommended in high-risk individuals. Its safety in pregnancy is not well established and should be considered carefully.

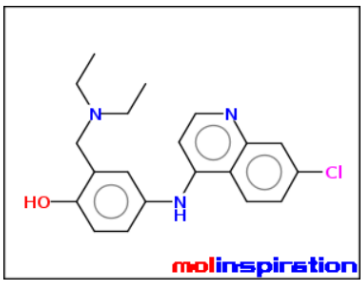
COMPARITIVE SAR OF MALARIAL DRUGS

SAR Feature	Chloroquine	Amodiaquine	Piperaquine
Core structure	4-aminoquinoline	4-aminoquinoline	Bis-4-aminoquinoline (dimer)
Position 4 substitution	Primary –NH_2 group essential for activity	Same as chloroquine	Same as chloroquine (on both quinoline units)
Side chain (at position 7 or 8)	Diethylamino-pentyl chain enhances lipid solubility	Mannich base (hydroxyanilino-propanol) improves binding & resistance profile	Two piperazine-linked bis-quinoline units increase potency
Substituents on ring	Chloro group at position 7 critical for activity	Same 7-chloro group	Both quinoline rings have 7-chloro substitution
Lipophilicity	Moderate	Slightly more polar (due to OH)	Highly lipophilic (increased tissue retention)

PHYSICOCHEMICAL PROPERTIES

molinspiration

miSMILES: CCN(CC)Cc3cc(Nc1ccnc2cc(Cl)ccc12)ccc3O
Amodiaquine



Molinspiration property engine v2022.08

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TPSA	48.38
natoms	25
MW	355.87
nON	4
nOHNH	2
nviolations	1
nrotb	6
volume	325.56

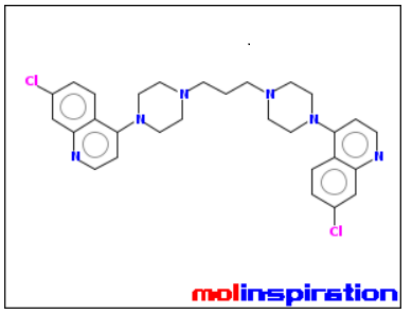
Get data as text (for copy / paste).

Get 3D geometry BETA

AMODIAQUINEPIPERAQUINE

molinspiration

miSMILES: Clc6ccc5c(N4CCN(CCCN3CCN(c1ccnc2cc(Cl)ccc12)CC3)CC4)ccnc5c6
Piperaquine



Molinspiration property engine v2022.08

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natoms	37
MW	535.52
nON	6
nOHNH	0
nviolations	2
nrotb	6
volume	476.47

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DOSAGE FORM V/S MARKETED AVAILABILITY

CHLOROQUINE

- **Dosage Forms:**
  - Tablets (usually 250 mg base)
  - Syrup (for pediatric use)
  - Injectable (intramuscular/IV, less common)
- **Marketed Availability:**
  - Available globally, but use is **limited due to widespread resistance**
  - Still used in areas with **chloroquine-sensitive Plasmodium vivax**
  - Available under brand names like *Nivaquine, Aralen*

AMODIAQUINE

- **Dosage Forms:**
  - Tablets (usually 150 mg base)
  - Most commonly used as a **fixed-dose combination** with artesunate (AS–AQ)
- **Marketed Availability:**
  - Widely used in **Africa and Southeast Asia**

- Common brand combinations: *ASAQ Winthrop*, *Coarsucam*
- Not usually available as monotherapy due to better efficacy in combinations

PIPERAQUINE

- **Dosage Forms:**
  - Primarily available in **fixed-dose combinations** with dihydroartemisinin (DHA–PQ)
  - Tablet form (varied strengths based on body weight)
- **Marketed Availability:**
  - Used in **artemisinin-based combination therapies (ACTs)**
  - Brands: *Eurartesim*, *Artekin*
  - Gaining popularity in **Asia and Africa** for treatment of uncomplicated malaria

SUMMARY OF CLINICAL USE BY POTENCY

1. PIPERAQUINE – Highest Potency

- Clinical Use: Used in combination with dihydroartemisinin (DHA–PQ) for uncomplicated falciparum malaria, especially in multi-drug resistant regions.
- Potency: Highest among the three due to strong binding to heme and prolonged half-life.
- Advantages: Effective against resistant strains, long-acting, well-tolerated.
- Limitation: Used only in combination therapy, not monotherapy.

2. AMODIAQUINE – Moderate to High Potency

- Clinical Use: Combined with artesunate (AS–AQ) for treatment of uncomplicated malaria in Africa and some parts of Asia.
- Potency: Higher than chloroquine, effective against chloroquine-resistant *P. falciparum* strains.
- Advantages: Cost-effective, short-course, effective in ACTs.
- Limitation: Mild risk of hepatotoxicity and neutropenia with repeated use.

3. CHLOROQUINE– Lowest Potency (due to resistance)

- Clinical Use: Still used in chloroquine-sensitive areas for vivax and ovale malaria, and sometimes for prophylaxis.

- Potency: Originally effective, but now low due to widespread resistance in *P. falciparum*.
- Advantages: Safe, cheap, and well-studied.
- Limitation: Not effective against resistant strains.

SCREENING METHODS FOR ANTIMALARIAL DRUGS

IN VITRO METHODS

1. 3H hypoxanthine uptake
2. Giemsa-stained slide method
3. Micro test
4. Flow cytometry
5. Measurement of Idh activity of p. Falciparum
6. Isobologram analysis

IN-VIVO METHOD

1. Plasmodium berghei 4-day suppression test
2. Hill's test for causal prophylaxix andresidual activity
3. Sporonoicidal activity testing
4. Plasmodium cynomolgi rhesus model

IN-VITRO METHODS

MICRO TEST

- Most commonly used method for Antimalarial testing for resistance.
- Provide information on the quantitative drug response of plasmodium falciparum
- Test can be carried out by several drugs, in a micro test kit with 12 x 8 wells, predosed with
- Chloroquine
- Mefloquine
- Amodiaquine
- Quinine
- Artemisinin
- Sulfadoxine
- pyrimethamine

Procedure

- Patients' blood sample is inoculated in the wells and incubated in the suitable medium
- The number of schizonts is counted and compared with control well
- For monitoring the level and spread of resistance, molecular diagnostic method for detecting resistant parasite have been proposed.
- These molecular tools are based on the detection by PCR of point mutation in the parasite genome responsible for invitro resistance.

3H HYPOXANTHINE UPTAKE TEST

- Most commonly used method for assessing antimalarial efficacy of a compound in vitro.
- Hypoxanthine used by parasite for purine salvage and DNA synthesis.
- Radiolabelled hypoxanthine uptake by parasite is an indicator of its growth and multiplication.
- Parasites are cultured in different concentration of test compounds in media containing reduced concentration of hypoxanthine.
- After which 3H Hypoxanthine is added.
- Measurement of radioactivity by a 1205 betaplate reader.
- Percent reductions are measured.

- Evalution % reduction = (mean cpm of test samples) 3H Hypoxanthine X 100
- Percent reductions are used to plot percentage inhibition of growth as a function of drug concentration.
- IC 50 are determined by linear regression analyses.

GIEMSA-STAINED SLIDE METHOD (MIC METHOD)

- Low-cost alternative for testing small number of compounds.
- Parasites are incubated with test compound.
- Parasitemia of control and treated groups are compared by counting Giemsa stained parasites by light microscopy.
- Parasites incubated in 5% suspension of erythrocytes at 370C.
- Change in the proportion of infected RBCs is assessed at end of 72 hr. at various concentrations of each drug.
- Drug is dissolved in 4% sucrose and fed ad libitum to the insects following the blood meal.
- The level of drug activity can be calculated from a comparison of mean oocyst counts in treated and control batches.

FLOW CYTOMETRY

- Parasites are fixed after appropriate period of incubation with test compounds.
- The parasite nuclei are stained with DAPI (42, 6-diamidino-2-phenylindole).
- Counts of treated and control cultures are then obtained by flow cytometry.

ADVATAGES OF INVITRO METHOD

- Precise and efficient
- Rapid
- Large number of compounds can be evaluated at the same time
- Synergism or antagonism of drug combinations can be studied
- Better assessment of intrinsic activity of drug

LIMITATION OF INVITRO METHODS

- Drug acting through active metabolite cannot be studied
- Non reproducibility of pharmacokinetic effect
- Toxic compounds also get selected
- Lack of clinical correlation.

IN-VIVO METHODS

- Compounds effective in invitro screening test are taken up for in vivo evaluation
- Plasmodium species that couse human disease are unable to infect non primate animal models
- Rodent malaria parasite plasmodium berghei, p.yoelii, p.chaboudi, p.vinckei have been used extensively in drug discovery and early development

PLASMODIUM BERGHEI 4 DAY SUPPRESSIO METHOD

- Most widely used preliminary test.
- Efficacy assessed by comparison of blood parasitemia and mouse survival time.
- Mice maintained at 220C at 50-70% humidity.
- Diet containing p-aminobenzoic acid 45 mg/kg and water ad libitum.
- On day o, mice are injected with 0.2 ml of aliquot (2X107 parasitized erythrocytes by Plasmodium berghei)
- Experimental groups are (five each):
- Vehicle treated (control group)
- Test drug treated group.
- Positive control group (chloroquine, reference drug) is administered.
- On day 1 to 3, the experimental groups are treated.
- On day 4, blood smears from all animals are prepared with Giemsa stain. Parasitemia is determined microscopically by counting 4 fields of approximately 100 erythrocytes per field.

- The difference between the mean value of the control group and those of the experimental groups is calculated. Untreated control mice typically die approximately one week after infection.
- For treated mice mean survival time is calculated in comparison with the untreated and standard drug treated group.
- Mice without parasitemia on day 30 of post-infection are considered cured.
- Compounds identified as active in this test are progressed through several of the following secondary tests. In the 'Dose ranging full four-day test', compounds are tested at four doses, by different routes of administration, to determine ED50 value. This test also leads to information about relative potency and bioavailability.

SUMMARY

Chloroquine, a 4-aminoquinoline, was once the cornerstone in the treatment and prevention of malaria, particularly *Plasmodium falciparum* and *P. vivax* infections. Its mechanism involves interference with heme detoxification in the parasite's food vacuole, ultimately leading to parasite death. However, the widespread emergence of chloroquine-resistant strains, especially of *P. falciparum*, has significantly reduced its efficacy in many regions.
This comparative study analyzes chloroquine alongside other 4-aminoquinolines such as amodiaquine, piperaquine, and nivaquine, focusing on parameters like:

- Efficacy
- Resistance profiles
- Pharmacokinetics
- Safety and tolerability

CONCLUSION

While chloroquine has played a historic role in malaria management, its reduced efficacy due to resistance limits its current use. In contrast, other 4-aminoquinolines like amodiaquine and piperaquine offer enhanced effectiveness when used in combination regimens. The future of malaria treatment lies in integrated strategies utilizing combination therapies, resistance monitoring, and region-specific drug policies. Chloroquine may still have niche applications, but newer analogs provide better clinical outcomes and improved resistance profiles.

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