NWU® | PharmaCen™

Molecular Hybridization in Anti-Infective Drug Discovery

David D. N'Da

INFECTIOUS DISEASES

MOLECULAR HYBRIDIZATION IN ANTI-INFECTIVE DRUG DISCOVERY

FUTURE PROSPECTS

MOLECULAR HYBRIDIZATION

RESEARCH HIGHLIGHTS

INFECTIOUS DISEASES - IFD

What is an infectious disease?

World Health Organization – WHO

Any disease caused by a pathogenic microorganism that can be spread directly or indirectly from one person to another

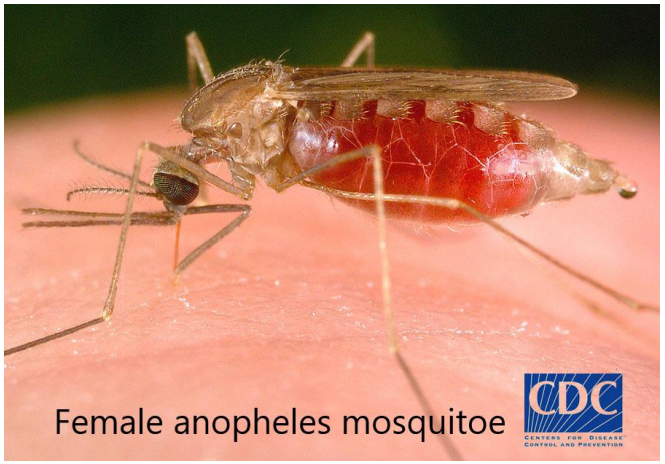
Classification

Nature of pathogen dependent

- Viral IFD: virus, *e.g.* AIDS, Ebola
- Bacterial IFD: bacterium, *e.g*. tuberculosis
- Parasitic IFD: parasite, *e.g.* malaria, leishmaniasis

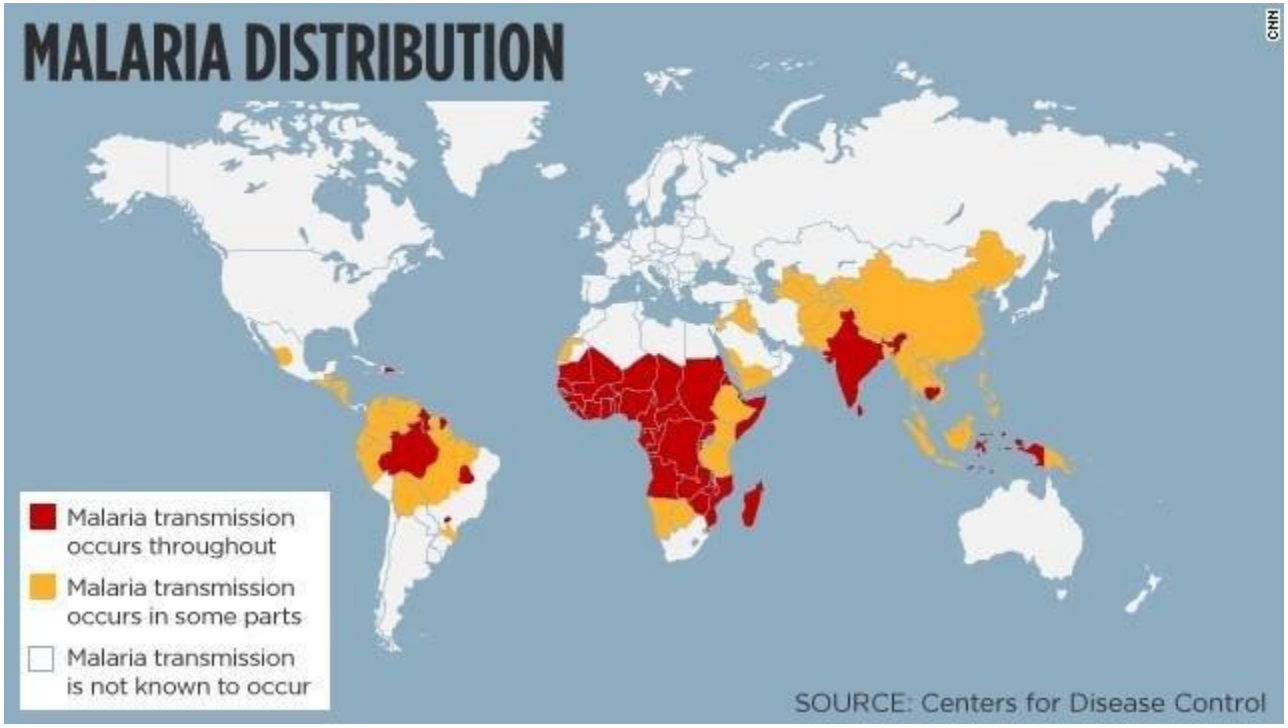
Malaria

Pathogenesis



- Vector-borne disease – protozoa of *Plasmodium* genus. Communication to mammals through bites of infected female anopheles mosquito.
- Five *P*. *spp*.: *falciparum*, *vivax*, malariae, *ovale,* knowlesi
- *P. falciparum* causes most fatal forms of malaria - majority of deaths.
- *P. vivax* – second leading cause of human malaria infection; ability to re-infect and cause relapse.

Geographical distribution



- Endemic in 87 countries: s-S Africa, S-E Asia, C. and S. America, E. Mediterranean, W. Pacific.
- 219 million (vs. 217 in 2016) new cases and 435 000 (vs. 449 000 in 2016) deaths in 2017 [1]. WHO African Region: 92 % of malaria cases and 93 % of malaria deaths.
- Children < 5: most vulnerable to malaria infection and death – WHO estimation: 1 child death every 2 min [1].Other vulnerable groups: pregnant women, HIV-AIDS infected individuals etc.

[1] WHO 2018. World malaria report 2018. http://www.who.int/malaria/publications/world-malaria-report-2018/report/en/

Pathogen prevalence / WHO region - 2017

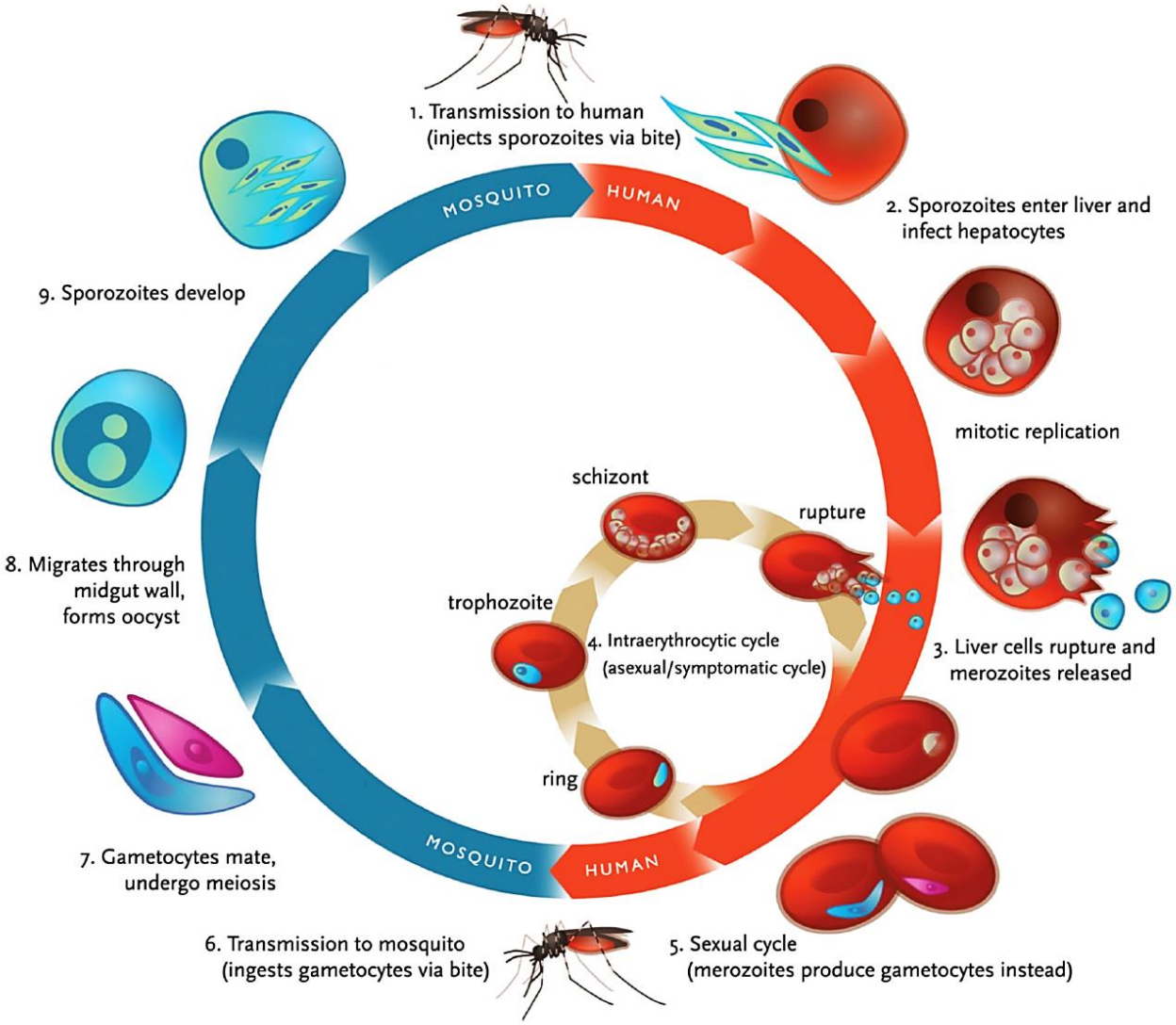
P. Falciparum

- s-S Africa - 99.7%
- S-E Asia - 62.8%
- Eastern Mediterranean - 69%
- Western Pacific - 71.9%

P. vivax

- Americas - 74.1%

Malaria parasite life cycle



Life cycle of the *Plasmodium* parasite
(Klein, 2013)

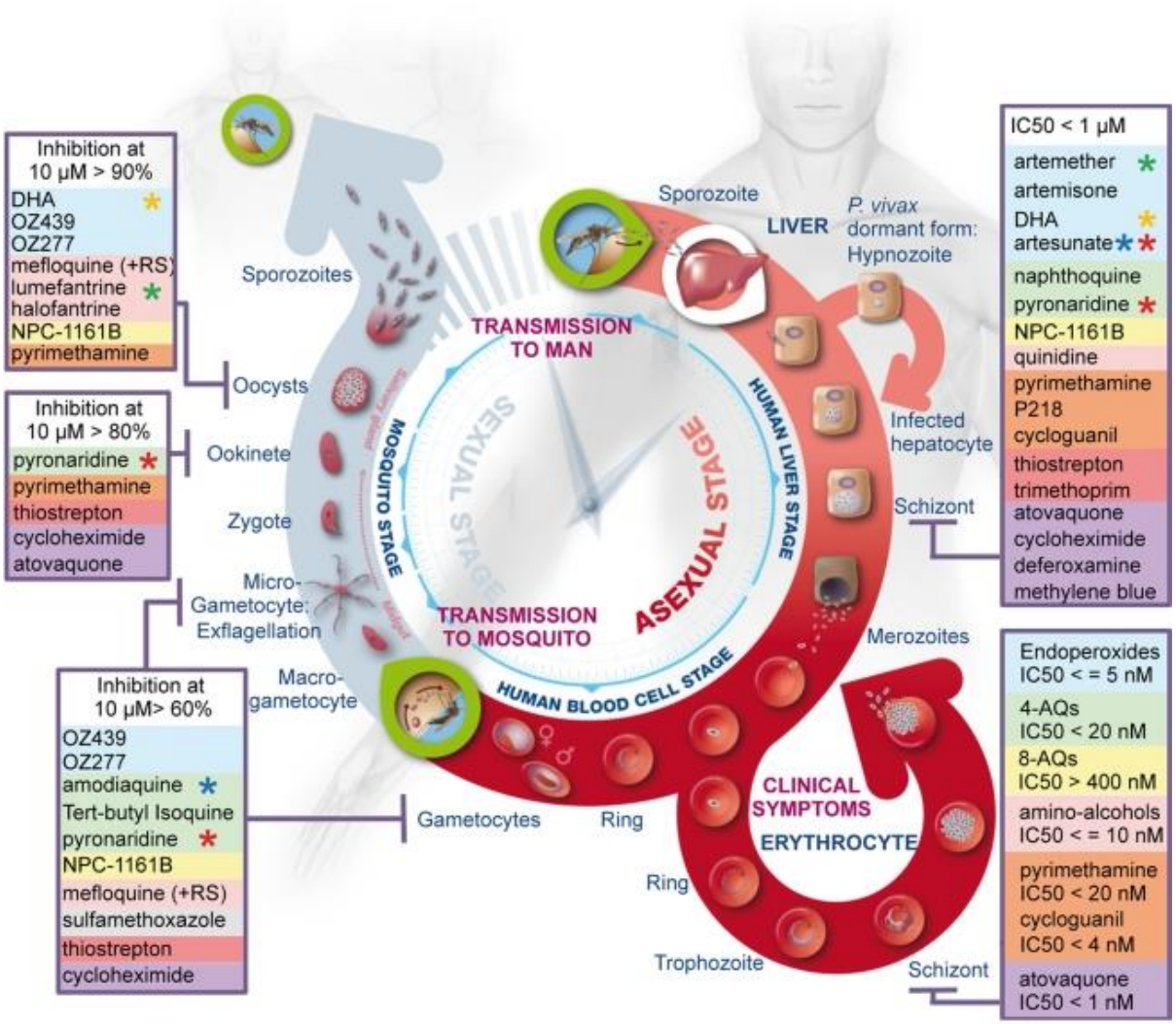
Sexual stage - mosquito

- 2 Asexual stages – human:
Liver – asymptomatic
and blood -symptomatic

- *P. vivax:* dormancy in liver
- hypnozoites for undetermined
period after infection – relapse

Klein E. Int. J. Antimicrob. Agents, 2013, 41, 311-317.

Chemotherapy



Summary of the activity of the most widely used antimalarials throughout the life cycle of *Plasmodium* [1].

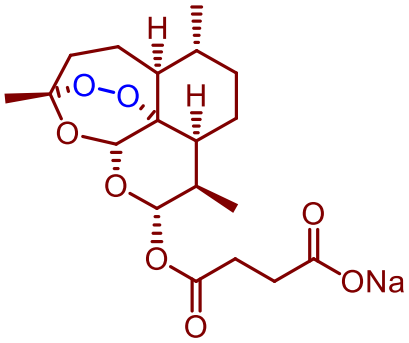
[1] Delves at al., PLoS Med. 2012, e1001169.

Current therapeutic armory

- Stage-specific drugs
- Few drugs are active against all 3 stages
- Widespread parasite resistance against most blood stage drugs except artemisinins

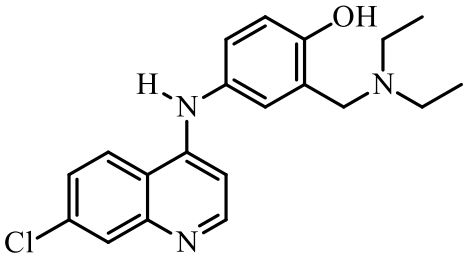
- WHO recommendation: malaria treatment – ACTs: Artemisinin combination therapies / 3 - 4 days course

e.g.



Artesunate

+



Amodiaquine = ASAQ (fixed or dual dose)

ACTs Main objective:

- Increase efficacy through drug combination
- Total parasitaemia clearance
- Suppression of resistance

Based on:

- Artemisinins induce *rapid reduction in* parasitaemia
- The partner in an ACT, because of its much longer half-life, continues to exert drug action once the plasma level of artemisinin falls below therapeutic levels.

- Most current clinical artemisinins used in ACTs; artemether (ARM) and artesunate (ARS) are prodrugs of DHA.
  - Chemical and metabolic instability

ARM $\rightarrow$ DHA - $t_{1/2}$ < 3 h p.o

ARS $\rightarrow$ hydrolysis to DHA ($t_{1/2}$ ~ 30 min)

DHA ($t_{1/2}$ ~1 h) $\rightarrow$ rapid ring opening in solution, thermally unstable

Low plasma levels, rapidly eliminated etc. – likelihood of repeated high doses use $\rightarrow$ Therapeutic effect.

- Safety - DHA is cytotoxic & neurotoxic at repeated high doses

- **Current artemisinins are inadequate AND**
- **Partial artemisinin resistance in S-E ASIA**

EMPHASIS ON DISCOVERY OF NEWS ANTIMALARIAL AGENTS

CRITERIA

MMV – Medicines for Malaria Venture:

A suitable drug candidate for malaria eradication should be able to kill gametocytes, hypnozoites and other liver stages, thereby inhibiting transmission, relapse, as well as providing prophylaxes for the disease → Eliminate human stages of *P.* life cycle.

LEISHMANIASIS

Pathogenesis


Phlebotomine sandfly

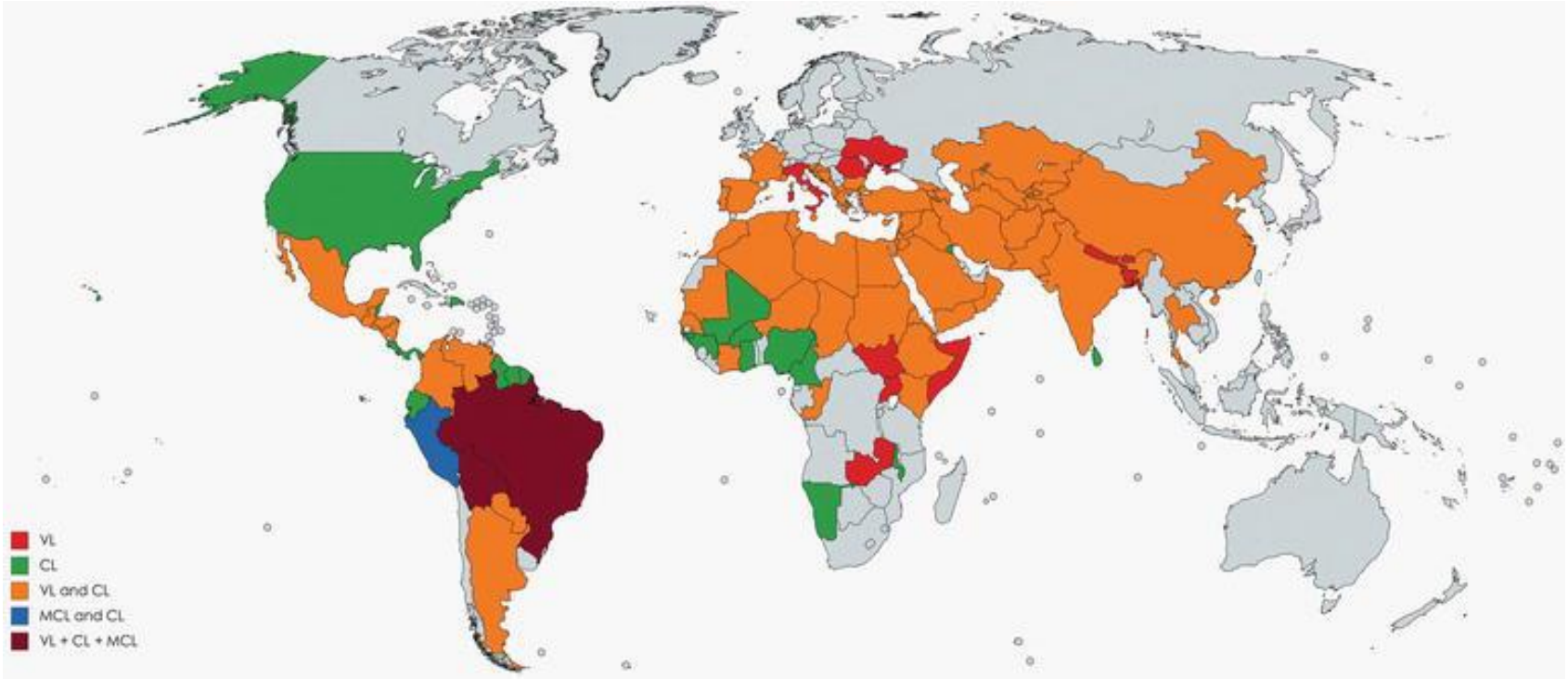

Leishmania sp.

- Poverty-related, NTD, vector-borne disease – protozoa of *Leishmania* genus.
  → to mammals through bites of infected female phlebotomine sandflies.
- 20 *Leishmania species*.

3 Clinical forms:

- Muco-cutaneous (MCL): lesions → partial/total destruction of the mucous membranes of the nose, mouth etc.
- Cutaneous (CL, Aleppo boil) - most prevalent: skin lesions → life-long scars
- Visceral leishmaniasis (VL, kala azar, black fever) – **most lethal** → internal organs, particularly the liver, spleen, bone marrow and lymph nodes.

Geographical distribution



- Wide distribution across 89 countries: Africa, Asia, Americas and Mediterranean region.
- 350 million people at risk of infection; 700 000 - 1 million new infection cases and 30 000 deaths in 2018 [1]: 50 000 - 90 000 new cases of V; 20 000 - 30 000 deaths due to VL; 600 000 - 1 200,000 new cases of CL
- 90% of new VL cases: Brazil, India, Ethiopia, Kenya, Somalia, South Sudan and Sudan.
- Majority of CL cases: Afghanistan, Algeria, Brazil, Colombia, Iran, and Syria

[1] WHO 2016. Leishmaniasis; http://www.who.int/news-room/fact-sheets/detail/leishmaniasis.

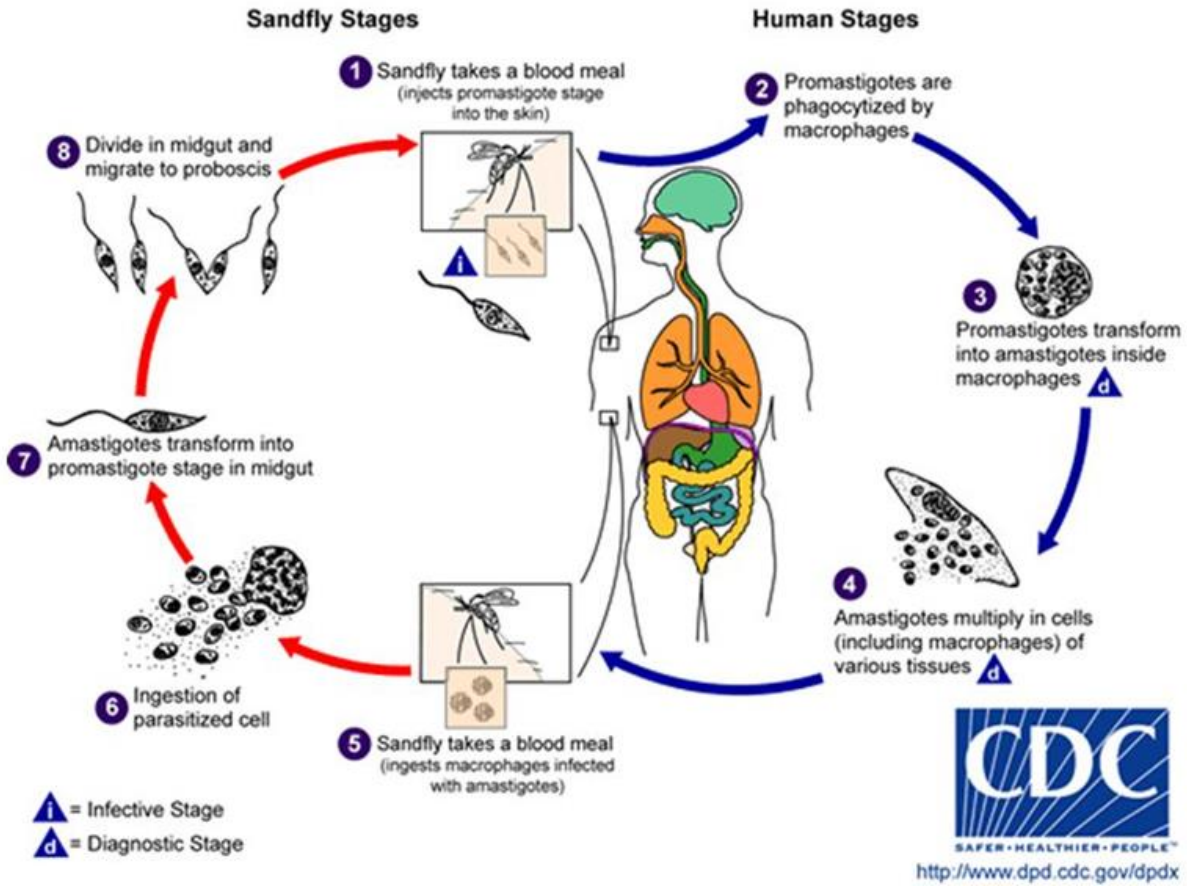
Pathogen prevalence / WHO region - 2017

- *L. (braziliensis*, *panamensis* and *amazonensis)* → MCL: Peru, Bolivia and Brazil - 35 000 cases annually [1]

- *L.* (*donovani* –India & Africa; *infantum/chagasi* - Mediterranean & New World regions) → VL

- *L.* (*aethiopica*, *major* and *L. tropica*)- Americas, Mediterranean basin, Middle East and Central Asia → CL:

Leishmania parasite life cycle



- 2-Stage cycle: 1 Infective (vector-insect) + 1 clinical/diagnostic (host-vertebrate: human, dog, baboon, rodent etc.).

2 Developmental forms: promastigote (infective) + amastigote (clinical)

Chemotherapy of Leishmaniasis

- Penta-antimonial drugs (all): effective BUT im/iv, toxic (liver, heart) & VL resistance
- Paromomycin (VL): effective (limited in Africa), cheap BUT im & toxic (liver).
- Miltefosine (CL & VL): effective BUT expensive, toxic (liver & kidney), GIT complications, pregnant ? (no - teratogenicity).
- Amphotericin B (VL): effective & safer BUT expensive & iv administration
- Pentamidine (Sb-resistant VL): near discontinuation – reduced efficacy & toxicity

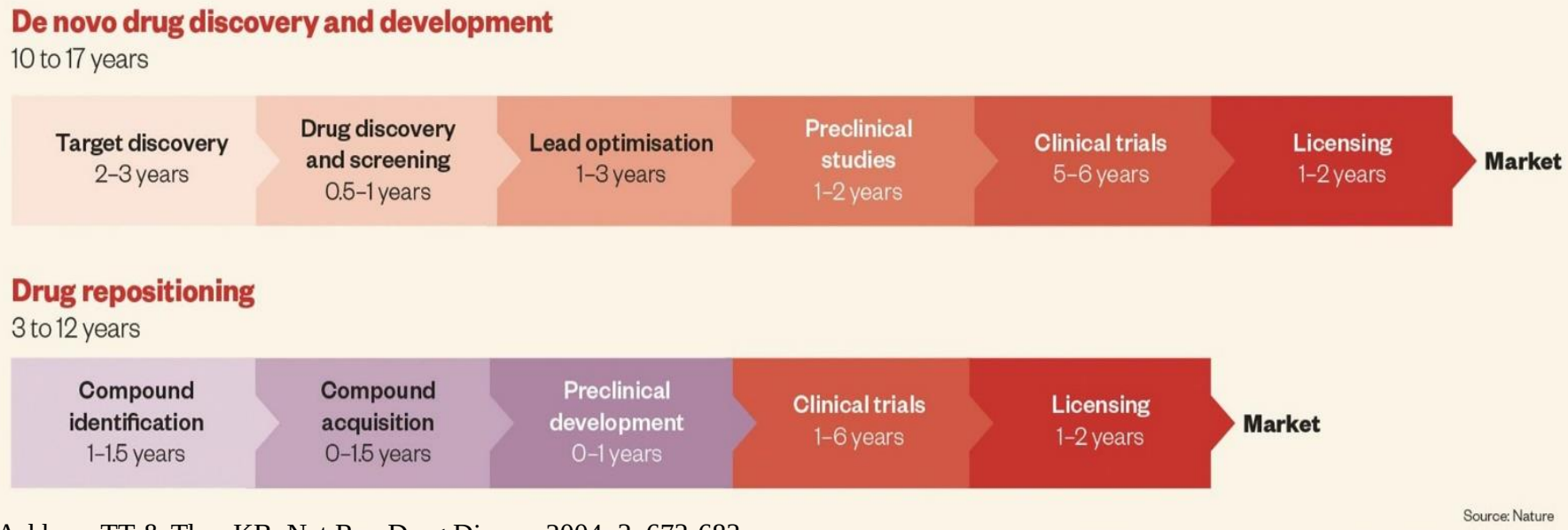
⇩

Discovery of news antimeishmanial drugs →

affordable, safe and effective

Traditional drug discovery versus repositioning

It is estimated that it takes on average 13.5 years to bring a new molecular entity to market, and the success rate for taking oncology drugs from phase 1 trials to regulatory approval is only about 7%. Drug repositioning builds on previous research and development, allowing compounds to progress through the drug development process more quickly as well as saving on the substantial costs associated with previous attrition.

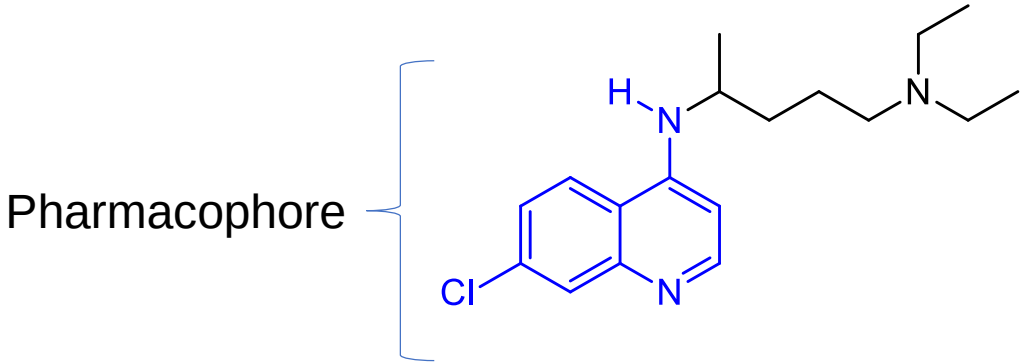


Ashburn TT & Thor KB. Nat Rev Drug Discov. 2004; 3, 673-683

Exploitation of SAR of existing drugs

MOLECULAR HYBRIDIZATION

- Pharmacophore: structural part of a molecule responsible for its biological property.



chloroquine

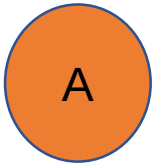
Molecular hybridization: concept/strategy in drug design and development based on the chemical combination of pharmacophoric moieties of different bioactive substances to produce a NCE (hybrid) with the following target features:

- improved affinity and efficacy
- modified selectivity profile
- different and/or dual MoAs
- reduced undesired side effects

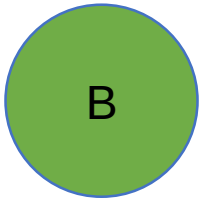
in comparison with:

- the individual parent drugs
- and the fixed ratio physical combination !!!

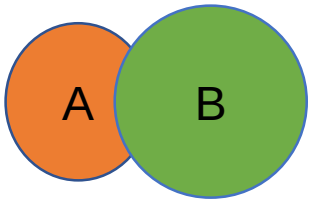
- Hybrid types



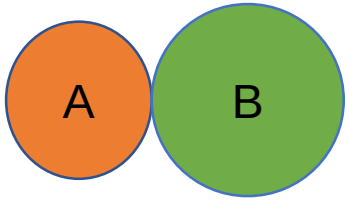
Pharmacophore A



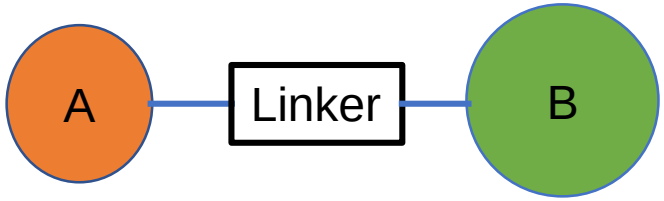
Pharmacophore B



fused hybrid



merged hybrid



conjugated hybrid

- Nature of linker: cleavable or metabolically resistant depending on sites of action
- A & B: Active or inactive (prodrug) forms

- Enhanced efficacy - dissociation is advantageous to allow independent actions at recognized sites within cell.

e.g. A acts in parasite DV and B inhibits a cytosolic receptor.

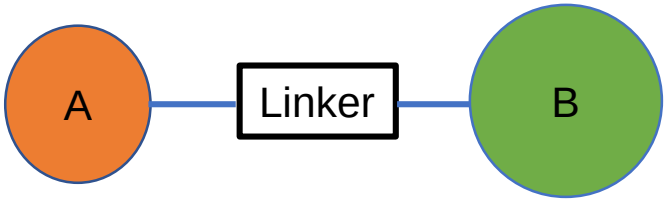
- Overcoming resistance - desirable for linker metabolic cleavage for the two components to work in tandem to produce an overall synergistic effect.

e.g. A exerts its pharmacological effect by overcoming the inhibitory actions of a resistance transporter of B.

- In principle, PK and PD profile of intact hybrid is easier defined than in situations where the linker is cleaved some time after administration.

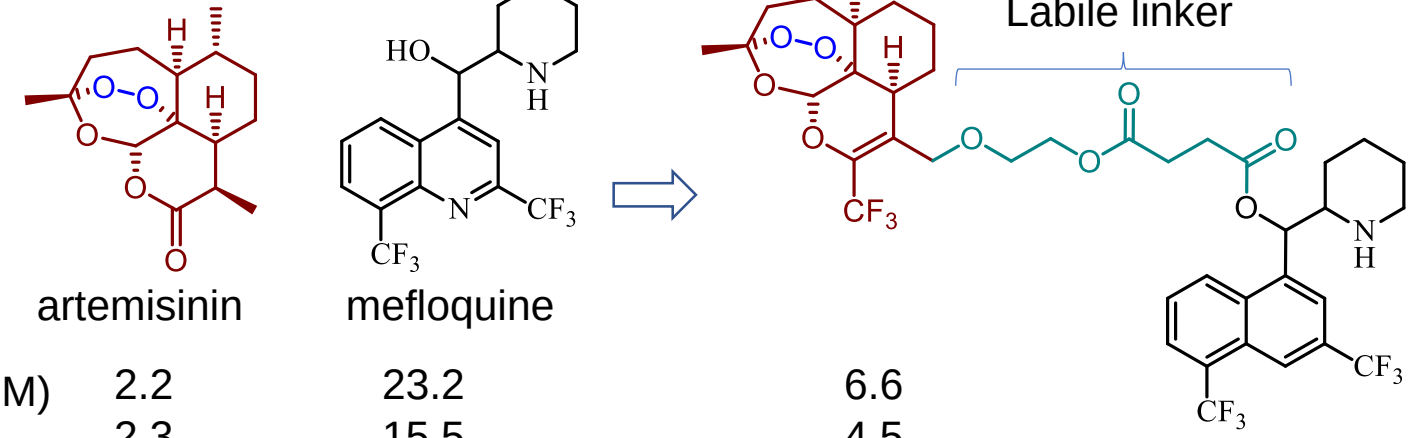
Molecular hybridization - Design

- **Option 1:** A and B are active forms and linker is labile - enhanced efficacy



Conjugated hybrid

e.g. artemisinin-mefloquine [1]

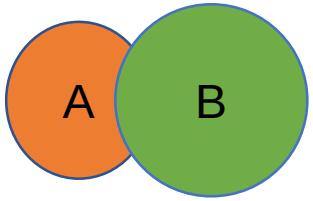


artemisinin mefloquine

Pf F32 IC_{50} (nM)	2.2	23.2	6.6
Thai	2.3	15.5	4.5
	ARM		

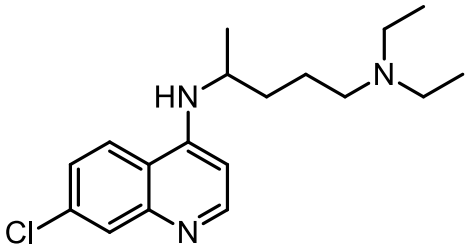
[1] Grellepois et al., Chembiochem 2005, 6, 648-652.

- **Option 2**: A and B are active forms – reverse resistance

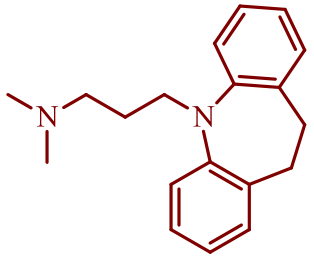


fused hybrid

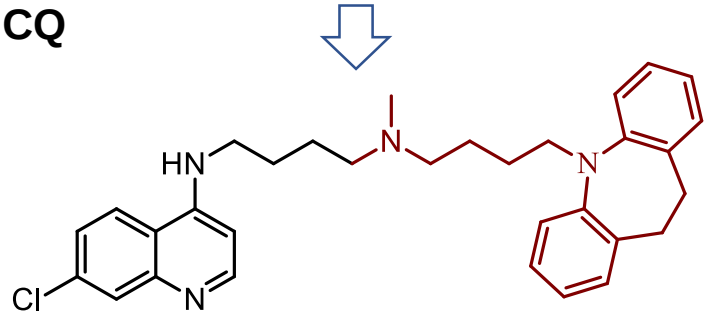
e.g. Chloroquine-imipramine [1]



chloroquine - CQ



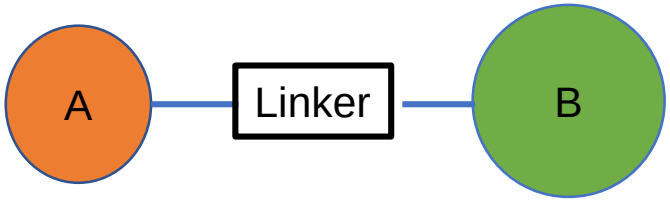
Imipramine



Reverse chloroquine (RCQ) hybrid
chloroquine-imipramine hybrid designed to overcome chloroquine resistance

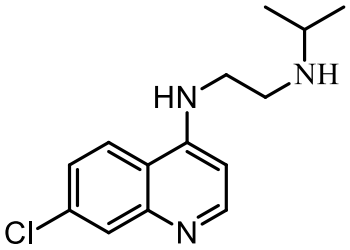
[1] Burgess et al., J Med Chem 2006; 49: 5623-5

- **Option 3**: A is in active form, B is presented in prodrug form and linker is labile – enhanced activity.



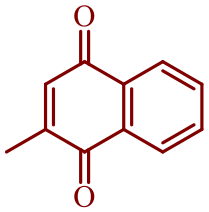
Conjugated hybrid

e.g. Aminoquinoline-naphthoquinone [1]

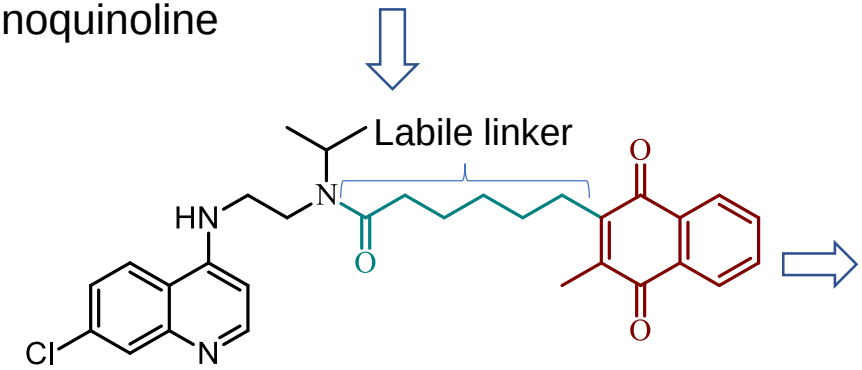


Pf IC_{50} 3D7 10.2 nM

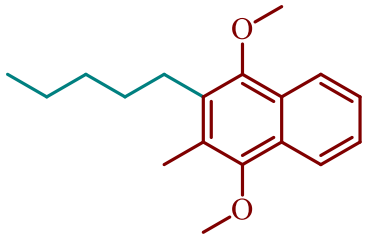
Aminoquinoline



1,4-Naphthoquinone



Pf IC_{50} 3D7 7.5 nM



1,4-dimethoxynaphthalene

Cleavable aminoquinoline-naphthoquinone hybrid

[1] Friebolin et al., J Med Chem. 2008; 51: 1260-77

Molecular hybridization - Benefits

Property	Separately dosed drugs	Drugs fixed-dose combination	Hybrid drug
Activity & safety	▪ ratio of two drugs can be adjusted for optimal activity and safety	▪ ratio fixed but optimal ratio can be chosen. ▪ combination partner may confer bypass of resistance to single agent	▪ ratio fixed usually at 1:1 (A:B) ▪ May confer superior/ inferior activity and safety. ▪ Hybrid may allow bypass of resistance to single agent.
Cost			▪ Potentially cheaper for single drug
Drug-likeness			▪ May be high-molecular weight
Patient compliance	▪ Potentially problematic	▪ Expected to be good	▪ Expected to be good
Pharmacokinetic properties	▪ Complex PK/PD relationship	▪ Complex PK/PD relationship	▪ More predictable PK/PD relationship
Solubility and stability	▪ May be different	▪ May be different	

RESEARCH HIGHLIGHTS

- Minimum inhibitory concentration - IC_{50}

Concentration of compound that is required to inhibit parasite growth by 50%. **The lower the IC_{50} the more potent the compound**

- Selectivity index – SI

Indicate how selective a compound in its antipathogenic action in the presence of mammalian cells.
The higher SI value the lesser cytotoxic the compound

- Hit compound

A molecule that shows the desired type of activity in a screening assay.

- Early Lead compound

Hit compound with improved cellular potency, selectivity and in vivo efficacy parameters in view of optimization as lead.

Infectious disease criteria for hits and leads - 2015:

- Japanese Growth Health Innovative Technology - GHIT
- Medicines for Malaria Venture - MMV
- Drugs for Neglected Diseases *initiative* - DND*i*
- TB Alliance
- Bill & Melinda Gates Foundation representatives

Two main types of criteria are considered:

Disease-specific criteria

- Potency
- Efficacy
- Pathogenicity

Compound-specific criteria

- Chemical scope of the compound
- Drug metabolism and pharmacokinetics (DMPK)
- Physical properties.

Malaria

Key selection criteria

- Hit

Cellular potency: IC_{50} <1 µM / drug-sens. & resis. strains
Selectivity: SI > 10
Synthesis ≤ 5 steps

- Early lead

Cellular potency: IC_{50} <100 nM/ drug-sens. & resis. strains
Selectivity: SI > 100
Activity across all mammalian stages of *P.* life cycle
In vivo efficacy with oral dose (< 50 mg/kg) inducing blood stage 90% parasites clearance
Efficacy in prophylaxis of malaria model at 50 mg/kg.

LEISHMANIASIS

Selection criteria

- Hit

Disease form: visceral leishmaniasis (VL)
Parasite strain: *Leishmania donavani* (*L. donavani*)
Developmental form: intracellular amastigote
Cellular anti-amastigote potency: $IC_{50} < 10\ \mu M$
Selectivity: SI > 10

- Early lead

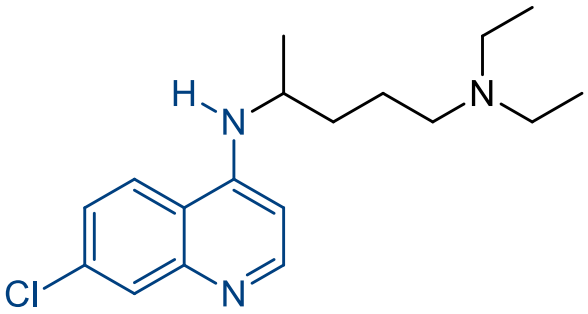
Efficacy: >70% reduction in liver parasite burden after 5 oral doses at 50 mg per kg,

Sir James W. Black, Nobel laureate, pharmacologist from the University of Glasgow, Scotland famously said:

"*The most fruitful basis of the discovery of a new drug is to start with an old drug*."

Sir James W. Black, Nobel laureate
in Physiology or Medicine 1988

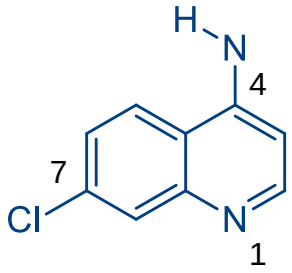
Quinoline-pyrimidine hybrids



Chloroquine - CQ

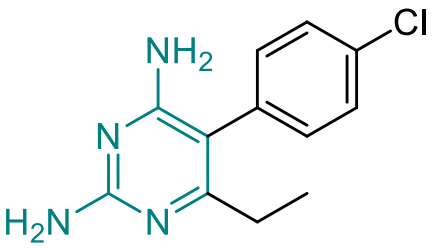
- First synthetic antimalarial drug used in clinics
- 4-Aminoquinoline class – slow acting, $t_{1/2}$ 3-5 days
- Curative
- Clinical use: discontinued - resistance

- Pharmacophore: 7-chloro-4-aminoquinoline



CQ pharmacophore

- MoA: heme binding ⇨ Toxic FP-CQ complex
  Cell death by lysis
- Target: hemazoin formation inhibition



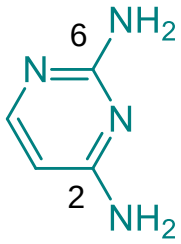
Pyrimethanine - PM

- Diamino-pyrimidine – class I antifolate
- Slow acting, $t_{1/2}$ 4 days
- Use – combination:

  + sulfadoxine ⇨ Fansidar

  Fansidar + ARS ⇨ ACT
- Full resistance & partial ACT

- Pharmacophore: 2,6-Diaminopyrimidine

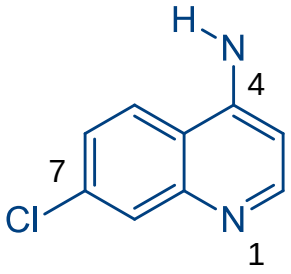


PM pharmacophore

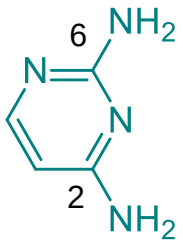
- Target: dihydrofolate reductase enzyme

⇩

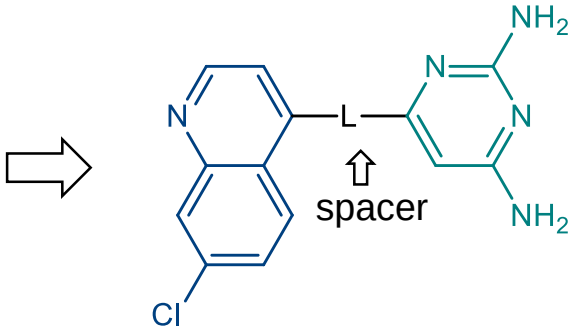
DNA, RNA synthesis inhibition



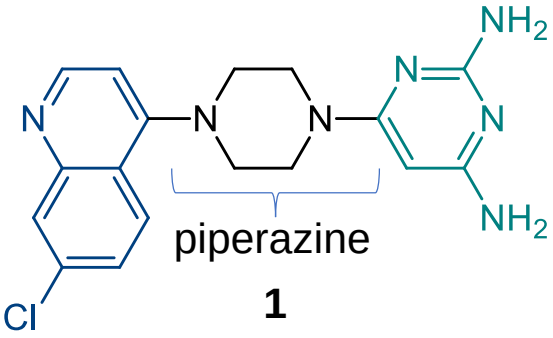
CQ pharmacophore



PM pharmacophore



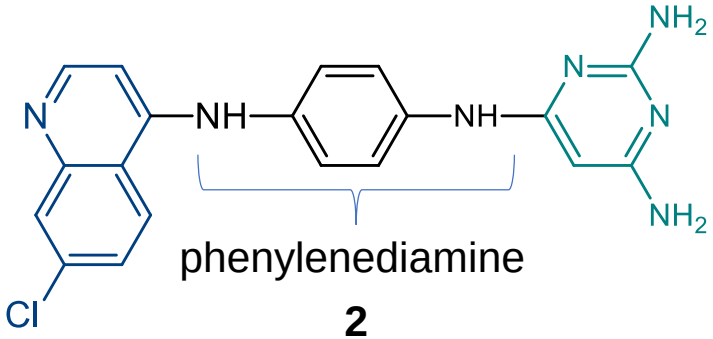
Quinoline-pyrimidine hybrids



1

- Potency: IC_{50} *Pf* D10 0.07; Dd2 0.2 µM; RI 2.3; *vs.* CQ D10 0.04; Dd2 0.4 µM; PM D10 0.07 µM ; Dd2 inactive.
- Cytotoxicity, SI: CHO 2000
- Synthetic steps: 2

HIT



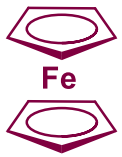
2

- Potency: *Pf* D10 0.2; Dd2 0.1 µM; RI 0.5
- Cytotoxicity, SI: CHO 2000

HIT

Pretorius et al., *Eur J Med Chem*. 2013, **21**, 269-277

Quninoline-ferrocene hybrids



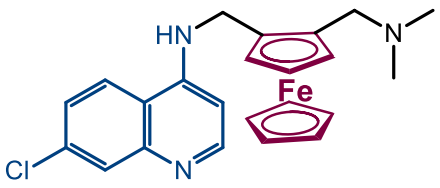
Ferrocene - Fc

- Organometallic compound
- Low toxicity
- Cheap
- Redox active: ROS generation ⇨ oxidative stress

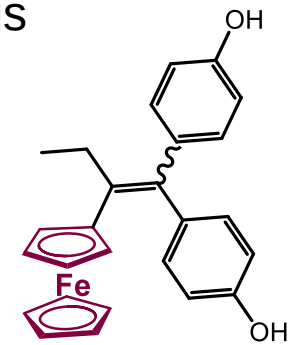
- Present in therapeutic agents

malaria: ferroquine BUT tedious synthesis

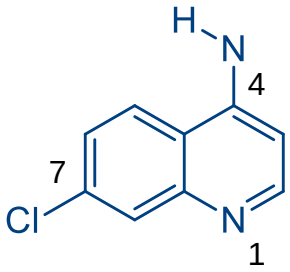
cancer: ferrocifen



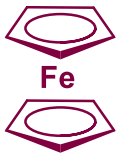
Ferroquine – FQ



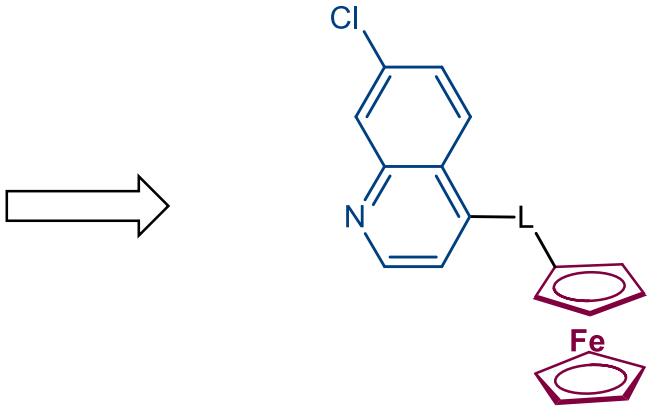
Ferrocifen



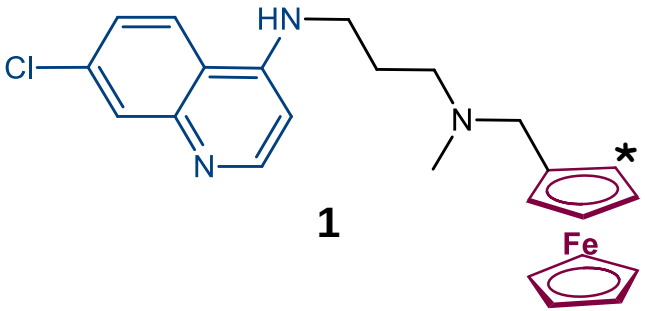
CQ pharmacophore



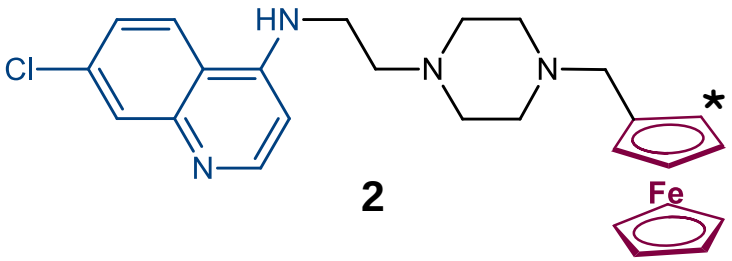
Ferrocene - Fc



Quinoline-ferrocene hybrids



1



2

- Potency: IC_{50} *Pf* D10 40; Dd2 10 nM; RI 0.25; *vs.* CQ D10 50; Dd2 160
- Cytotoxicity, SI: CHO 1300
- Fc disubstitution

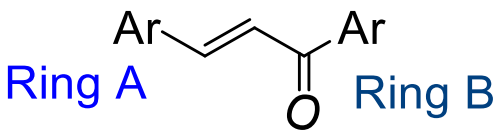
POTENTIAL EARLY LEAD

- Potency: *Pf* D10 50; Dd2 20 nM;
- Cytotoxicity, SI: CHO 1700
- Fc disubstitution

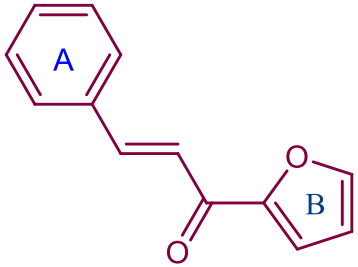
POTENTIAL EARLY LEAD

N'Da et al., *Med Chem Res*. 2014, **23**, 1214-1224

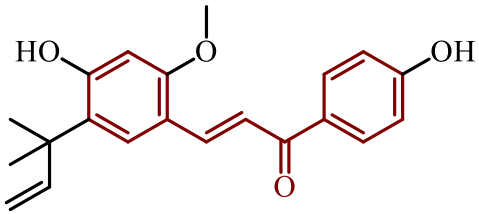
Quinoline-chalcone hybrids



Chalcone – general structure



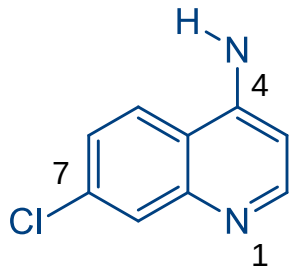
- An aromatic ketone and an enone
- Pharmacophore of important biological compounds known as chalconoids



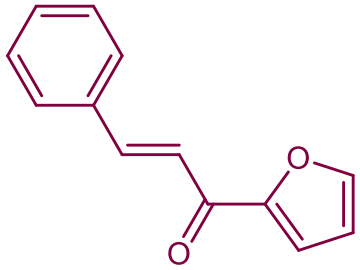
Licochalcone A

- Natural product
- Extraction from licorice root

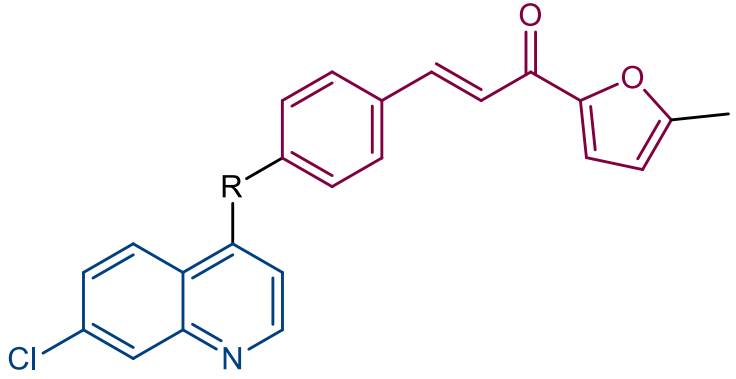
- Biological properties of chalconoids: antimalarial, anticancer, antibacterial, antiviral



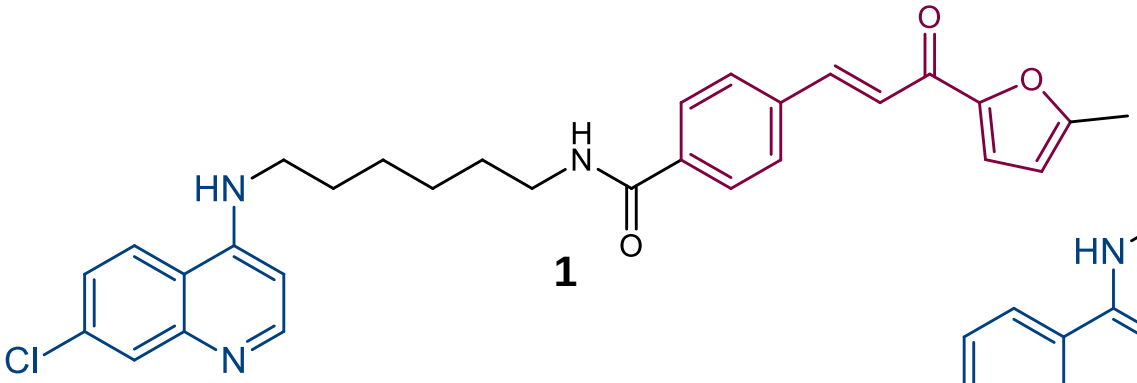
CQ pharmcophore



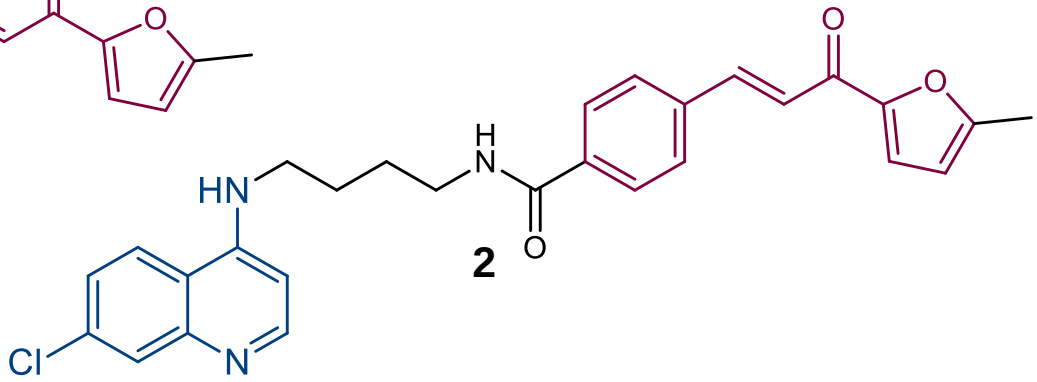
Chalcone



Quinoline-chalcone hybrid



1



2

- Potency: *Pf* D7 40; W2 70 nM; RI 1.5; *vs.* CQ D7 50; W2 120 nM
- Cytotoxicity, SI: HFLF 435
- Further derivatization

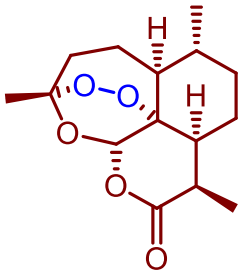
POTENTIAL EARLY LEAD

- Potency: *Pf* D7 50; W2 100 nM; RI 2
- Cytotoxicity, SI: HFLF 435

POTENTIAL EARLY LEAD

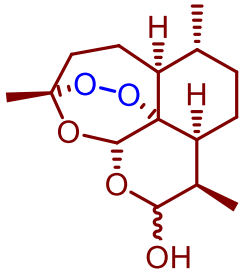
Frans et al., *Bioorg Chem Med*. 2014, **22**, 1128-1138

Artemisinin-quinoline hybrids

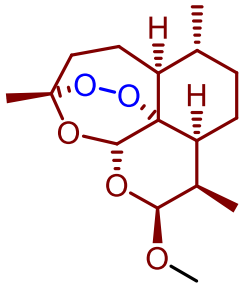


Artemisinin

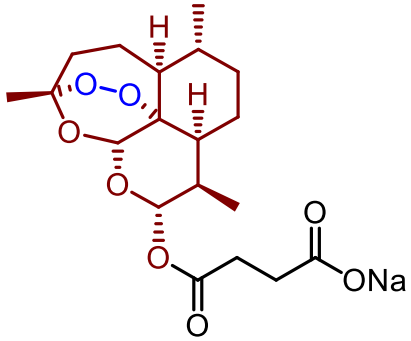
- Natural product – extracted from *Artemisia annua* used in ACT.
- Hemiacetal derivatives are mainstay antimalarials – *uncomplicated* malaria



Dihydroartemisinin
DHA



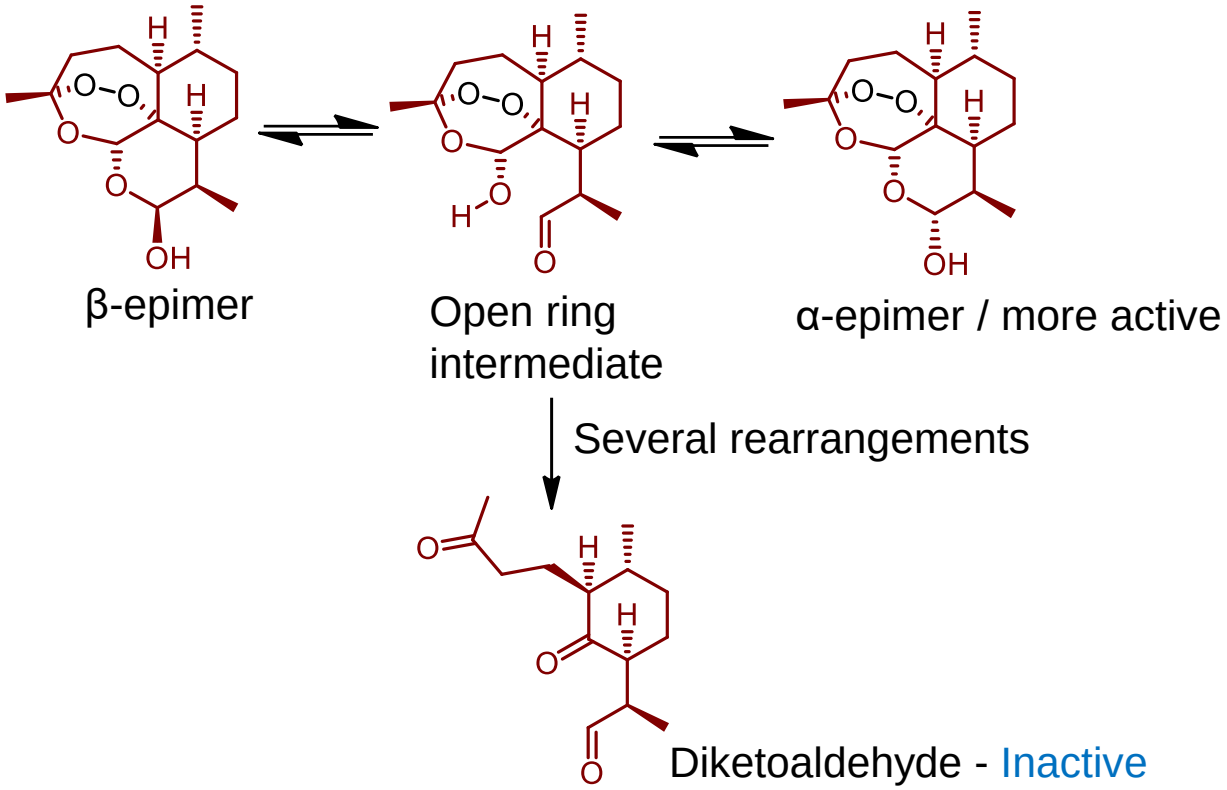
Artemether
ARM



Artesunate
ARS

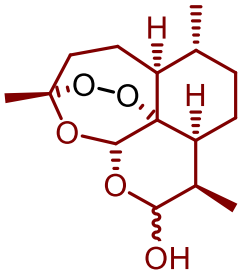
- Chemical & enzymatic instability – ARM & ARS are prodrugs of DHA
- DHA major active metabolite of current clinical artemisinins BUT

  has intrinsic chemical instable – ring opening

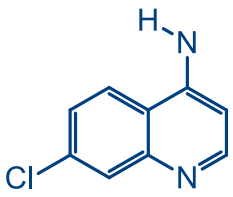


- FAST acting BUT rapid metabolism – short half-lives, 0.5 – 3h
- Redox active: ROS generation – oxidative stress
- Pharmacophore: endoperoxide O-O bridge
- Clinical use: combination – ACT BUT partial resistance in S-E Asia

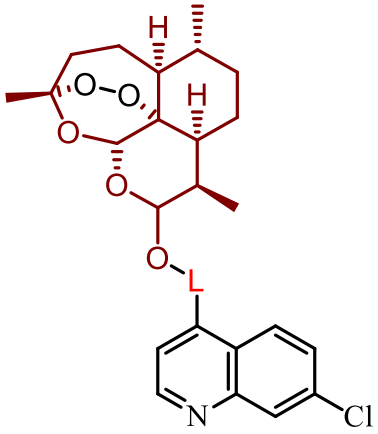
ACTs JEOPARDY



DHA

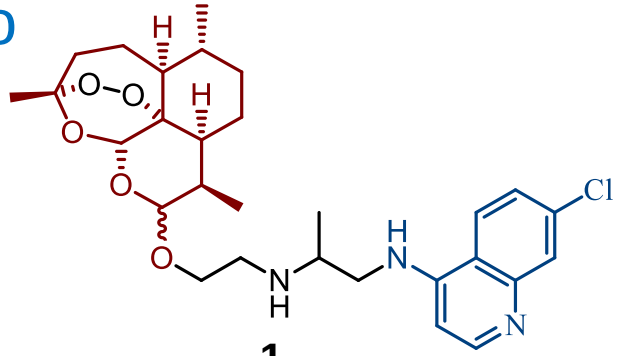


CQ pharmcophore

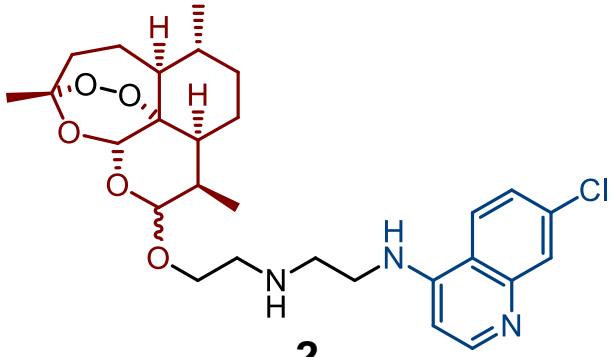


Artemisinin-quinoline hybrid

In vitro



1



2

- Potency: *Pf* D10 12; Dd2 7 nM; RI 1; *vs.* DHA D10 5; Dd2 2; CQ D10 22; Dd2 160 nM.
- Cytotoxicity: CHO SI 435

⇩

Potential EARLY LEAD

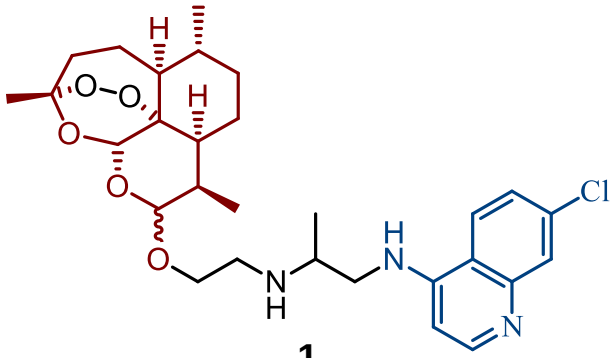
- *Pf* D10 22; Dd2 25 nM
- CHO SI **77**

⇩

EARLY LEAD? NO
SI<100

Lombard et al., *Bioorg. Med. Chem. Lett.* 2011, **21**, 1683–1686

In vivo: *P. vinckei* infected mice – 4-day treatment

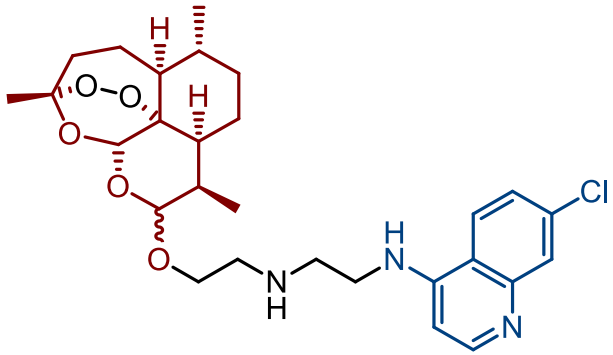


1

- ED_{50} 1.1 (ip); 12 mg/kg (os)

Cure - no recrudescence)

15 *vs.* ARS 30 mg/kg (po)



2

- ED_{50} 1.4 (ip); 16 mg/kg (os)

Cure - no recrudescence

20 *vs.* ARS 30 mg/kg (po)

Snapshot PK: 20 mg/kg (po)

- Half-life $t_{1/2}$ 4 *vs.* 25 min (DHA)
- % B: 0.3 *vs.* 19-35 (DHA)

⇨ **NOT EARLY LEAD**

- Short half-life and poor oral bioavailability

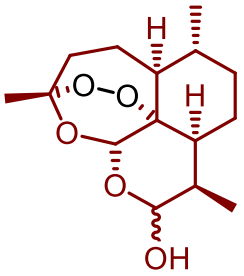
BUT

- Malaria cure in mice at very low dosages

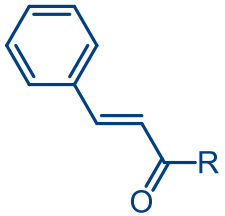
⇨ **Metabolism into several unstable active metabolites** ⇨ **Inconsistent results during trial**

Lombard et al. *Malaria J.* 2013, **12**:71

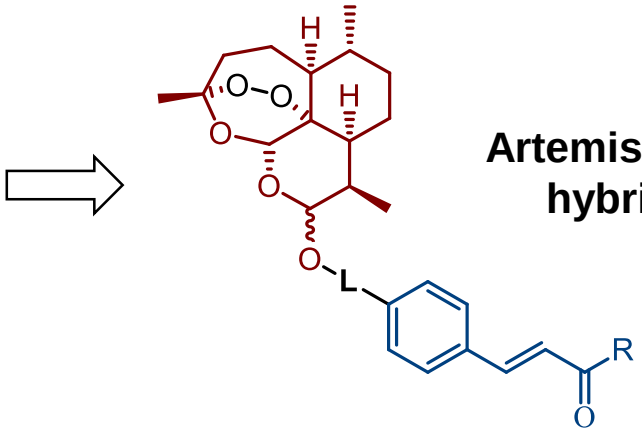
Artemisinin-chalcone hybrids



DHA

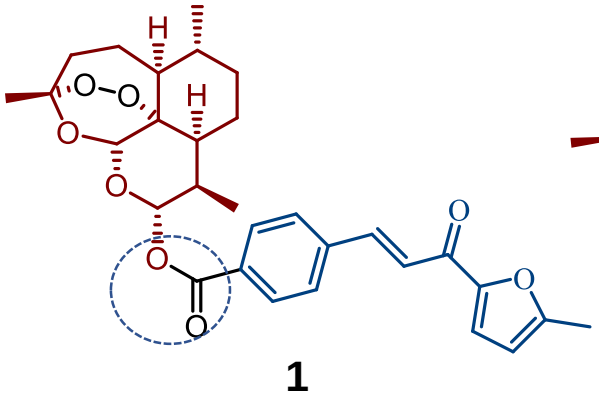


Chalcone



Artemisinin-chalcone hybrid (merged)

R: aromatic ring

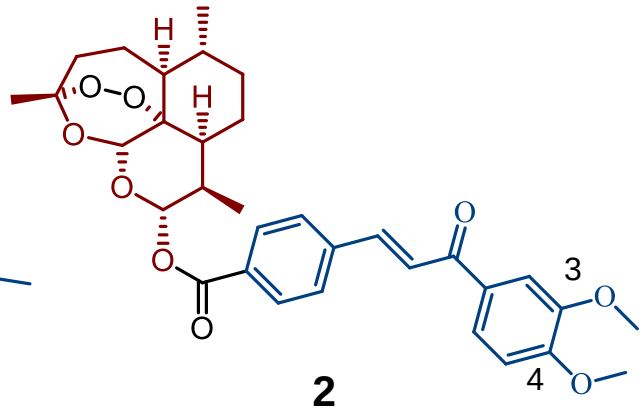


1

- *Pf* 3D7 2; W2 1.5 nM

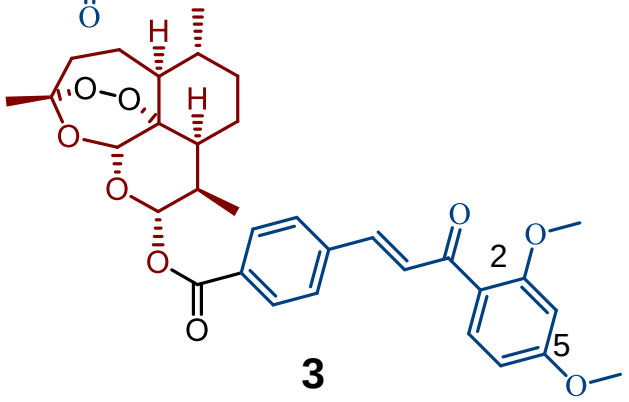
RI 0.8; *vs.* DHA 3D7 1.5; W2 1.3 nM.

- HFLF SI 36 000



2

- *Pf* 3D7 3; W2 1.5 nM; RI 0.5;
- HFLF SI 30 000



3

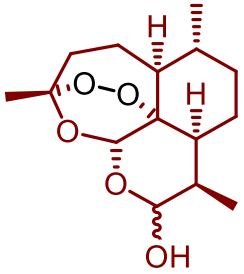
- *Pf* 3D7 3; W2 2 nM; RI 0.7
- HFLF SI 33 000

3 POTENTIAL EARLY LEADS ⇨ CHALLENGE – FURTHER DERIVATIZATION

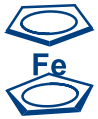
NWU® | PharmaCen™

Frans et al., *Eur J Chem Med*. 2015, **90**, 33-34.

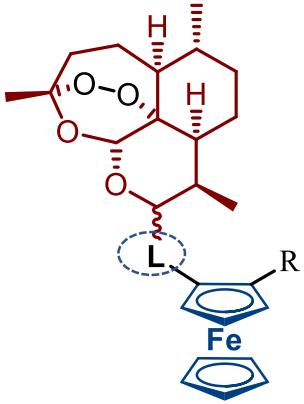
Artemisinin-ferrocene hybrids



DHA



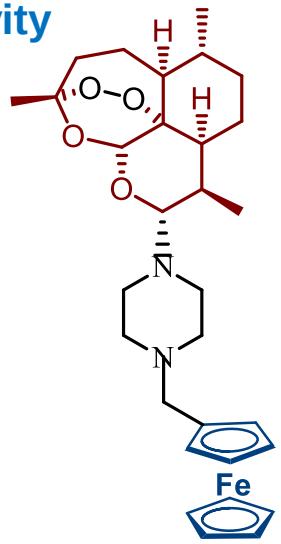
Ferrocene - Fc



Artemisinin-ferrocene hybrids

R: H, alkyl etc.

Blood stage activity

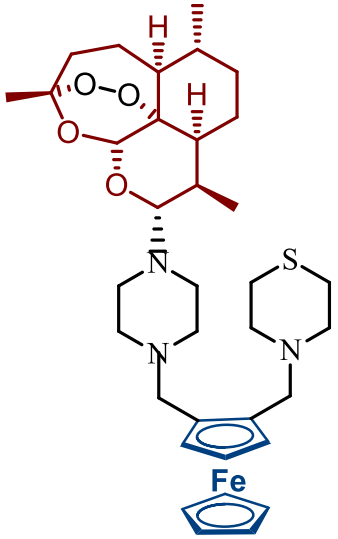


1

- *Pf* NF54 5; K1 3; W2 3 nM

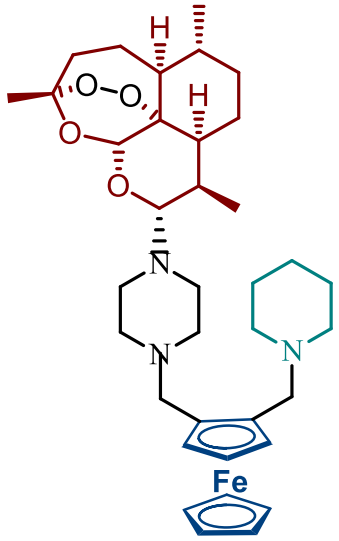
vs. DHA NF54 3; K1 2; W2 1.3 nM

- HEK-293 SI 11 500



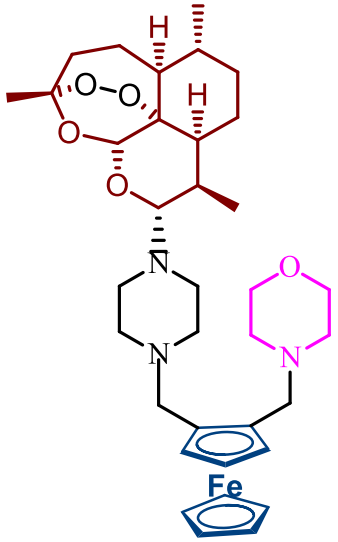
2

- NF54 3; K1 0.8; W2 3 nM
- SI 11 500



3

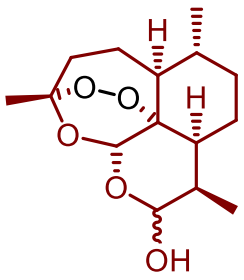
- NF54 4; K1 1; W2 2 nM
- SI 15 000



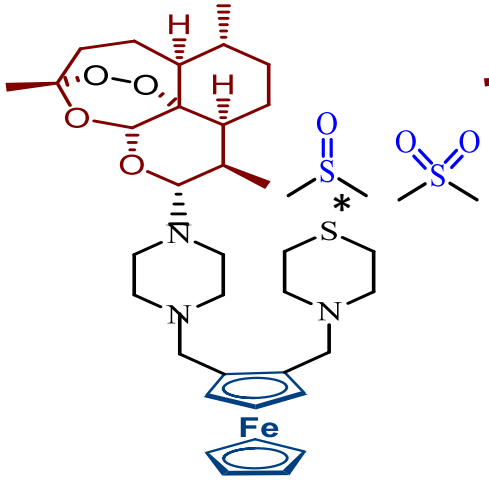
4

- NF54 3; K1 0.8; W2 1.4 nM
- SI 300

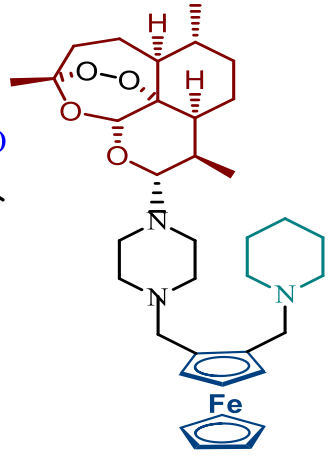
4 POTENTIAL EARLY LEADS



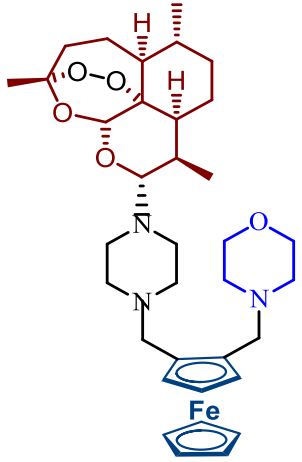
DHA



2



3



4

Gametocytocidal activity

Compd.	Early stage (I-III) gametocyte, % inhibition		Late stage (IV-V) gametocyte, % inhibition	
	1 µM	100 nM	1 µM	100 nM
DHA	97.1 ± 0.5		72.0 ± 6.7	
2	95.7 ± 0.33	93.8 ± 0.7	86.5 ± 3.56	84.8 ± 0.5
3	95.8 ± 0.21	95.9 ± 0.3	88.7 ± 2.04	87.0 ± 0.4
4	96.1 ± 0.37	99.1 ± 0.2	88.4 ± 0.96	87.6 ± 0.8

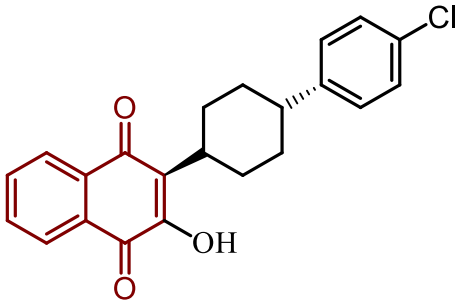
- Transmission blocking potential
- Ferrocene impact - *in vivo* testing
- Optimized Synthesis

- Further derivatization

⇩

Hybrid 2 ⇨ **Potential Early LEAD**

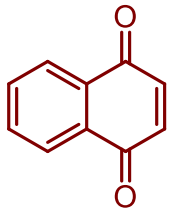
Naphthoquinone-triazole hybrids



Atovaquone

- Naphthoquinone antimalarial
- Multi-stage activity: oocyst, liver schizont and trophozoite
- MoA: parasitic mitochondrial bc1 complex inhibitor
- Clinical use: Combination with proguanil

  malaria chemoprophylaxis & curative



1,4-Naphthoquinone

- Pharmacophore: 1,4-naphthoquinone
- Redox active

1,2,3-Triazole

- Physicochemical properties

High aqueous water soluble;

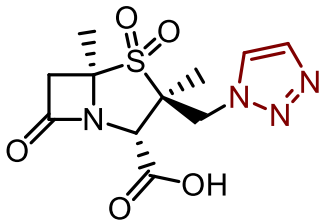
chemical stability: acidic and basic media;

enzymatic stability: oxidative and reductive conditions;

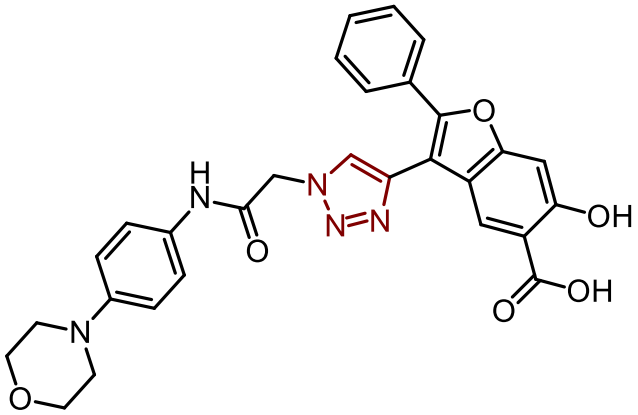
hydrogen bonding capacity

- MoA: cell wall biosynthesis inhibitor - lipid biosynthesis blocker

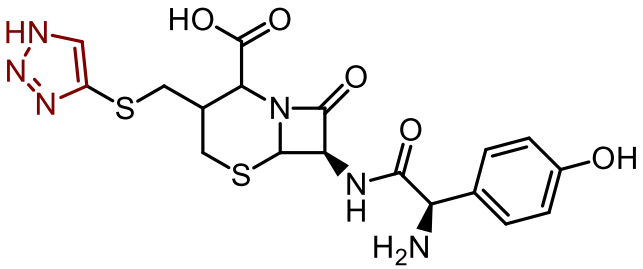
- 1,2,3-triazole containing drugs:



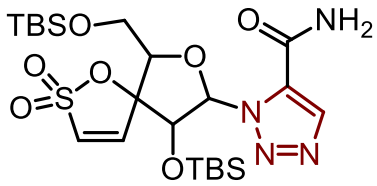
Tazobactum [antibiotic]



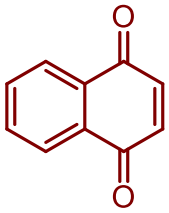
I-A09 [anti-TB in clinical trials]



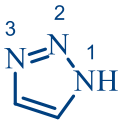
Cefatrizine [antibiotic]



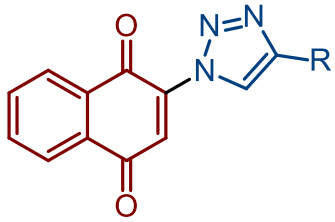
TSAO [Anti-HIV]



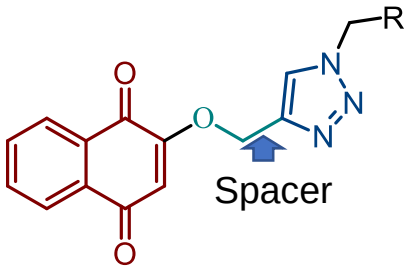
1,4-Naphthoquinone
AV - pharmacophore



1,2,3-Triazole



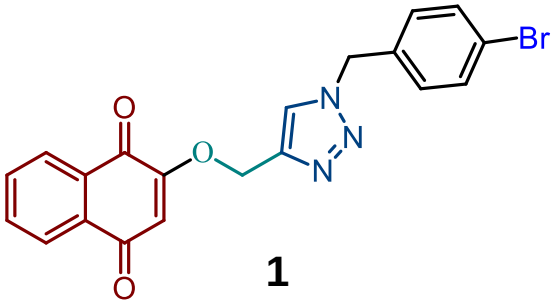
Merged hybrids



Conjugated hybrid

Malaria

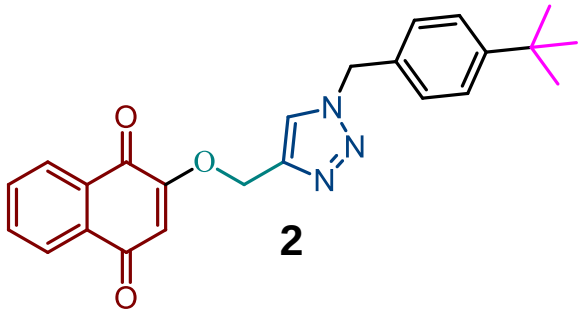
- All merged hybrids – inactive <u>BUT</u> all conjugated hybrids – active → flexibility critical



1

- Potency*: Pf* 3D7 IC_{50} 0.18; Dd2 0.17 µM; RI: 0.9
- Cytotoxicity, SI HEK-293 >550.

HIT



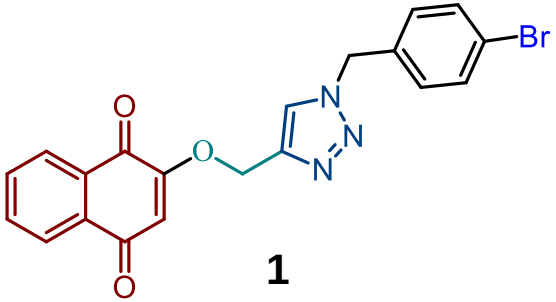
2

- Potency: *Pf* 3D7 IC_{50} 90 nM; Dd2 80 nM; RI 0.9
- Cytotoxicity, SI 405.

Potential EARLY LEAD

Leishmaniasis

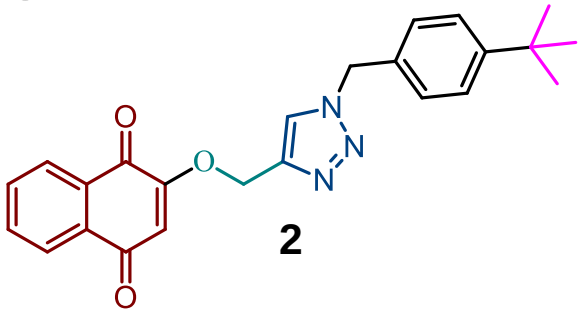
- Promastigotes - infective



1

- Potency: *L. don* 9515 IC_{50} 3 µM *vs.* AV. IC_{50} 5 µM (VL)
- Cytotoxicity: HEK-293 SI 33

- Potency: *L. m* IR-175 IC_{50} 2 µM *vs.* AV. IC_{50} 17 µM (CL)
- SI 41



2

- Potency: *L. don* 9515 IC_{50} 0.8 µM (VL)
- Cytotoxicity: HEK-293 SI 40

- Potency: *L. m* IR-175 IC_{50} 1.5 µM (CL)
- SI 22

Figure: Cytospin slide images of *L. major* promastigotes treated for 72 hours. (a) staurosporine (positive control), (b) hybrid **1** and (c) hybrid **2 -** 5x magnification.

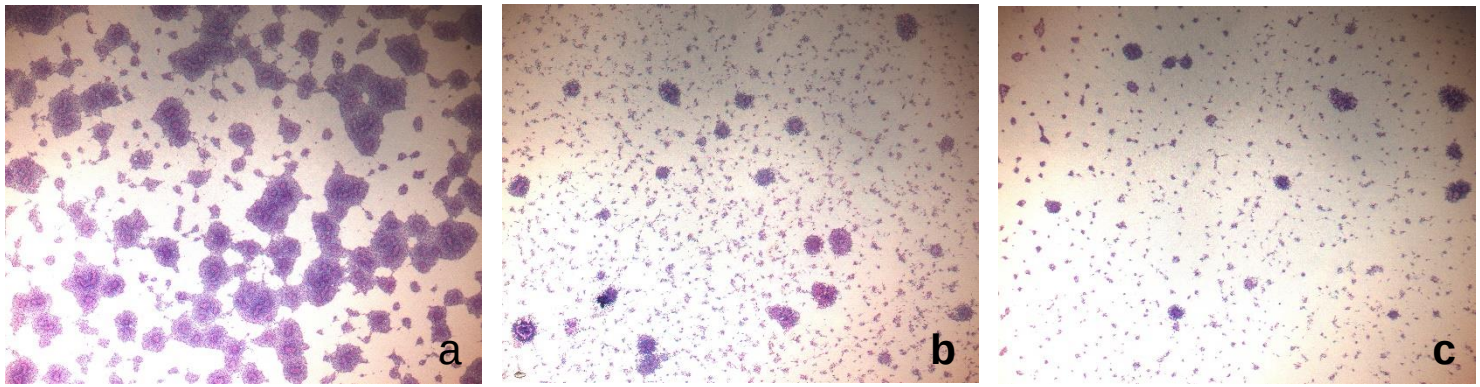
Hybrids **1** and **2**

⇩

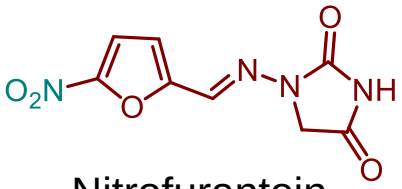
Higher parasite Antiproliferative effect

⇩

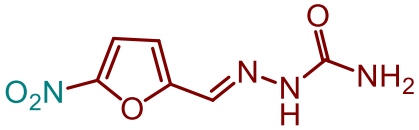
L. don amastigote screening



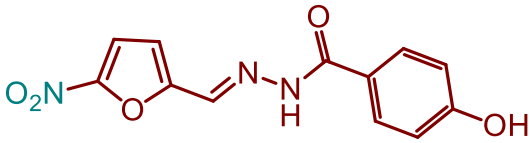
Nitrofurantoin-triazole hybrids



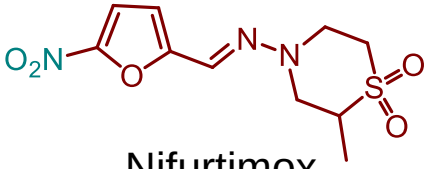
Nitrofurantoin



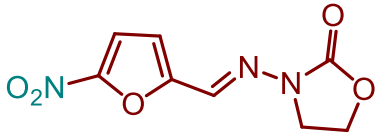
Nitrofurazone



Nifuroxazide



Nifurtimox

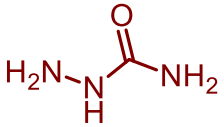


furazolidone

- Class: nitroaromatic
- Family: nitrofuran
- Use: human anti-UTI
- EU/USA collective ban as veterinary medicines – risk of carcinogenicity of semicarbazide metabolite in edible tissues.

SEM natural sources: crustaceans (shrimp, prawns and crab) and honey

- cheap



SEM

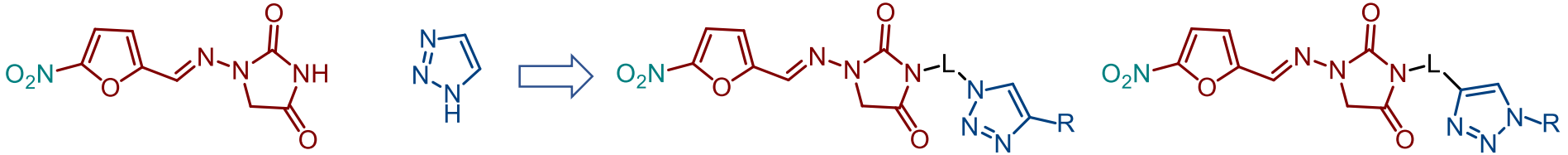
- Redox active: ROS generation
- Multi-activity: azoreduction, nitroreduction Type I (anaerobic) and II (aerobic).
- Multiple targets – critical metabolic pathways: replication, transcription, translation, Krebs cycle

→ no resistance

- Pharmacophore: 5-nitrofuran

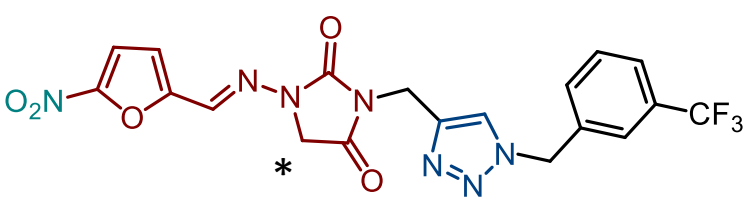
- Key liabilities:

poor water or oil solubility,
short half-life – 30 min
poor bioavailability



Nitrofurantoin - NFT

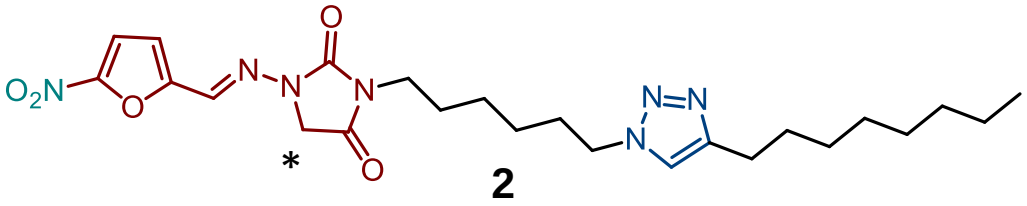
Malaria



1

- Potency: *Pf* 3D7 0.6, Dd2 0.18 µM;
  RI 0.3; *vs.* CQ 3D7 0.01; Dd2 0.16 µM
- Cytotoxicity, SI: HEK-293 >345

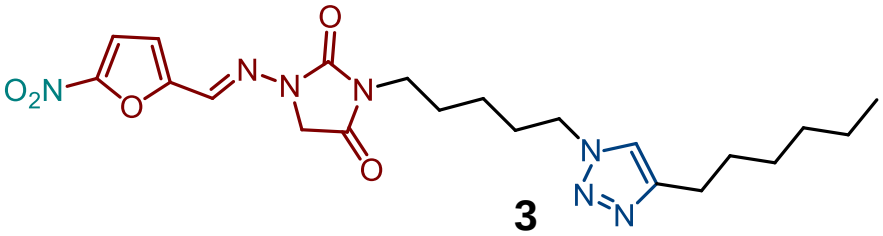
HIT



2

- Potency: *Pf* 3D7 0.6; Dd2 0.7 µM; RI 1.2
- Cytotoxicity, SI: HEK-293 55

HIT



3

- Potency: *Pf* 3D7 0.9; Dd2 0.5 µM; RI 0.6
- Cytotoxicity, SI: HEK-293 51

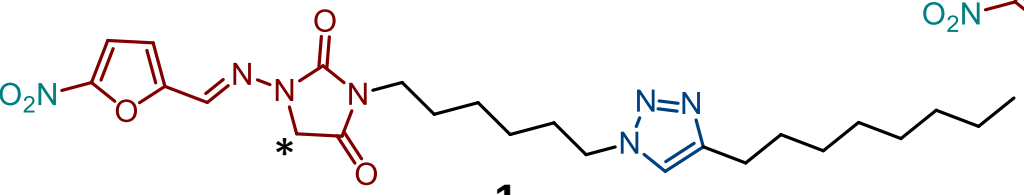
HIT

- 3 HITS

Possible derivatization to leads

Leishmaniasis

Promastigotes - infective



1

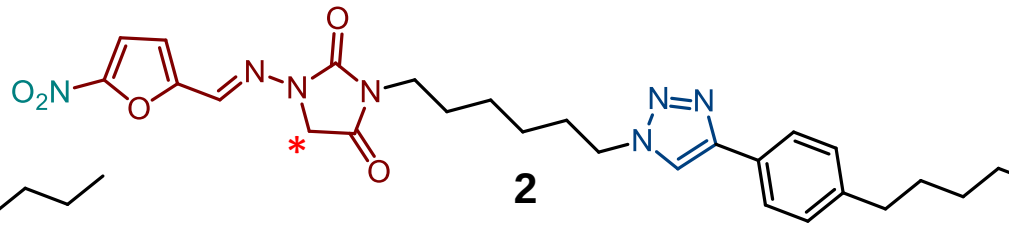
- Potency: *L. don* 9515 IC_{50} 59 nM (VL) vs. NFT 26 µM; SI 2
- Cytotoxicity: HEK-293 SI 559

- Potency: *L. m* IR-175 3 µM (CL)
- Cytotoxicity: SI 9

Nanomolar antiproliferative effect against *L. don* Promastigote

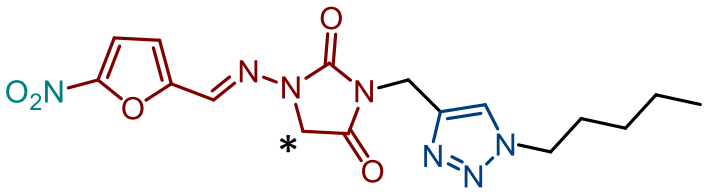
⇩

Amastigigote screening



2

- Potency: *L. don* 9515 IC_{50} 48 nM (VL)
- Cytotoxicity: HEK-293 SI 112



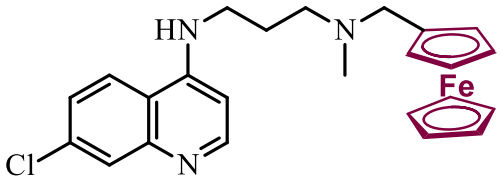
3

- Potency: *L. m* IR-175 90 nM (CL) *vs.* NFT 57 µM
- Cytotoxicity: SI 1111

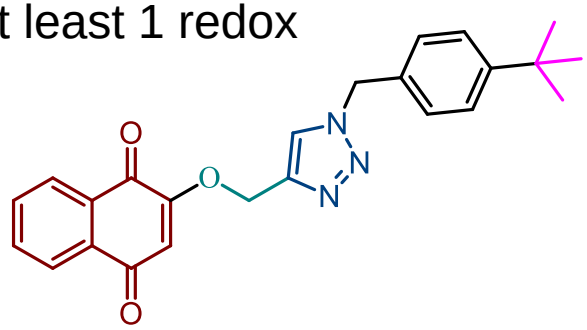
SUMMARY

Application of molecular hybridization strategy:

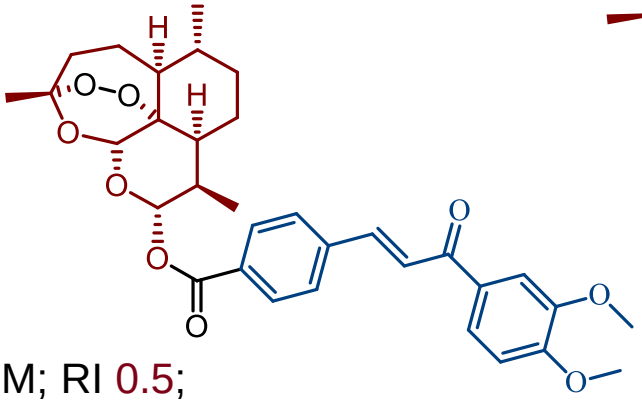
- Several antimalarial hits and potential early hybrids
- Most potential early leads contained at least 1 redox active pharmacophore



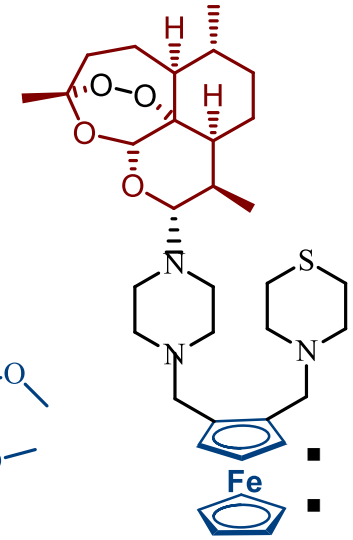
- Potency: IC_{50} *Pf* D10 40; Dd2 10 nM
- SI: CHO 1300



- Potency: *Pf* 3D7 IC_{50} 90; Dd2 80 nM
- SI 405



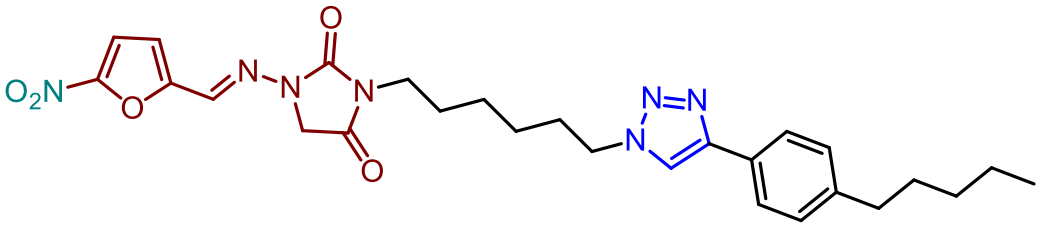
- *Pf* 3D7 3; W2 1.5 nM; RI 0.5;
- HFLF SI 30 000



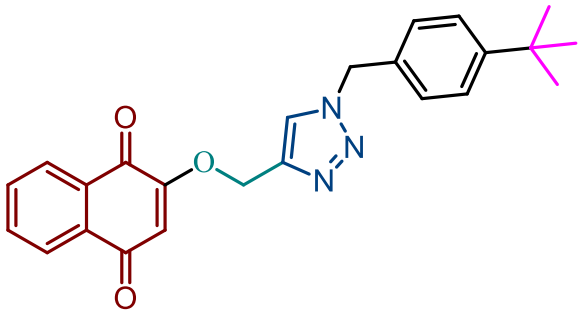
- NF54 3; K1 0.8; 2 3 nM
- SI 11 500

- Novel hybrids possessing high antiproliferative effect on the infective form of *Leishmania* parasite communicating the lethal form of the disease

- These hybrids also contain redox active pharmacophores



- Potency: *L. don* 9515 IC_{50} 48 nM (VL)
- Cytotoxicity: HEK-293 SI 112



- Potency: *L. don* 9515 IC_{50} 0.8 µM (VL)
- Cytotoxicity: HEK-293 SI 40

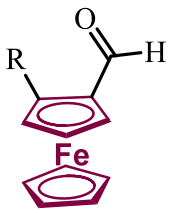
- Screening data are required on the clinical form of the parasite to judge of the standing of these hybrids as potential antileishmanial hits.

Future prospects

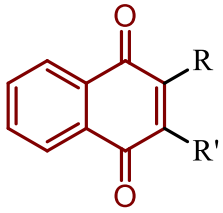
- Confirmation of the antimalarial early leads → In vivo studies: efficacy, DMPK
- Selection of antileishmanial hits based on anti-amastigote screening
- Continuation of molecular hybridization based on redox active pharmacophores

Redox active squad of pharmacophores:

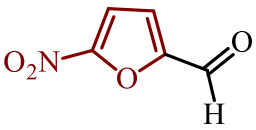
- Ferrocene
- Quinone
- Nitroaromatic
- Truncated artemisinin



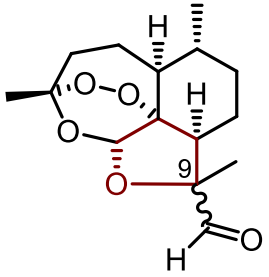
Ferrocene carboxyaldehyde



e.g. 2,3-Disubstituted naphthoquinone



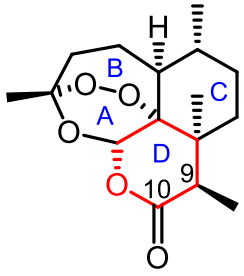
e.g. 5-Nitro furfural



TA 9-aldehyde

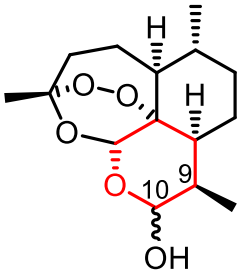
Current artemisinins: inappropriate!!!

- Chemical & enzymatic instability → $t_{½}$ < 2 h
- Partial resistance – parasite dormancy



Artemisinin (lactone)

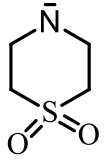
[Red]



DHA

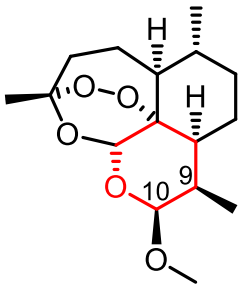
chemical instability
Rapid ring D opening

Met
NO
X

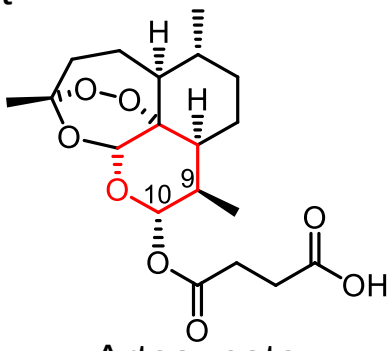


Artemisone: $t_{½}$ ≈ 3 h – still too short

Met



Artemether

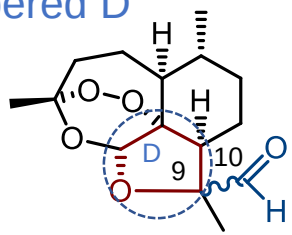


Artesunate

Enzymatic instability

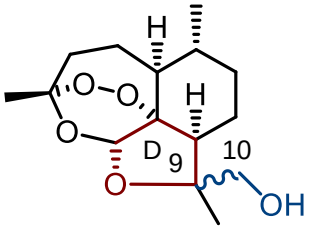
Truncated artemisinin [1]

- Stronger 5-membered D (THF) ring
- Exocyclic C-10



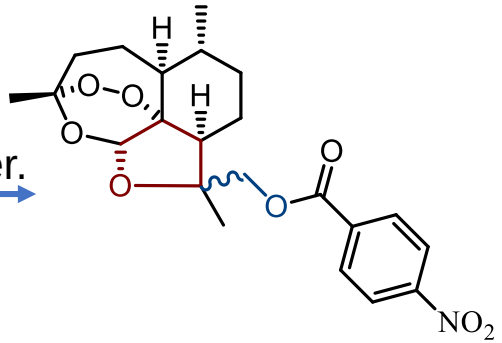
Aldehyde (TAA)

[Red]



Alcohol (TAL)

Ester.



IC_{50} *Pf* NF54 4; Dd2 7 nM
SI: HFLF 14 500

IC_{50} *Pf* NF54 7; Dd2 4 nM
SI: HFLF 24 400

IC_{50} *Pf* NF54 7; Dd2 2.6 nM
SI: > 14 900

NWU® | PharmaCen™

Zuma et al., *Eur J Med Chem*. 2016, 122, 635-646

Thank you

ACKNOWLEDGEMENTS & APPRECIATIONS

I want to make use of the opportunity to express my gratitude to the following:

- First and foremost - I thank God, my Lord for His love and grace in my life, for giving me such a wonderful Grand-Mother, surely she is with you in Heaven
- My wife Clarina for your love, unwavering support and motivation in our shared lives. You always make me laugh when I need it most.
- My daughter Palmovia. You arrived and rescued me at the right time, and I am always delighted to hear you speaking Afrikaans. You know much difference you have already made in our lives.
- My friends for always being there for me and making the road a little easier and certainly a whole lot more fun.
- My fellow parishioners from St Michael Catholic Church for your prayers and support.
- My students from whom I always learnt something new and reminding me of myself during postgraduate years.

- My colleagues at the School of Pharmacy, Pharmacen and the NWU for the opportunity to pursue a research career in such a conducive environment.

- Prof Lesetja Legoabe, my “brother” with whom I share so many memories, good and bad alike.

- The Dean of Health Sciences, Prof. Awie Kotzé and the Deputy-Dean for Research & Innovation, Prof Jeanetta du Plessis, who made this evening possible.

- My collaborators at UCT, UP, Obihiro University (Japan) and Justus Liebig University (Germany) for the shared my research interests.

- A special thanks to Yolande Avenant for all the arrangements and excellent taste.

I would like to thank three people who played a tremendous role in my career and life:

- Prof Jeanetta du Plessis for being my entry point to the NWU in 2006, my support ever since, and always there to advise me.

- Prof Jaco Breytenbach, my mentor for his support, encouragement, advice and guidance during the working together in a spirit of collaboration to increase the quality of research. Thank you wholeheartedly for putting me on the right career path. May God bless and reward you immensely.

- Prof Frikkie for his compassion and assistance to others. From you, I learnt that small acts of kindness can go a long way.