Antiprotozoal Drugs (Lecture 16)
Dr Chishti

Knowledge Objectives

1 .Know the organisms responsible for malaria.

2. Know the mechanisms of action of the antiprotozoal drugs discussed in the lecture.

3. Know the life cycle of Plasmodium, and how this affects malaria treatment. Know which drugs are useful for which life stages.

4. Know the mechanisms of action of the major antimalarial drugs.

5. Know the major adverse effects of antimalarial drugs.

6. Understand how resistance to , and relapse from, antimalarial drugs occurs.

Drug List

metronidazole
primaquine
mefloquine
chloroquine
haloxantrine
quinine
pyrimethamine
doxycycline
artemisinin
proguanil
dapsone
sulfadoxine

Antiprotozoal Drugs

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Classification and Strategy

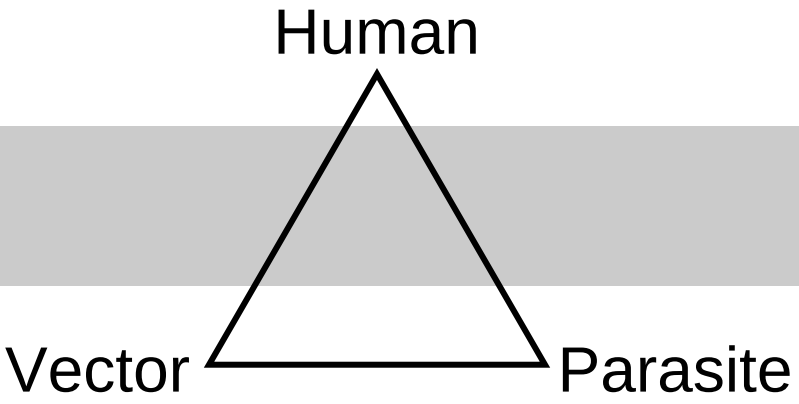
- **Kingdom---Protista---Eukaryotic unicellular organisms. Protozoa---One celled solitary heterotrophs**

- **Anti-parasite chemotherapy (Biochemical analysis of essential processes). Differences between parasite and host. (a) Enzymes (b) Membrane transporters (c) Microtubules (d) Synaptic transmission (e) Unknown targets**

Major Parasite Infections

- **Protozoan**
  - **Malaria**
  - **Amebiasis**
  - **Giardiasis**
  - **Leishmaniasis**
  - **Trypanosomiasis**
  - **Trichomoniasis**
  - **Toxoplasmosis**
  - **Pneumocystosis**

Parasites



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  - **Malaria**
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  - **Giardiasis**
  - Toxoplasmosis
  - Trichomoniasis
  - **Leishmaniasis**
  - **Trypanosomiasis**
    - Chagas' Disease =American Trypanosomiasis
    - African Sleeping Sickness

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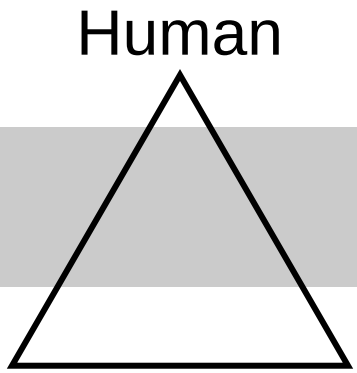
Water + Food

Insect

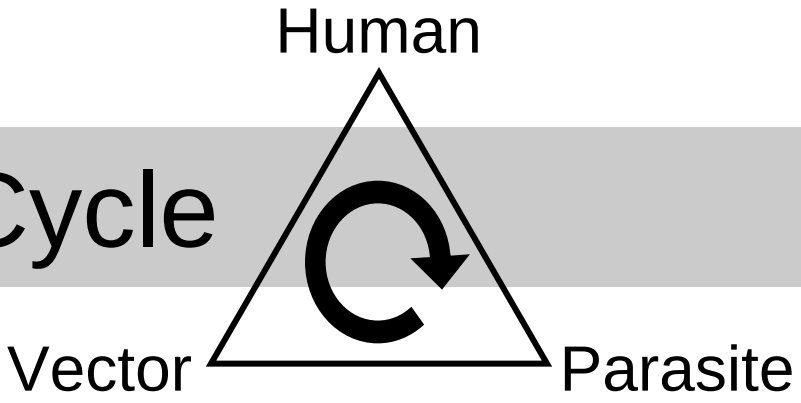


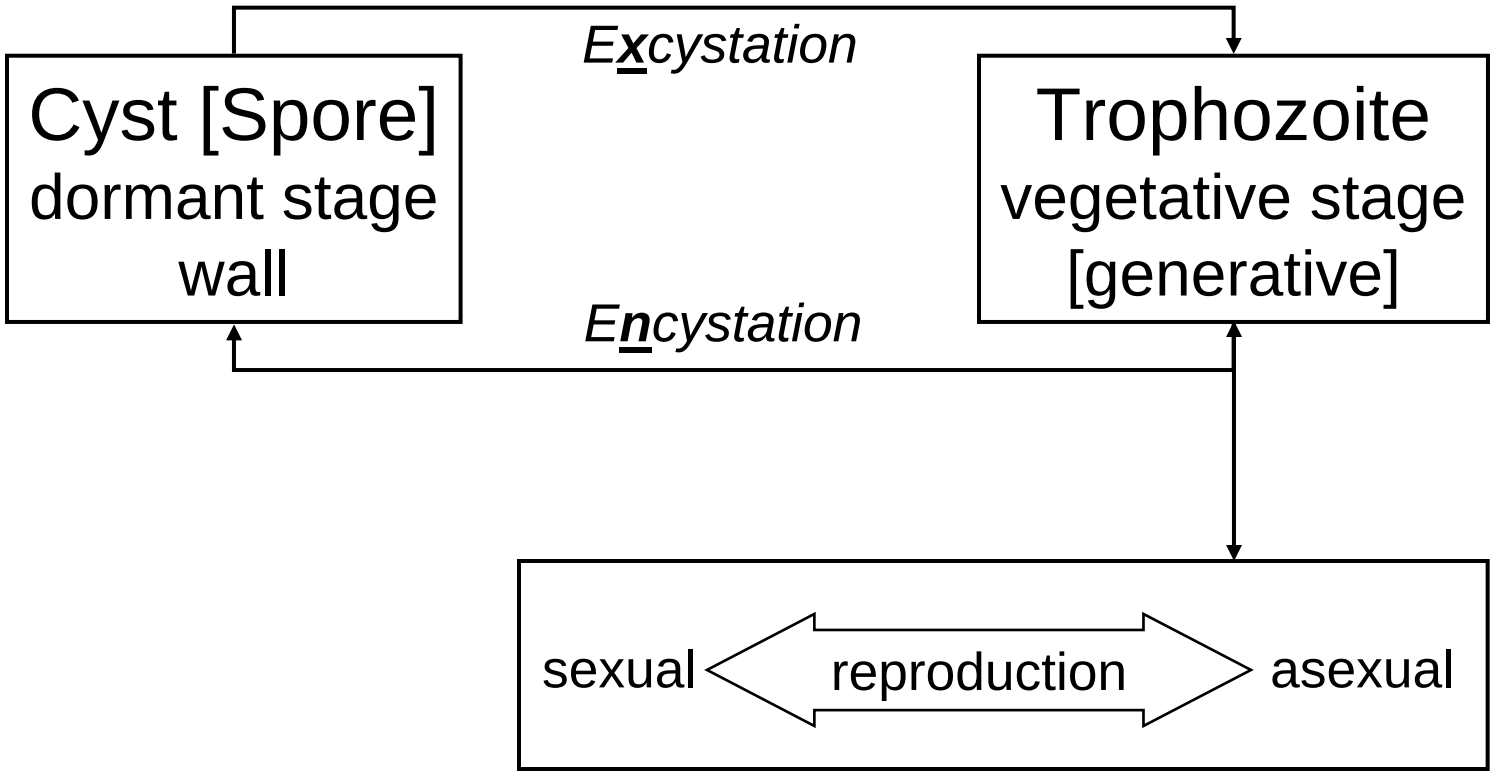
Parasitism

Symbiotic relationship, in which the host is injured...

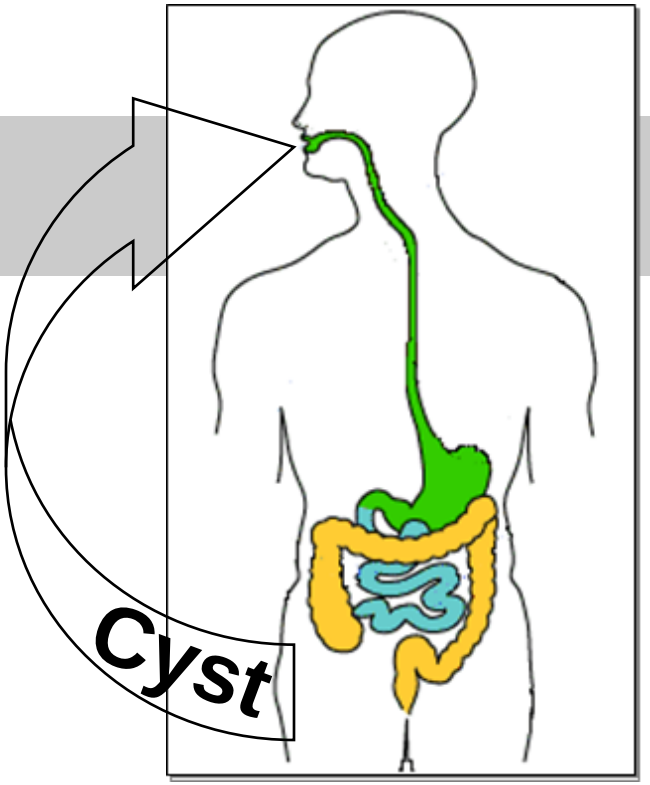
...or

Protozoan Life Cycle

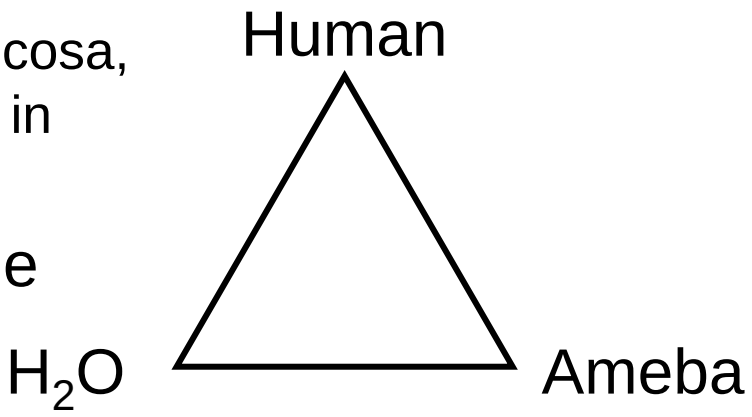




Amebiasis Life Cycle



- Infection by ingestion of cysts
  - fecal contamination
- Excystation in the ileum
- **Trophozoites** multiply by binary fission in the large intestine
  - Most remain in the lumen of the intestine
  - Some may invade the intestinal mucosa, enter the bloodstream and develop in extraintestinal sites
- **Cyst** formation is triggered by the dehydration of gut contents



PCR diagnostic test for detection of *Giardia* DNA

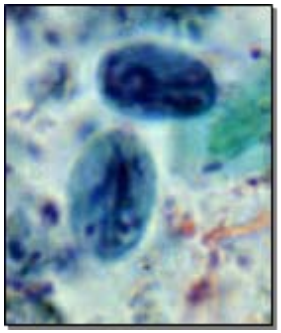
Amebiasis & Giardiasis

- *Entamoeba*
  - one-celled parasite
  - *E. histolytica*
  - *E. hartmanni*
    - motile trophozoites
    - dormant cyst
  - affects large intestine (colon)

Cysts

Trophozoites

- *Giardia intestinalis*
  - cysts are hardy (pH, Cl_2)
    - survive several months in cold water
    - most common intestinal parasite in the US (16% prevalence)
  - affects small intestine (ileum)



Cysts

Trophozoites

Amebiasis & Giardiasis

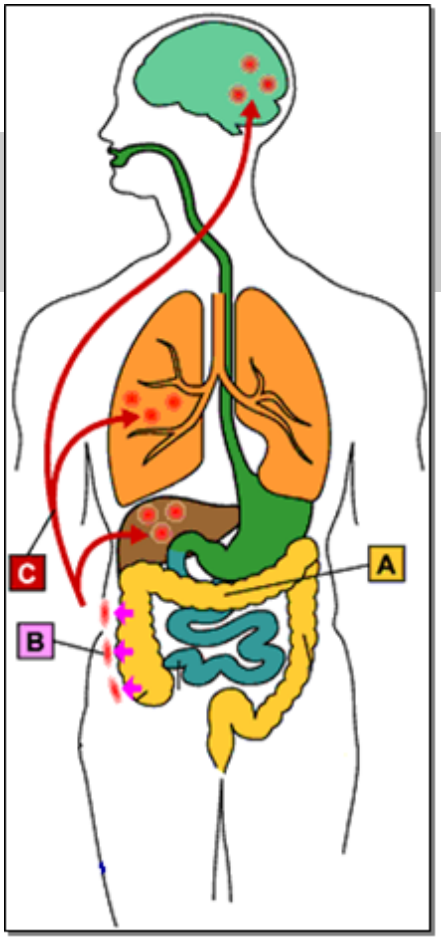
Signs and Symptoms

For some people, symptoms of Amebiasis can begin within days to weeks of swallowing food or water contaminated by amoebas. For other people, symptoms of Amebiasis either take months to appear or never appear at all.

When people do become ill, they experience abdominal pain that begins gradually, along with frequent loose or watery bowel movements, cramps, nausea, and a loss of appetite. In some cases they develop a fever and, possibly, bloody stools.

- Amebiasis

Drugs



A • Non invasive colonization
  – asymptomatic cyst passer
  – luminal agent (85-94% cure)

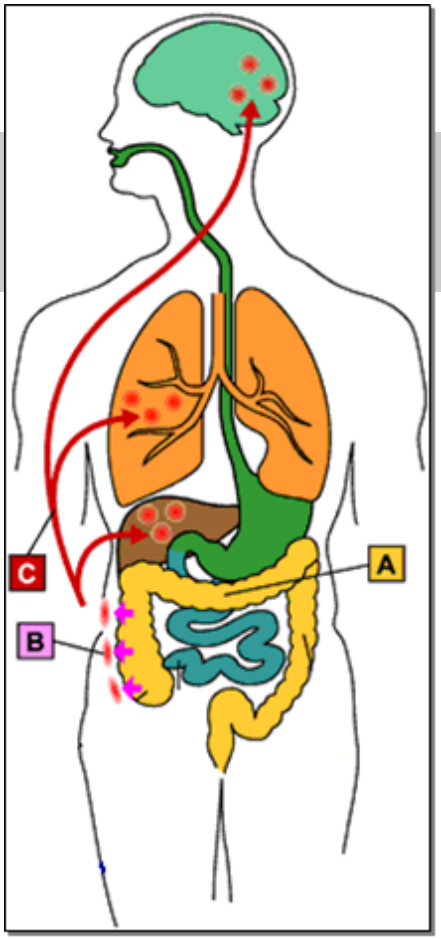
B • Intestinal Disease

C • Extra-Intestinal Disease
  – 1. systemic; 2. luminal agent

luminal agents	**systemic agent**
poorly absorbed	well absorbed
paromomycin 25 mg/kg/d, 3x/d, 7d iodoquinol 650 mg 3x/d, 20d diloxanide furoate 500 mg, 3x/d, 10d	**Metronidazol** 750 mg, 3x/d, 7d

Giardiasis (Beaver Fever)

Drugs



A • Non invasive colonization
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B • Intestinal Disease

C • Extra-Intestinal Disease
- 1. systemic, 2. luminal agent

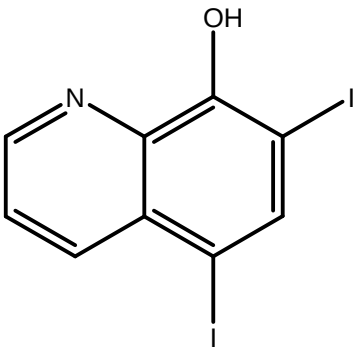
85-95% cure

luminal agents	systemic agent
poorly absorbed	well absorbed
Paromomycin 25 mg/kg/d, 3x/d, 7d iodoquinol 650 mg 3x/d, 20d diloxanide furoate 500 mg, 3x/d, 10d	**Metronidazol** ~~750~~ 250 mg, 3x/d, 7d

Yodoxin^{R}

Furamide^{R} (CDC)

Iodoquinol



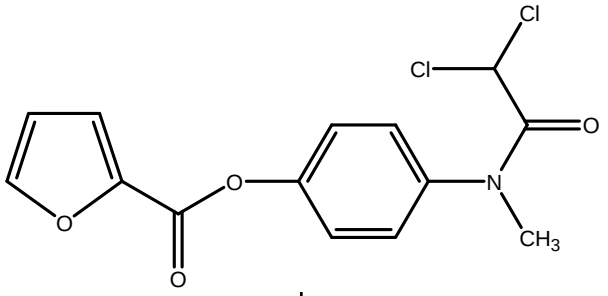
GlcA

10% unabsorbed
luminal amebicide

urine
90%

Dosage ↑ — neurotoxic, iodine toxicity

Diloxanide Furoate



furoic acid

diloxanide

GlcA

10% unabsorbed
luminal amebicide

urine
90%

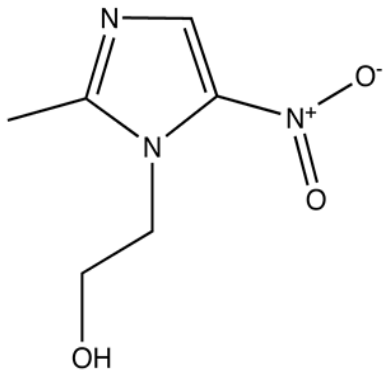
- mechanism unknown

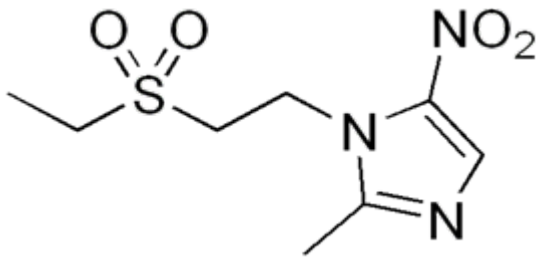
Flagyl

Fasigyn

Metronidazole

Tinidazole





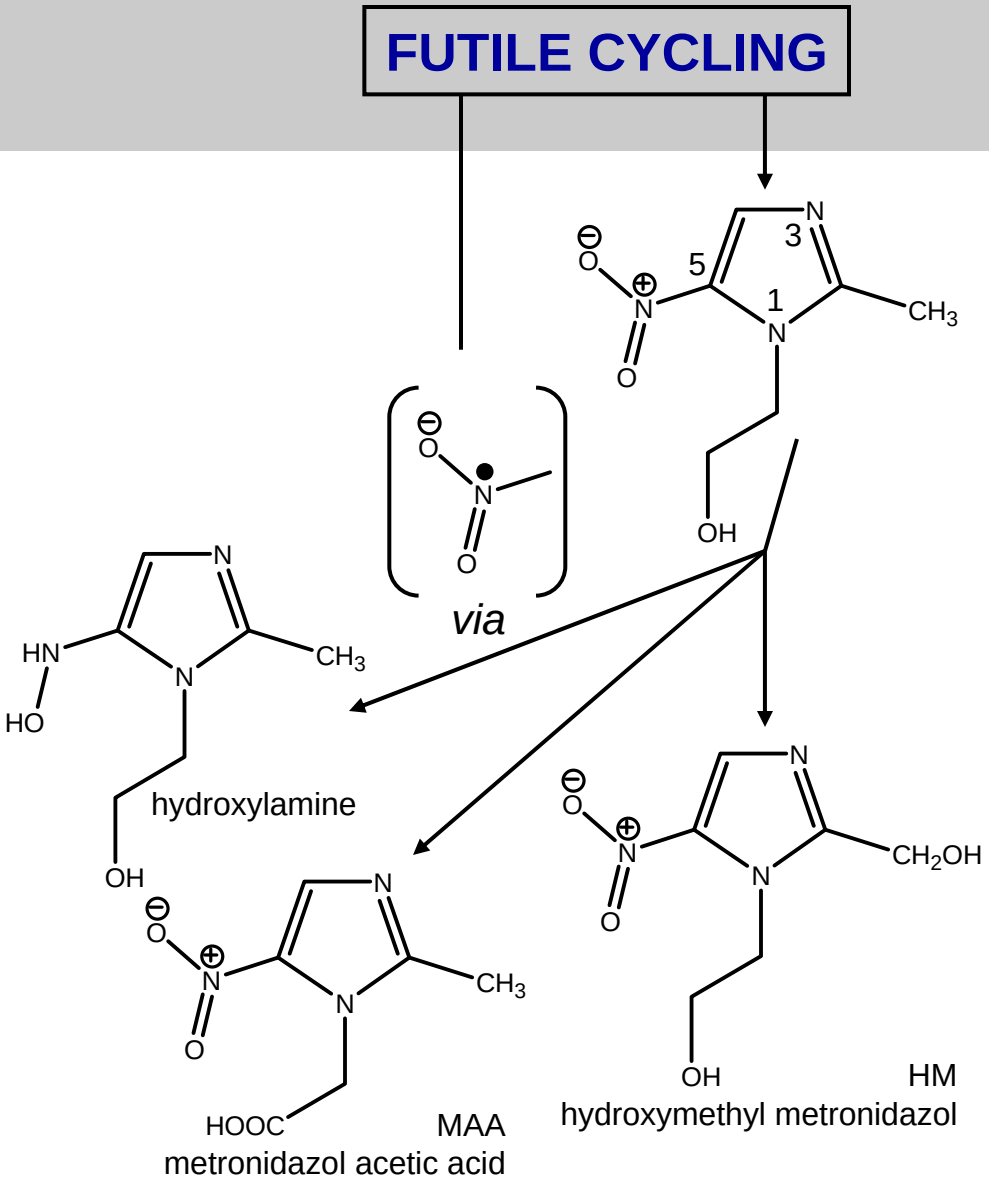
- **Acute invasive intestinal disease; hepatic amebiasis.**
- **Kills the trophozoites but not cysts.**
- **Oral. Bitter metallic taste. Nausea, headache, itchiness, dry mouth, diarrhea, and dizziness.**
- **Interferes with alcohol metabolism.**
- **Mutagenic in bacteria. Not recommended in pregnancy.**

Flagyl^{R}, Metryl^{R}, Satric^{R}

Mechanism of action

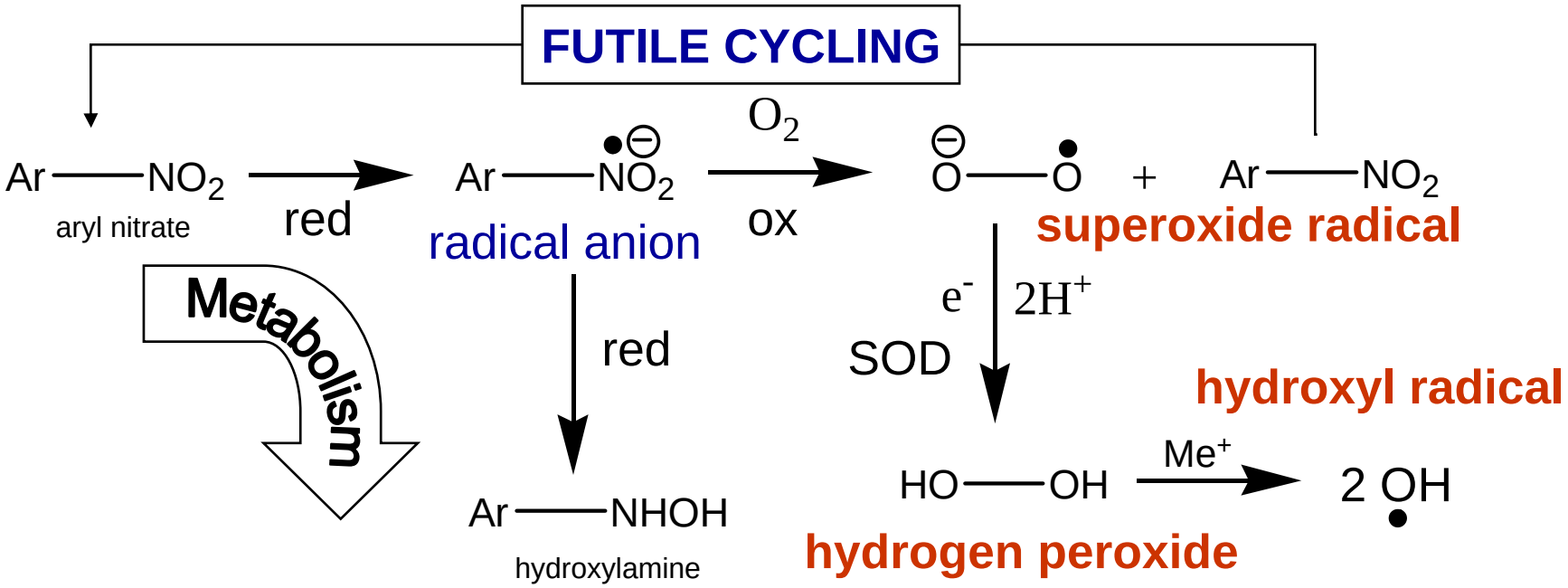
Metronidazole

- 5-nitroimidazole derivatives
  - Tinidazole
- pro-drug
- NO_2 reduced by anaerobic organisms
- yield hydroxylamine and nitroso derivatives
- destructive to parasite



Reactive **O**xygen **S**pecies

- Oxidation as active principle



LEISHMANIASIS

- Mainly tropical and subtropical. About one million cases each year. A serious infection in patients with AIDS.

- Flagellated form (female sand fly) and non-flagellated form (bitten host). Mononuclear phagocytes. Cutaneous form and Visceral form (kala azar).

- Kala azar (Visceral Leishmaniasis or Black Fever) is a potentially fatal infection, where the parasite spreads through blood stream causing hepatomegaly, splenomegaly, anemia, and fever.

LEISHMANIASIS

Parasites

- *L. braziliensis*
- *L. mexicana*
- *L. tropica*
- *L. donovani* (kala azar)

LEISHMANIASIS

Therapies:

Antimony compounds (pentavalent antimonials).

Resistance is increasing.

Drugs:

Sodium stibogluconate (Pentostam^{R}) and Meglumine antimonate (Glucantim^{R}) for visceral disease. Mechanism? Oxygen free radicals?

Intramuscular or intravenous injection. Side effects include gastrointestinal symptoms, fever, headache, and rash. Electrocardiographic changes may occur such as T wave changes and QT prolongation, thus leading to dangerous arrhythmias.

Pentamidine isethionate—Antimony-resistant Leishmaniasis. Highly toxic.

Miltefosine (Impavido^{R})—Orally effective against cutaneous and visceral leishmaniasis.

Paromomycin, amphotericin, metronidazole—cutaneous lesions.

TRYPANOSOMIASIS

Three species of parasites cause human disease:

- **Trypanosoma (brucei) gambiense and T. rhodesiense (Sleeping sickness in Africa)**
- **Trypanosoma cruzi (Chagas' disease in South America)**

TRYPANOSOMIASIS

- About 50,000 infections of sleeping sickness are reported each year and almost 50 million people are at risk.
- Sleeping sickness infection begins with the bite of tsetse fly in sub-Saharan Africa. In both infections, local lesions are followed by parasitemia and fever.
- Damage to organs is caused by toxins released by the parasite. CNS (Sleeping sickness) and other organs including heart (Chagas' disease).

TRYPANOSOMIASIS

- **Drugs for sleeping sickness: Suramin, Pentamidine**

- **Suramin--**given by slow intravenous injection. Binds to host plasma proteins and enters the parasite by endocytosis. Released by parasite proteases and inhibits key parasite enzymes. **Does not kill parasites but clears them from circulation.** Side effects include vomiting, dermatitis, and kidney damage.

- **Pentamidine isethionate--**an alternative to suramin for hemolymphatic stage of sleeping sickness. Drug is rapidly taken up by the parasite and interacts with its DNA. Serious side effects include blood pressure drop and kidney damage.

- **Melarsoprol--**good for the late stage infection with CNS involvement.

TRYPANOSOMIASIS

- **Drugs for Chagas' disease:**

  Primaquine, Puromycin, Nifurtimox, Benznidazole

  Nifurtimox and Benznidazole are used in the acute stage disease. In essence, there is no effective treatment for this condition.

Other Protozoal Infections

TOXOPLASMOSIS

Parasite---*Toxoplasma gondii* (cats). Pregnancy, AIDS

Drugs---Pyrimethamine + Sulfadiazine

TRICHOMONIASIS

Parasite---*Trichomonas vaginalis*. Inflammation of the vagina.

Drugs---Metronidazole. Tinidazole (high doses)

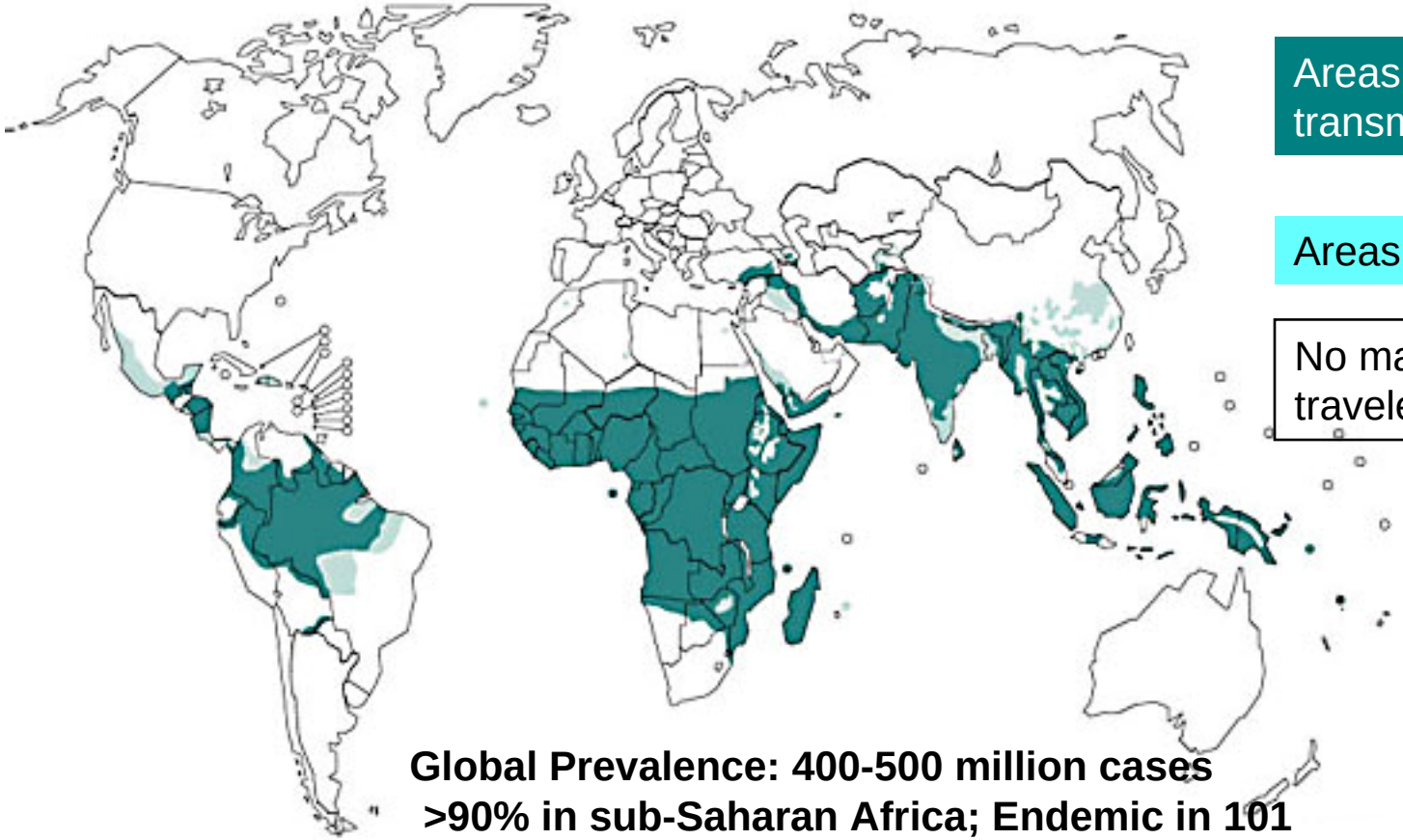
Pneumocystis Pneumonia (*P. carinii*). A very serious infection in AIDS patients.

Drugs---High dose of Trimoxazole with Pentamidine.


MALARIA
(mala–aria = bad air)

Epidemiology

WHO



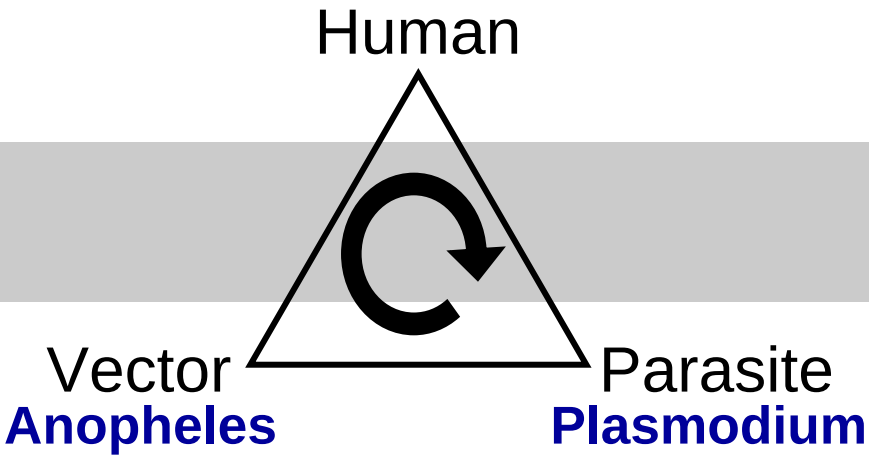
Areas where malaria transmission occurs

Areas with limited risk

No malaria (except travelers)

Global Prevalence: 400-500 million cases >90% in sub-Saharan Africa; Endemic in 101 countries. Mortality >1.5 million/year (mostly young children)

Malaria



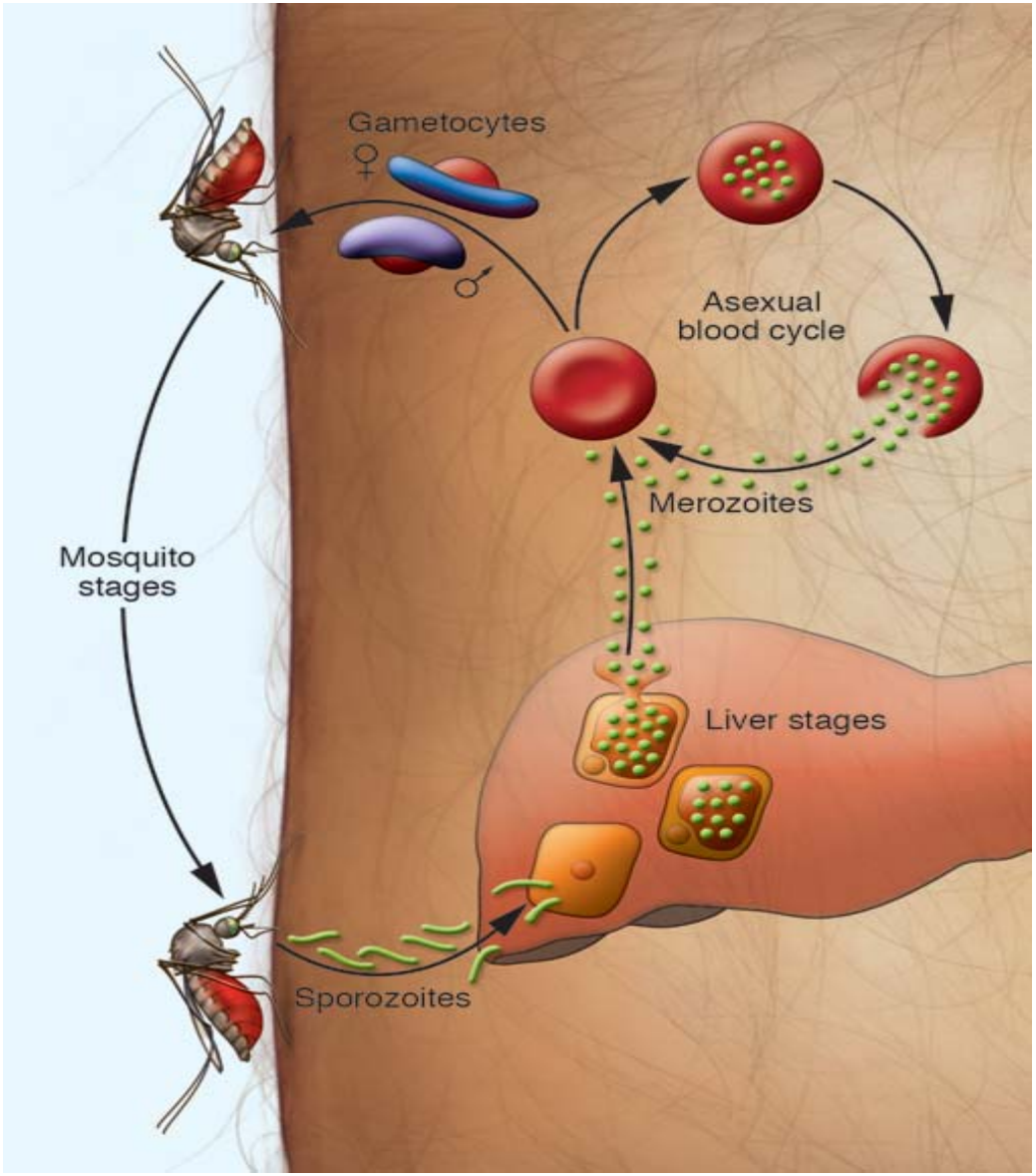
Phylum: *Apicomplexa*

Genus: *Plasmodium*

1. P. falciparum	**M. tropica**
2. P. vivax	**M. tertiana**
3. P. ovale	**M. tertiana**
4. P. malariae	**M. quartana**

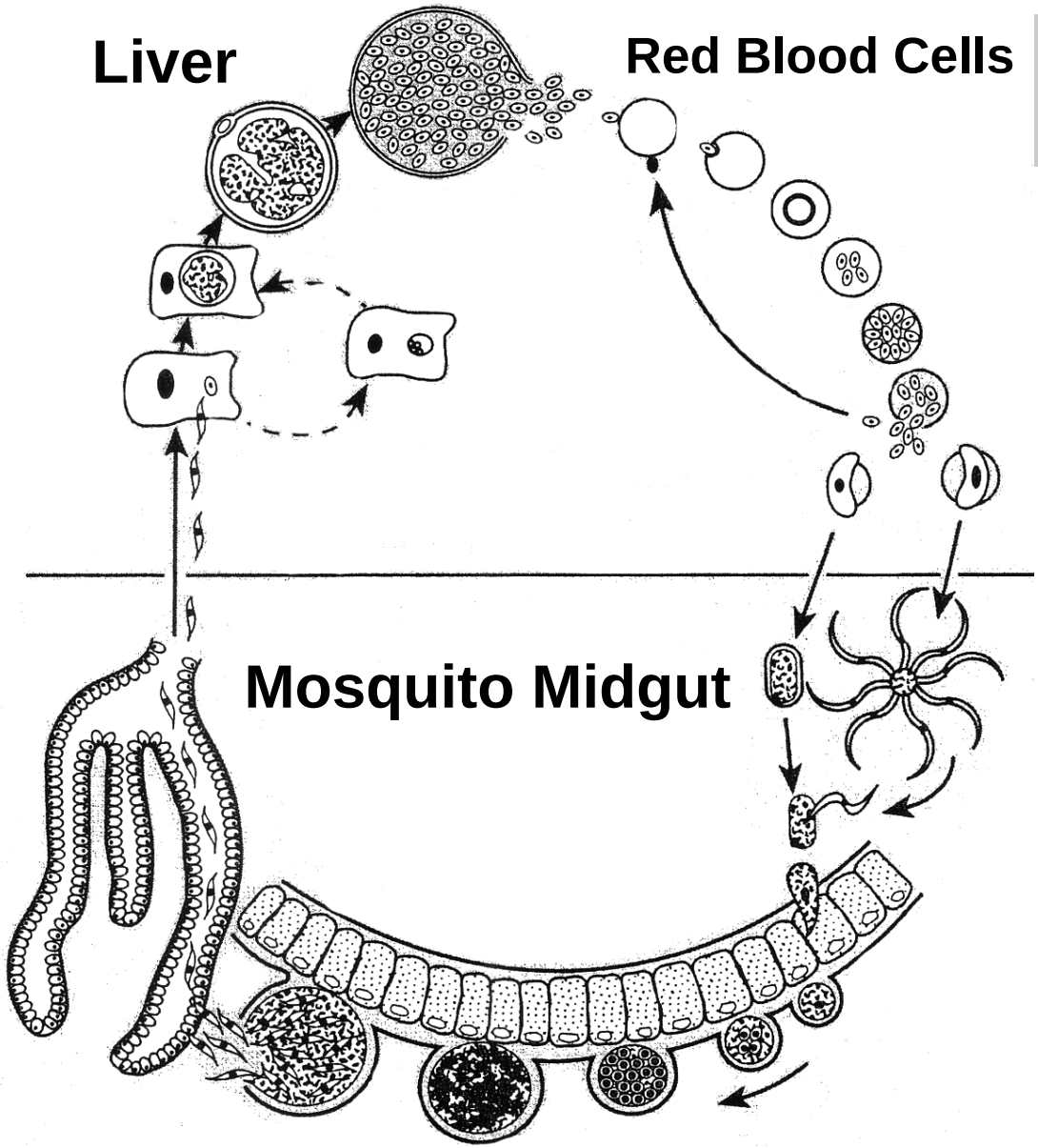
***P. knowlesi* (monkey malaria parasite)**

Life cycle of *Plasmodium falciparum*



J. Clin. Invest. 2009 (119) 2496-2505

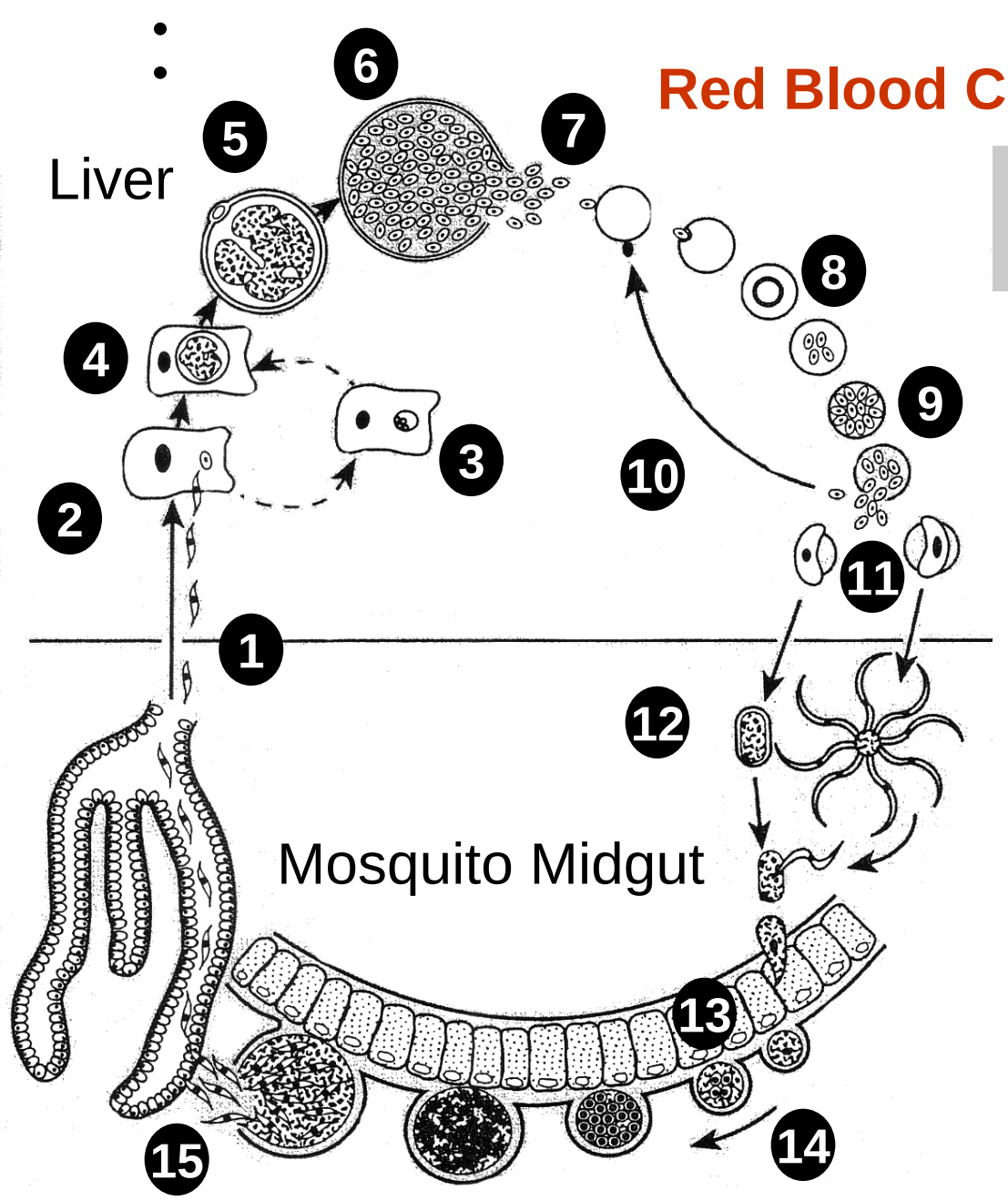
Understand Drugs?
Malaria Life Cycle!



HUMAN

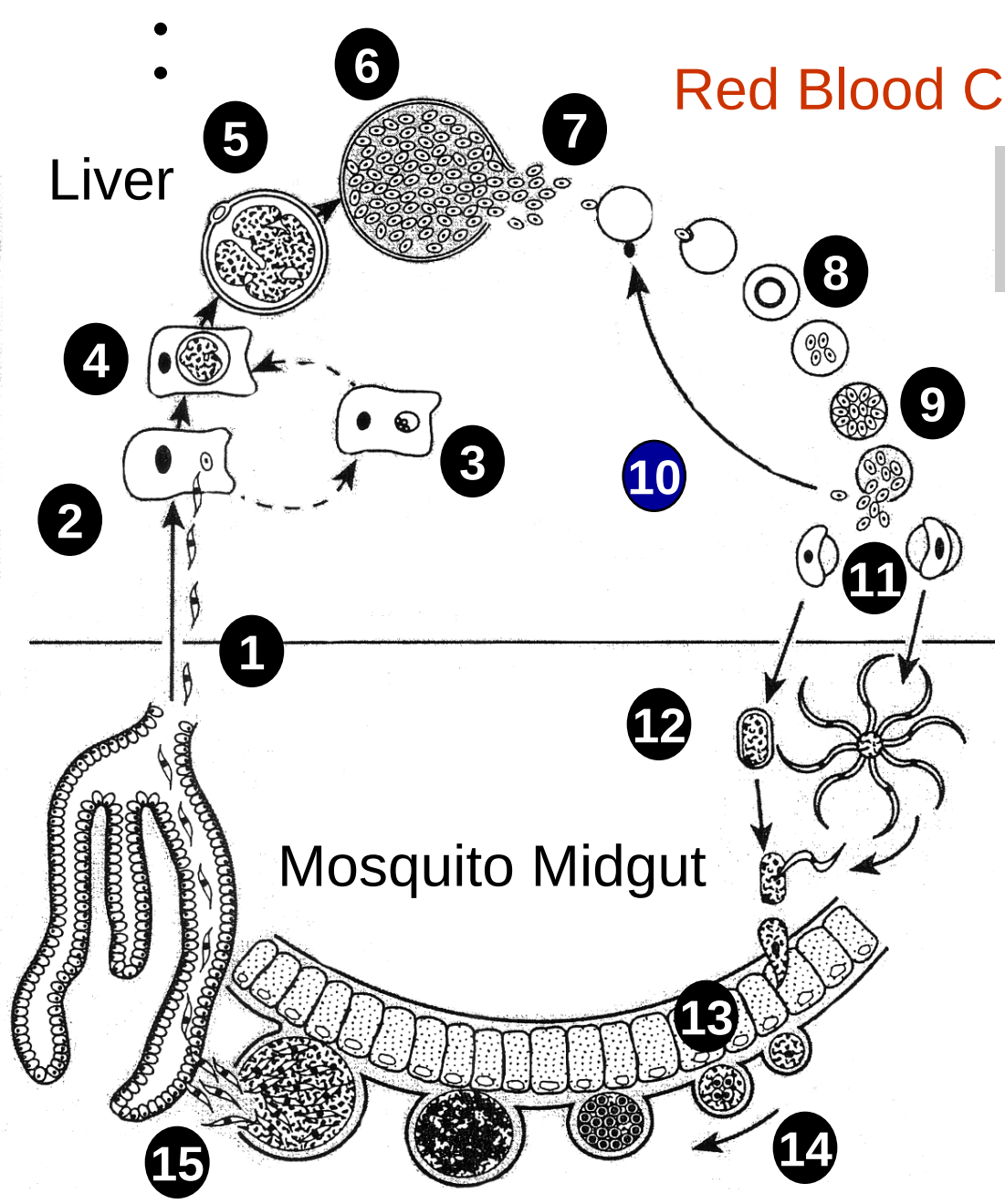
MOSQUITO

Malaria Life Cycle



1 Sporozoite injection through skin
2 Sporozoite infected hepatocyte
3 Hypnozoites (*P. vivax/ovale*)
4 Liver stage parasite
5 Tissue schizogeny
6 Exoerythrocytic = liver schizonts
7 Merozoites blood release
8 Ring stage, trophozoites
9 Schizogeny to blood schizonts
10 Merozoites‘ release and erythrocyte re-infection
11 Female and male gametocytes (macro-) (micro-)
12 Mature gametocytes
13 Ookinete penetrates gut wall
14 Oocyst development
15 Sporozoite penetrate salivary gland

Malaria **Fever**



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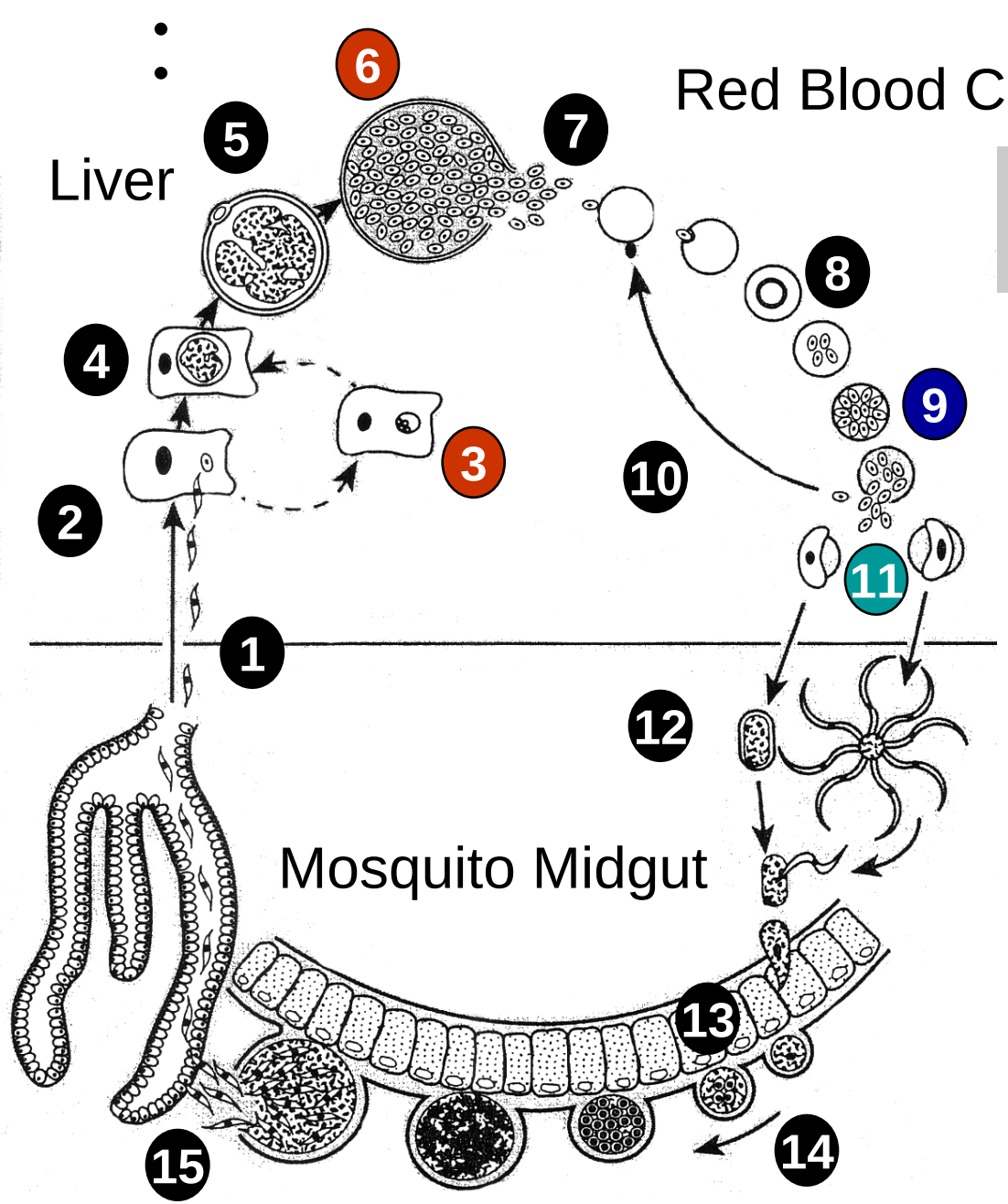

Drug Targets

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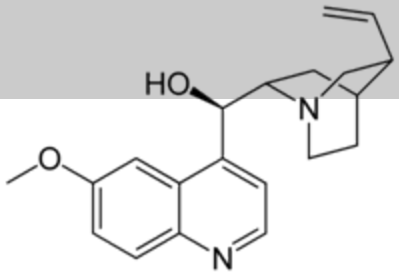
3 Targets → 3 Major Groups of Drugs

	Liver		Blood		
	Hypnozoiticides	**Schizontocides**	**Schizontocides**		**Gamtocytocides**
quinolones					
artemisinins					
anti-folates (***sulfa drugs***)					

Selectivity of Antimalarial Drugs

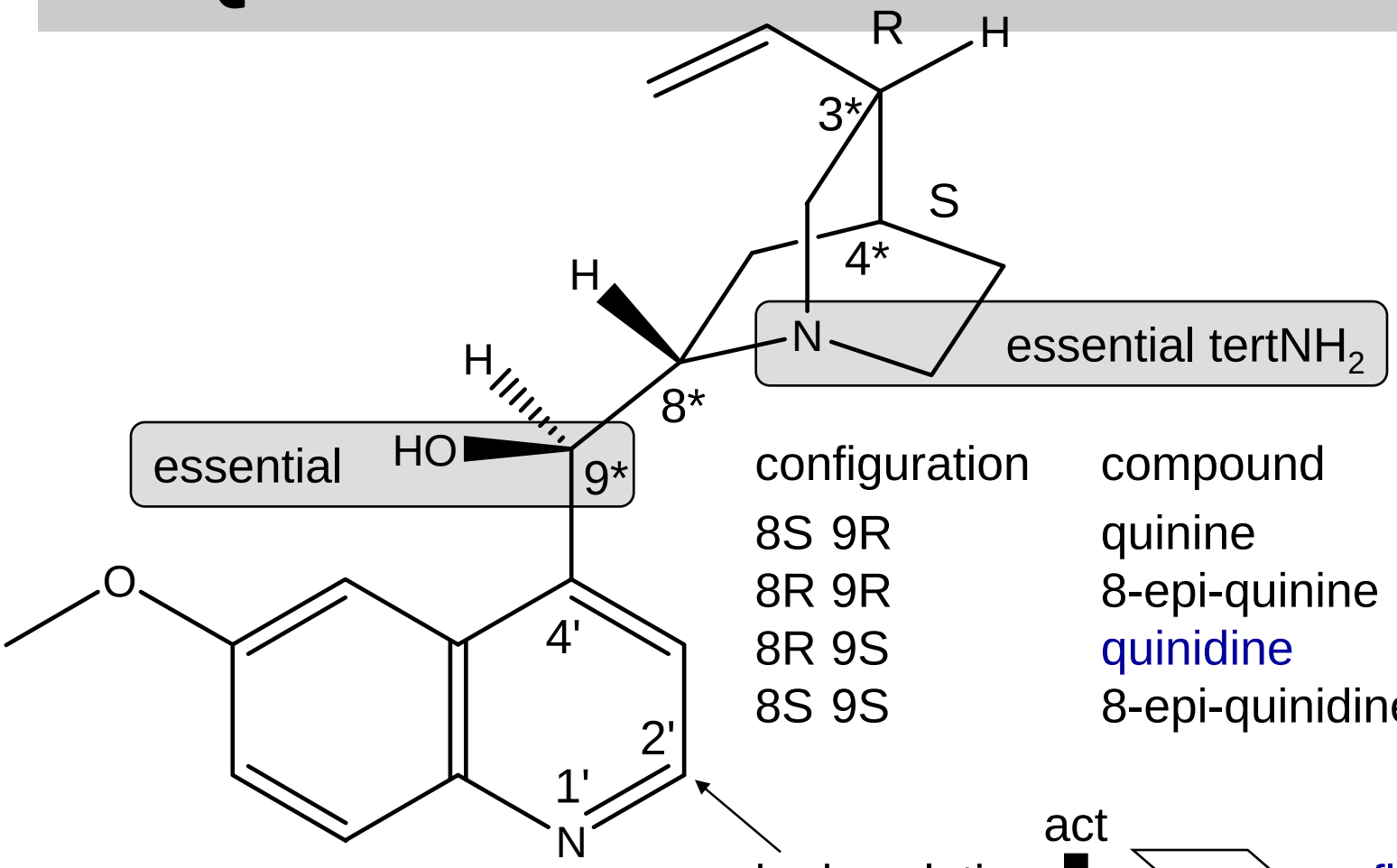
	Liver		Blood		
	Hypnozoiticides	**Schizontocides**	**Schizontocides**		**Gametocytocides**
			QCsens	QCresist	
4-substituted quinolones (**QC**)	–	–	+	–!	–
- quinine	–	–	+	+	–
- mefloquin					
8-substituted quinolones	+	+	weak	weak	+
artemisinins	–	–	+	+!	+
anti-folates (***dhps*** inhibition)	supplemental	supplemental	$+_{slow}$	$+_{slow}$	–
anti-folates (***dhfr*** inhibition)	–	–	weak	weak	–

Quinine



- **Natural alkaloid isolated from the bark of cinchona tree in Peru. Has been used as an anti-malarial drug since 17th century.**
- **Quinine salts are given orally or intravenously. Quinine sulfate tablets (Qualaquin). Not recommended as prophylaxis.**
- **Extremely bitter taste. Causes red cell hemolysis in G6PD deficiency. May cause birth defects and abnormal heart rhythms. QT interval prolongation has been a consistent finding with oral or parenteral quinine administration.**

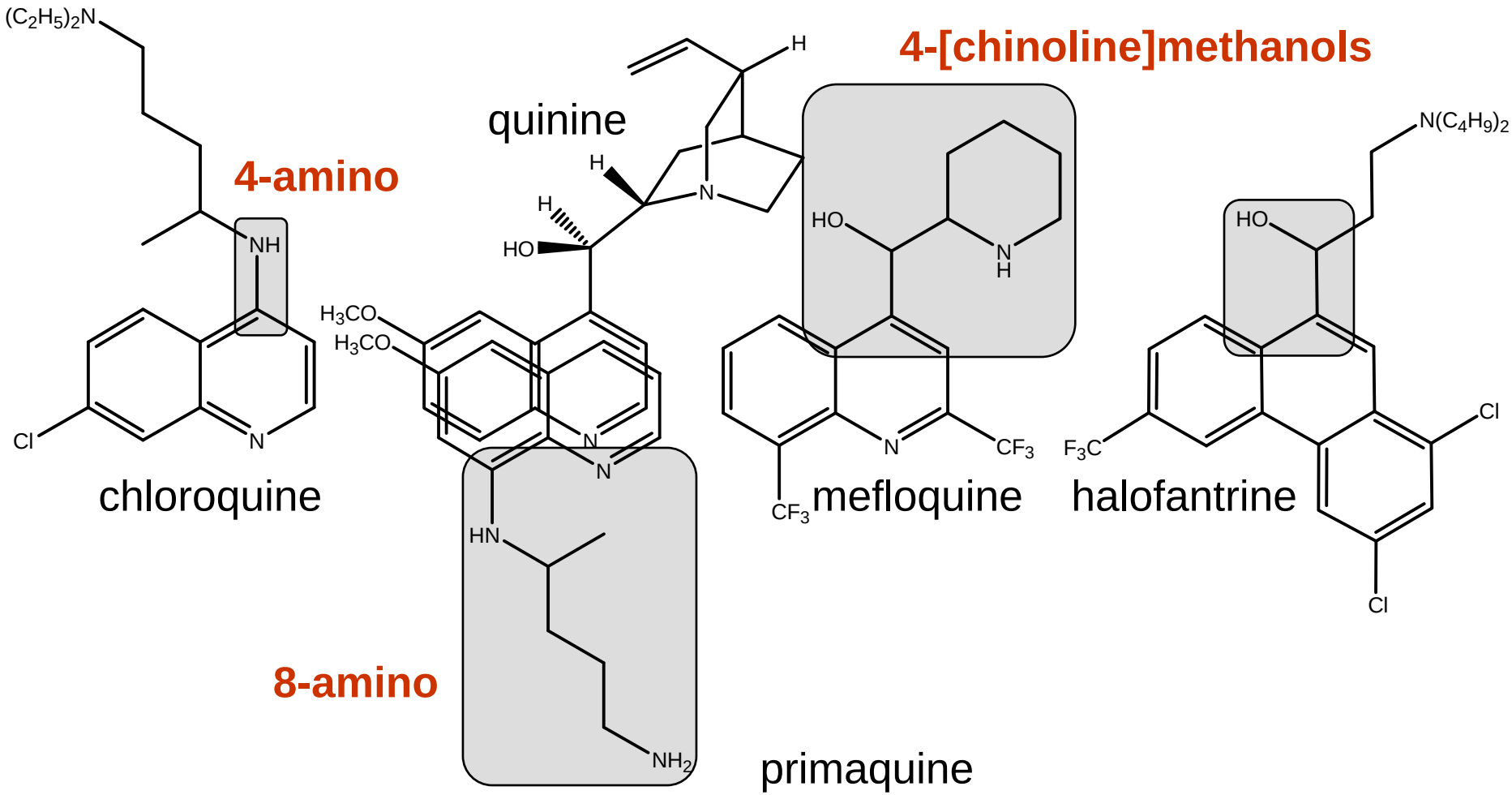
Quinine



configuration	compound	activity
8S 9R	quinine	+
8R 9R	8-epi-quinine	–
8R 9S	quinidine	+
8S 9S	8-epi-quinidine	–

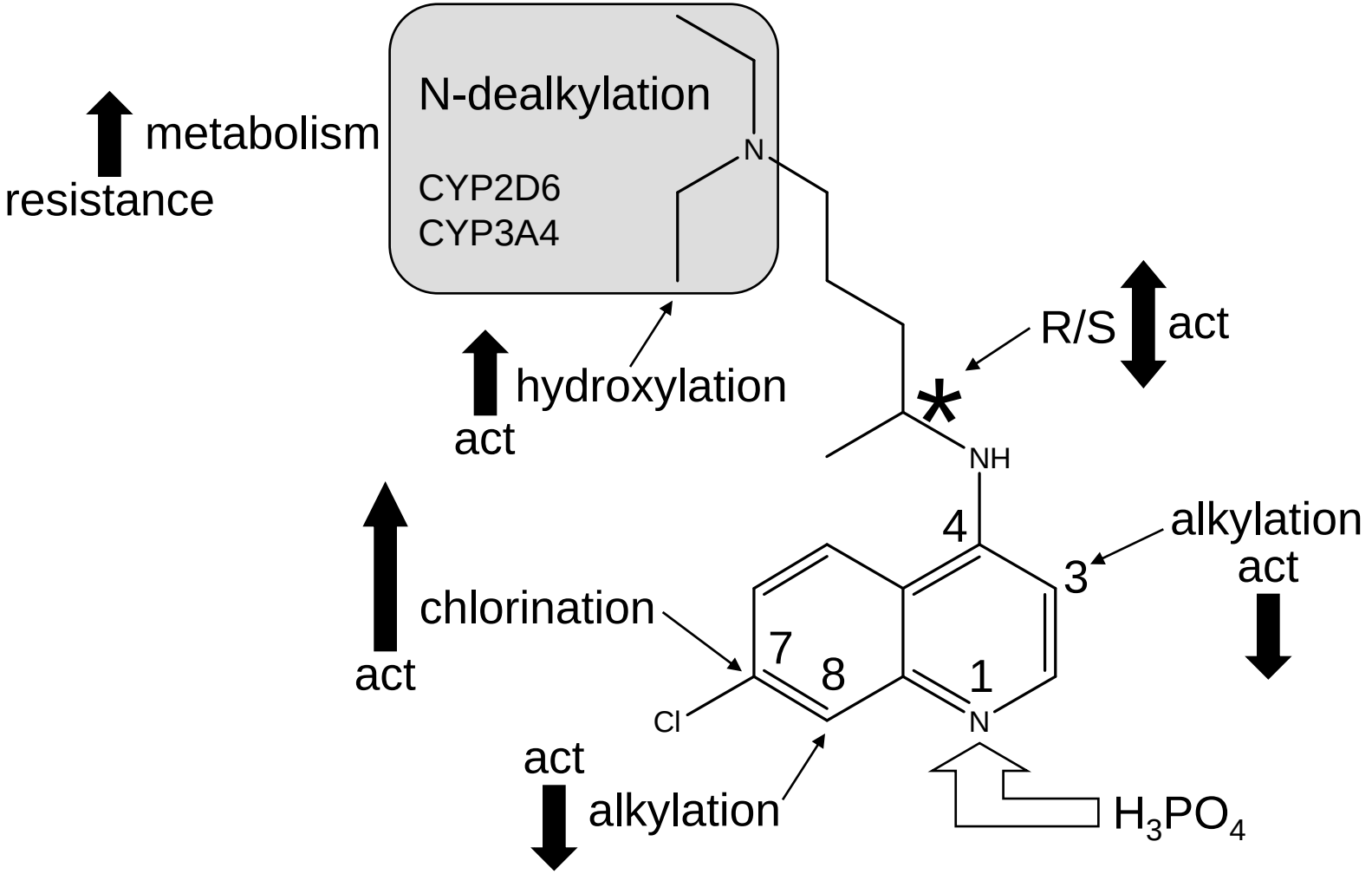
hydroxylation → act → Dev → mefloquine

Quinoline-derived Antimalarials



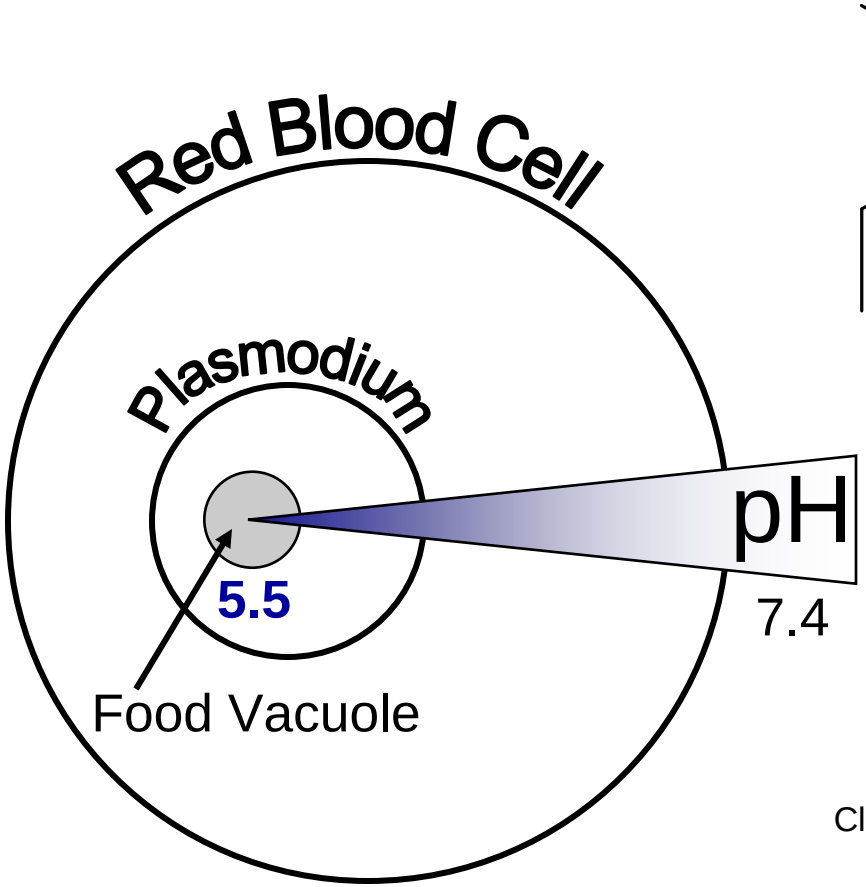
Aralen^{R}, Resochin^{R}, generic

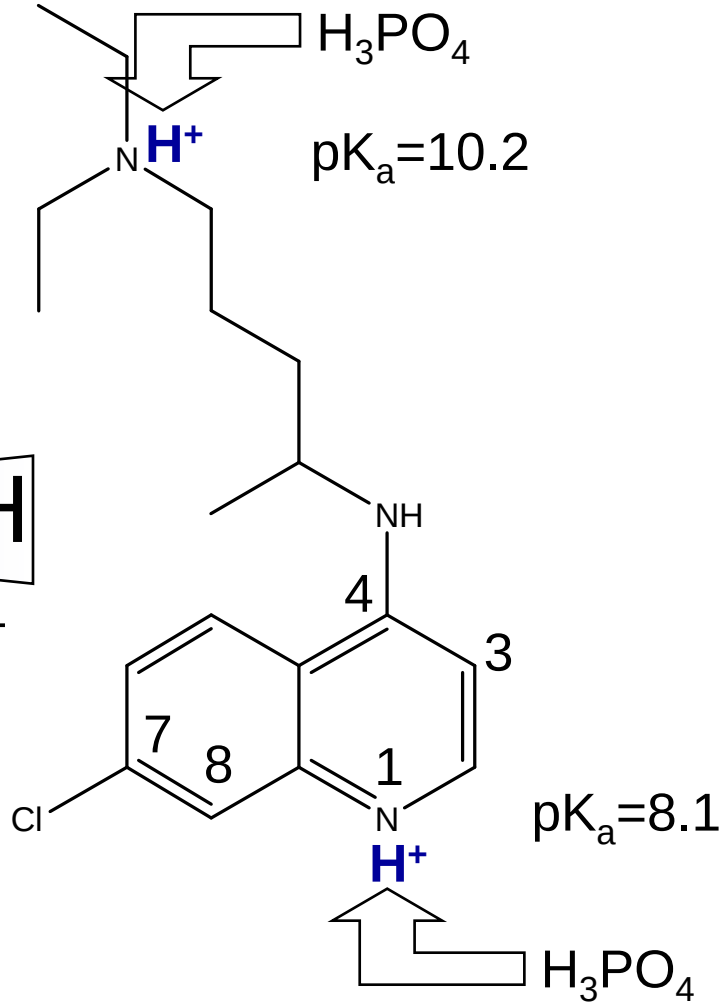
Chloroquine SAR



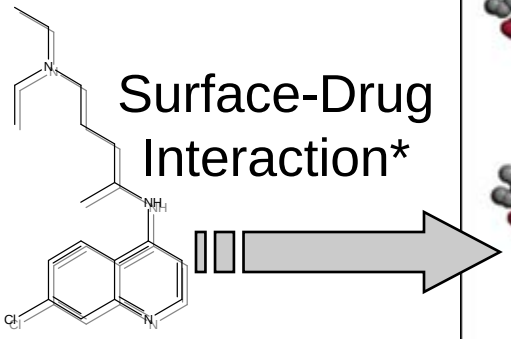
Aralen^{R}, Resochin^{R}, generic

Chloroquine Ion Trapping

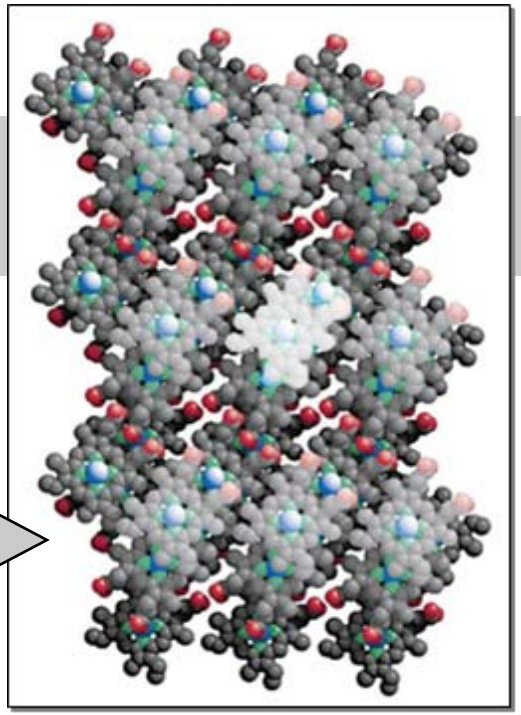


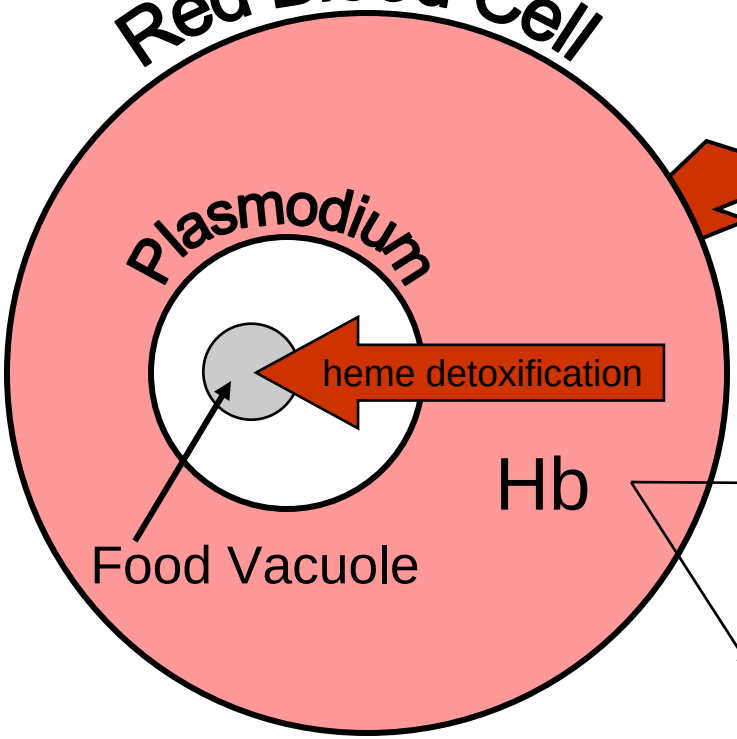


MOA Quinolines



Surface-Drug Interaction*



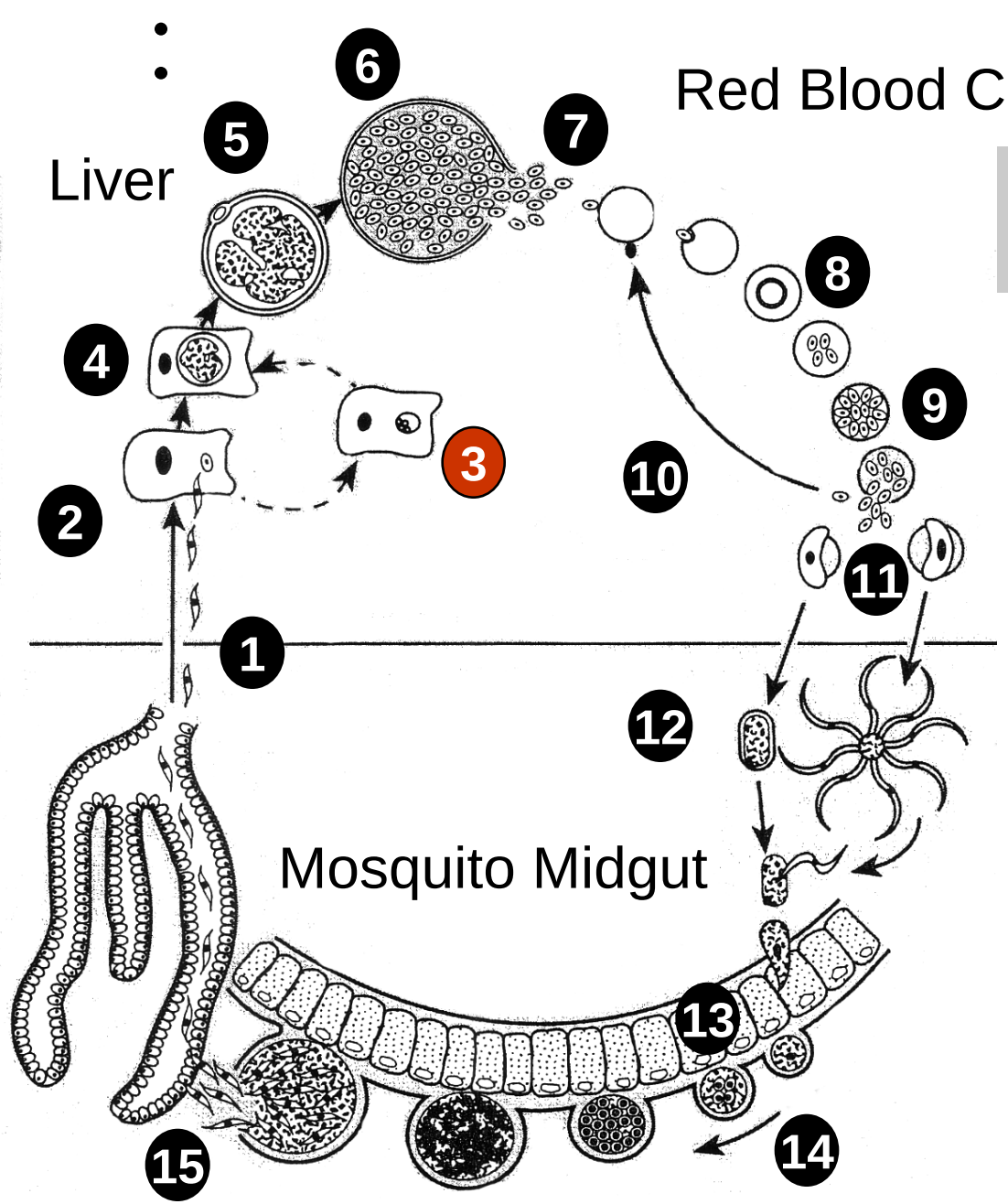


heme → β-hematin *syn.* ferriprotoporphrin-IX FPIX $\xrightarrow{n=\infty}$ hemozoin *syn.* malaria pigment

protein

*Pagola, S. et al. (2000). Nature **404**(6775): 307-10.

Malaria Life Cycle



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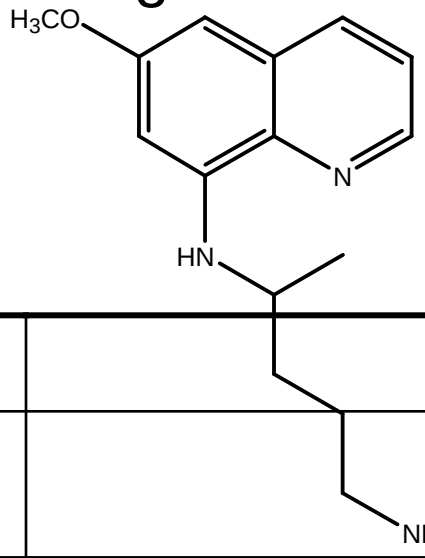
6 Exoerythrocytic = liver schizonts
7 Merozoites blood release
8 Ring stage, trophozoites
9 Schizogeny to blood schizonts
10 Merozoites‘ release and erythrocyte re-infection
11 Female and male gametocytes (macro-) (micro-)
12 Mature gametocytes
13 Ookinete penetrates gut wall
14 Oocyst development
15 Sporozoite penetrate salivary gland

Why has M. tertiana tendency to relapse ?

- First line quinolones = blood schizontocides only
- BUT are ineffective vs. dormant hepatic stage
  3 hypnozoites!



Prevention of *P. vivax* and *P. ovale* relapses		
Primaquine (base/phosphate)	15 mg base(=26.3mg salt)/d x 14d 45 mg base/w x 8w	
	CI: G6PD deficiency (hemolysis, methemoglobinemia)	glucose-6P-dehydrogenase, fava beans allergy (favism)

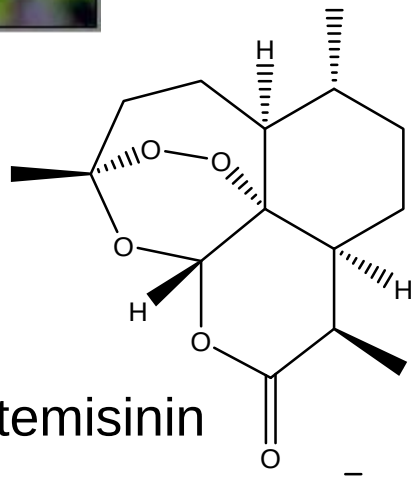
QC Resistance

- Spontaneous gene mutations
  - *pfcrt* transporter protein* on digestive vacuole membrane
    - *Plasmodium falciparum* Chloroquine Resistance Transporter
  - enhanced drug efflux against ion trapping
  - 8 or 9 point mutations, e.g., Lys 67→Tyr (K76T)
- Increased plasmodial P450 metabolism

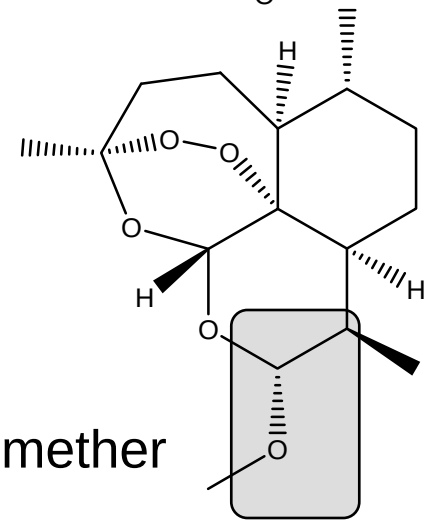
*Sidhu, A. B., D. Verdier-Pinard, et al. (2002) Science **298**(5591): 210-3
Hastings, I. M., P. G. Bray, et al. (2002). Science **298**(5591): 74-5

Quinhaosu

- Sesquiterpene lactone **Artemisinin**
  - *Artemisia annua* (Chinese Materia Medica since 168 BC)
  - Semi-synthetics: arteether, **artemeter**
  - Artemisinins kill nearly all asexual parasite stages in blood. Also effective against young sexual gametocytes. Not effect against *Plasmodium vivax* and *P. ovale* in the liver.



artemisinin



artemether

Artemisinin

Mechanism of action

- Free radical formation (endoperoxide bridge), oxidative damage to parasite's membrane.
- Ion-dependent alkylation. Sarcoplasmic ER calcium ATPase (PfATPase) is one target.
- Artimisinin interacts with heme and inhibits Hb degradation and heme polymerization.

White NJ, Science 2008

Araujo JQ, Bioorg. Med. Chem 2008

Anti-malarial drugs

Mefloquine (Larium^{R}): used in the treatment of chloroquine-resistant falciparum malaria and as a prophylaxis. Serious side effects include depression, anxiety, paranoia, aggression, nightmares, insomnia, seizures, peripheral motor-sensory neuropathy, vestibular (balance) damage and CNS problems. In mice, it blocks brain connexin-36. Other alternate drugs are Malarone and Doxycycline.

Primaquine: is used to treat the *P. vivax* and *P. ovale* malaria. Effective against hypnozoites in the liver with a 14 day course (called radical cure). Generally given with quinine. Side effects include nausea, vomiting, and stomach cramps. CI: G6PD deficiency because of hemolytic anemia.

Proguanil: a prophylactic drug that inhibits both falciparum and vivax dihydrofolate reductase in infected RBCs. Taken in combination with Atovaquone (Malarone) or Chloroquine.

Anti-malarial drugs

Pyrimethamine: (Daraprim^{R}) is used for both treatment and prevention of malaria. Generally given with Sulfonamide. Blocks folic acid synthesis by inhibiting dihyrofolate reductase.

Malarone: Fixed combination of Atovaquone and Proguanil. Atovaquone is a hydroxy-1,4-naphthoquinone. One tablet contains 250 mg Atovaquine and 100 mg Proguanil.

Fansidar: Combination of Pyrimethamine and Sulfadoxine. Effective against chloroquine-resistant falciparum malaria. Not recommended for prophylaxis.

Coartem: Combination of Artemether-Lumefantrine. >95% effective. Acute falciparum malaria. Lumefantrine is a fluorene derivative (2,3-benzindene) that belongs to the amino-alcohol class. WHO essential drug list. Novartis and China.

for the Prevention Regiment see: http://www.cdc.gov

Treatment of Malaria

Infection and Clinical Setting	***Drug Therapy***
CQ sensitive *P. falciparum* and *P. malariae*	**CQ phosphate**, 1 g at 0h, 500 mg at 6h, 500 mg/d for 2d
P. vivax and *P. ovale*	**CQ phosphate**, 1 g at 0h, 500 mg at 6h, 500 mg/d for 2d Then **primaquine** 26.3mg/d x 14d
Uncomplicated CQ resistant *P. falciparum*	**quinine sulfate**, 650 mg 3x/d, 3-7d PLUS doxycyclin OR clindamycin OR Fansidar^{R} (sulfamethoxazol/pyrimethamine)
Severe and complicated CQ resistant *P. falciparum*	**quinidine gluconate** 10 mg/kg IV 1-2h, then 0.02 mg/lg IV/min OR **artemether** 3.2 mg/kg IM, then 1.6 mg/mg/d IM; **Coartem**