

# Complex Diseases, Success and Failure

Finding the „right“ target → valid targets

which constraints are limiting factors?

Dosage, bioavailability, Actual drug concentration in the respective compartment (cell, organelles)

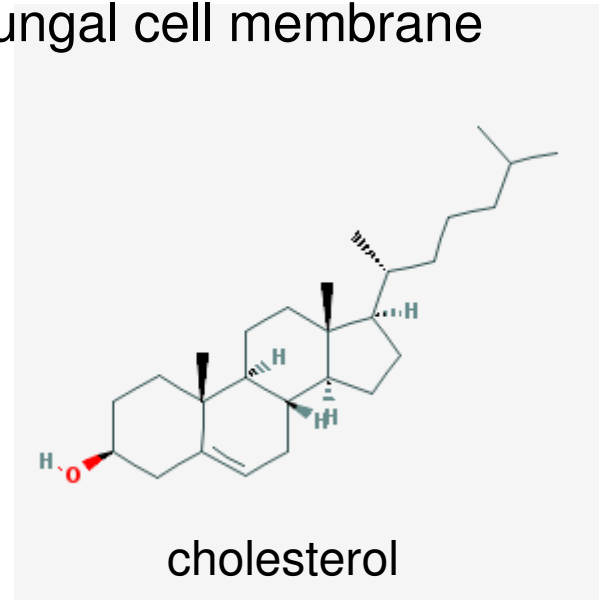
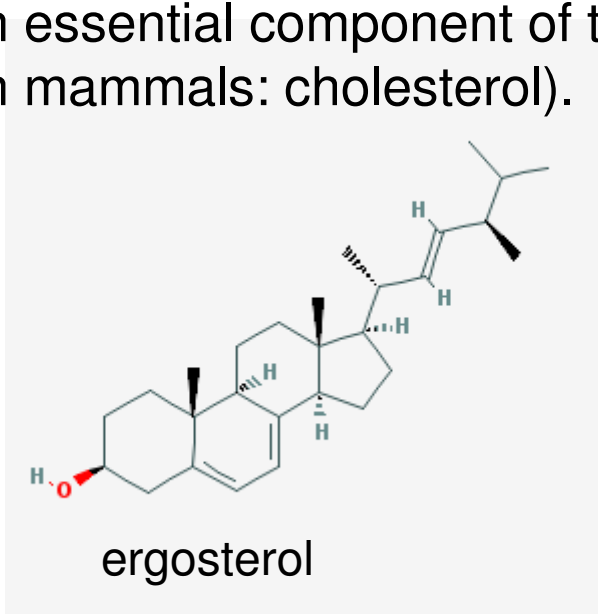
Are we competing against a natural substrate,  
e.g. ATP ? (concentration in the cell: ca. 4 mMol)

# Success

## Antifungals

Ketoconazole, Fluconazole, Itraconazole, Clotrimazole, ...

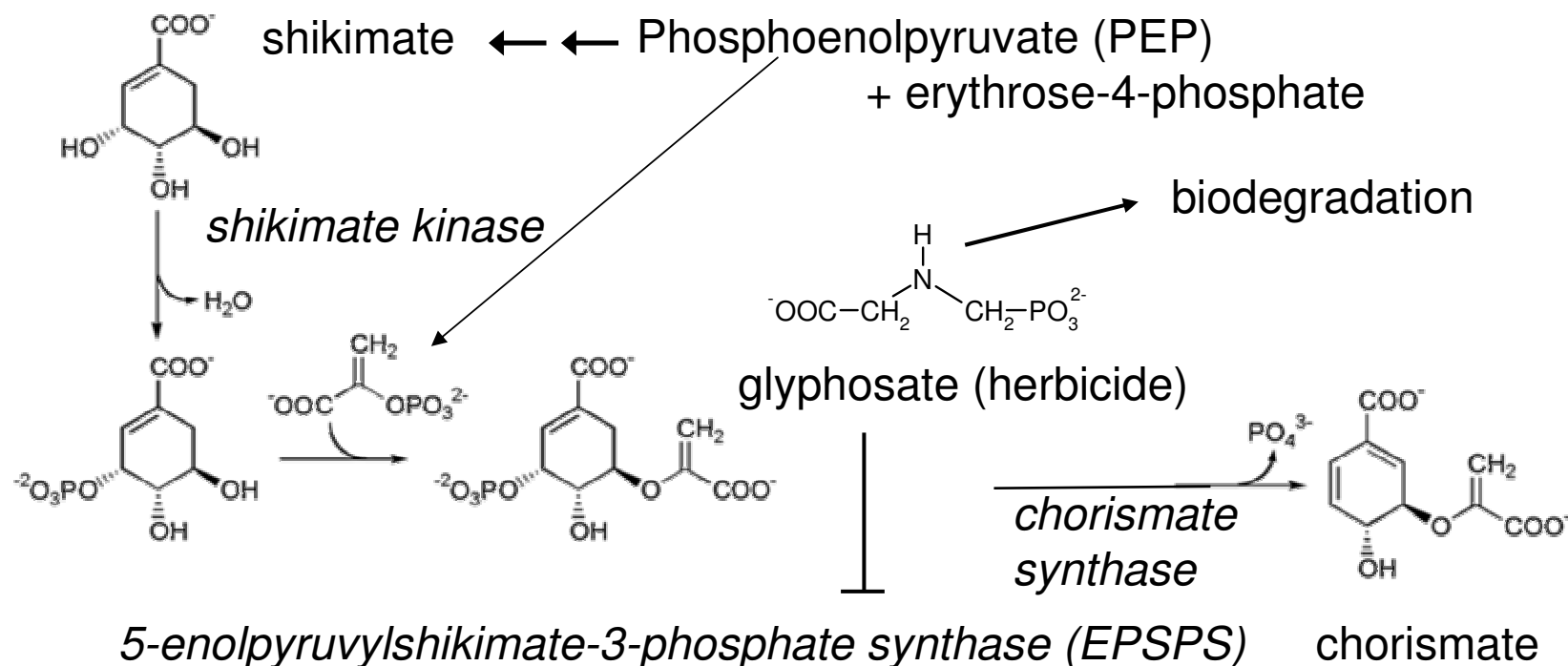
Mechanism of action: Inhibition of 14- $\alpha$ -demethylase (CYP51) that is part of the biosynthesis pathway of ergosterol, which is an essential component of the fungal cell membrane (in mammals: cholesterol).



Cons: Inhibition of Cytochromes causes hepatotoxicity (Ketoconazole). Other conazoles are more specific.  
Development of resistances (overexpression of efflux proteins).

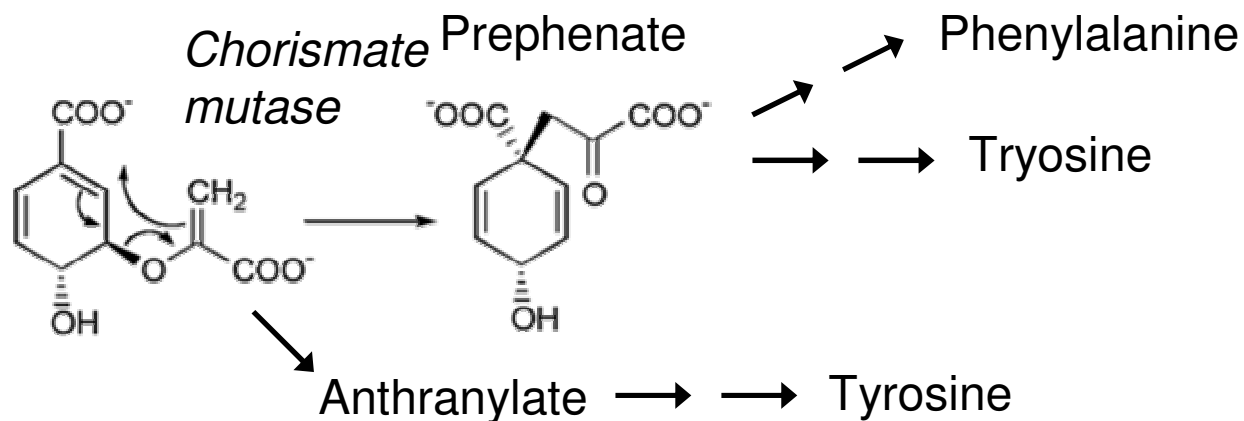
# Failure (so far) (I)

Antibacterial agents targeting enzymes of the Shikimate pathway (responsible for the synthesis of the amino acids Phe, Tyr, and Trp).



Pro: Those enzymes are only found in plants, fungi, algae, and bacteria but not in mammals. Thus interference can be ruled out.

## Failure (so far) (II)



Pathogens such as *Oxoplasma gondii*, *Plasmodium falciparum*, and *Cryptosporidium parvum* contain the Shikimate pathway and the seven enzymes involved.

Lit. C.W.Roberts et al. *J.Infect.Dis.* **185** (2002) Suppl.1:S25-36.

Con: Obviously the necessary inhibitor concentration in the respective compartment could not be achieved.

For comparison:

The cellular level of phosphoenolpyruvate (PEP) is ca. 4 mMol

# Complex Diseases

malaria is the tropical disease no.1

300-500 millionen infections per year  
causing 1-3 million fatalities

clinical symptoms:

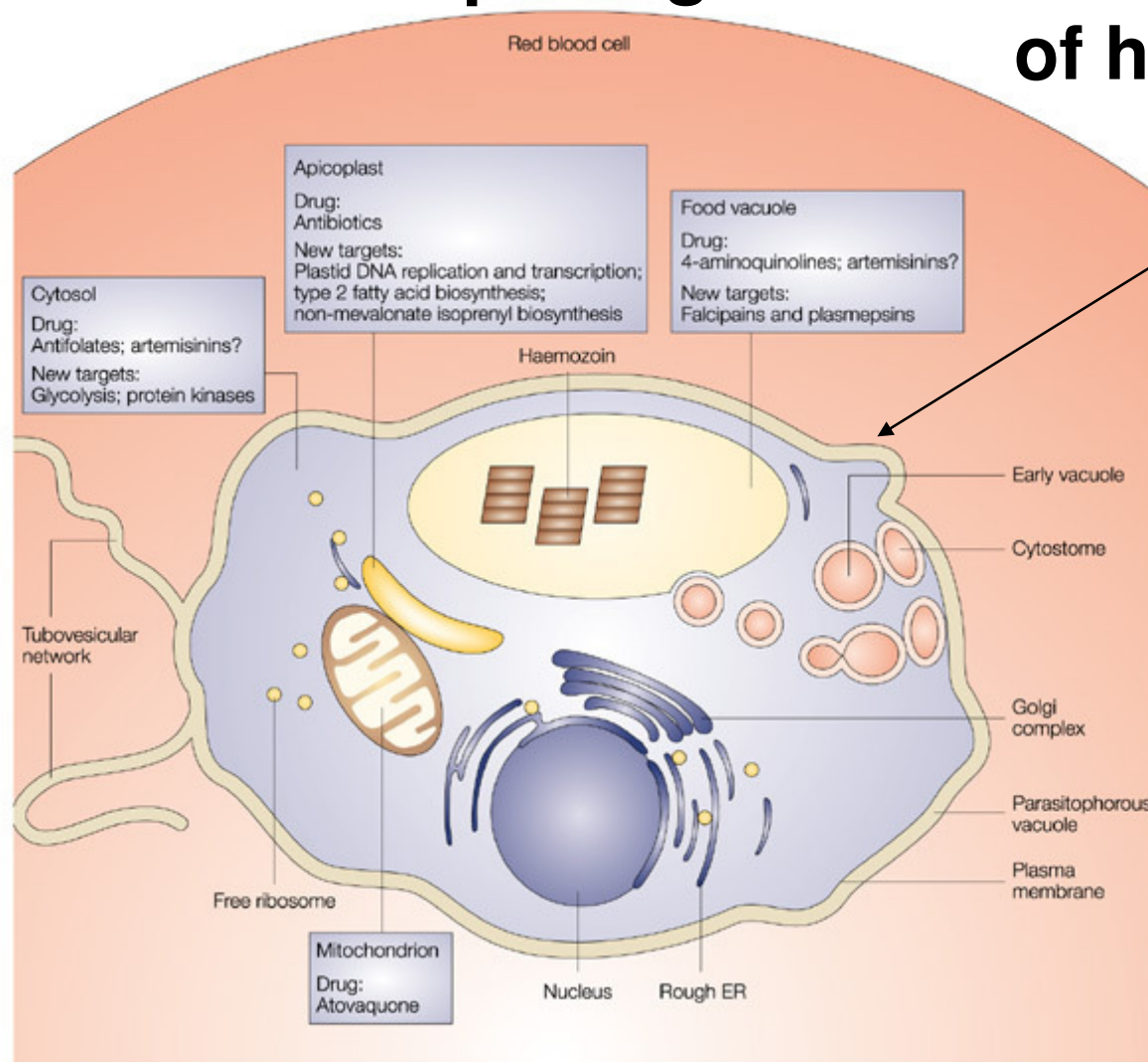
Strong fever, anemia, acidosis,  
multiple failure of organs



Due to the life cycle of the pathogen *Plasmodium flaciparum* and the transmission by the *anopheles* fly, there are several starting points for control and therapy.

Lit. D.A.Fidock et al. *Nature Rev. Drug Disc.* **3** (2004) 509

# malaria pathogens cause degradation of hemoglobin



*Plasmodium falciparum* trophozoite

Further pathogens in human:

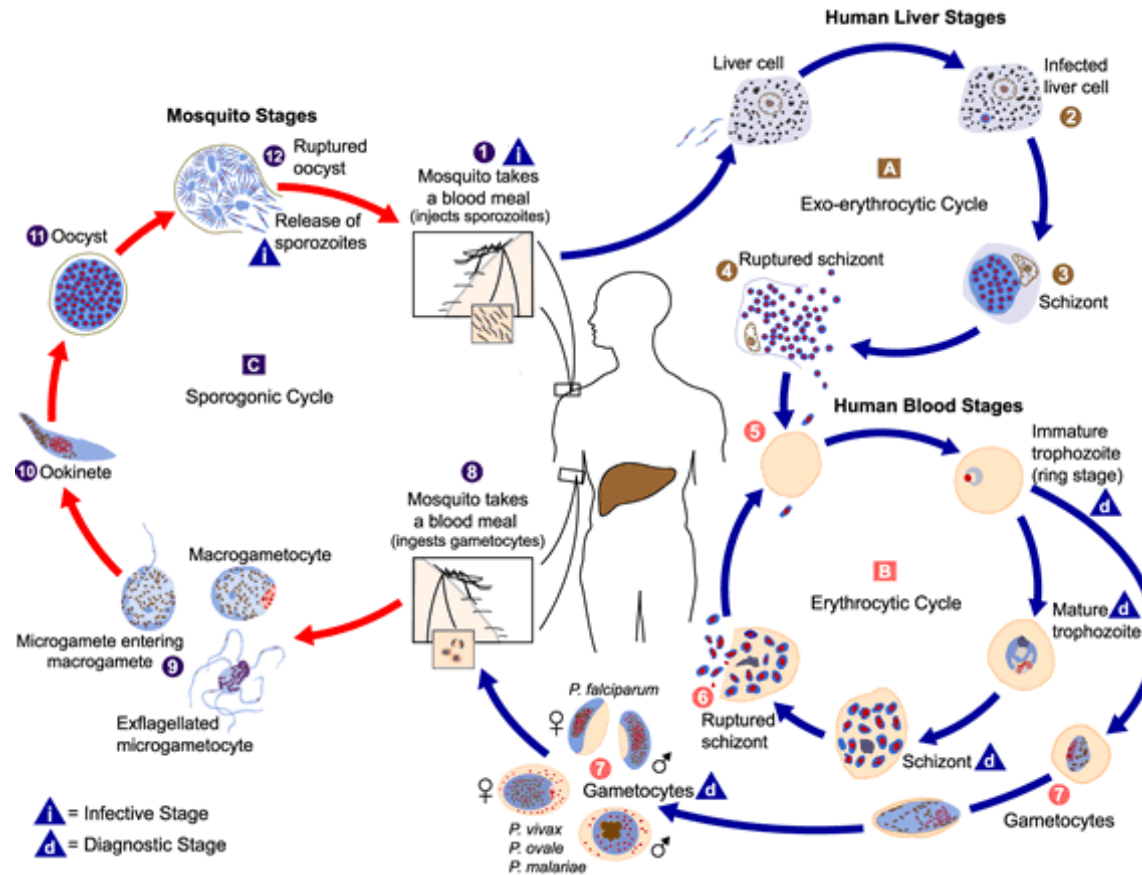
*P. vivax*

*P. malariae*

*P. ovale*

and about 56 more species of *Plasmodium*

# Lifecylce of the malaria pathogens



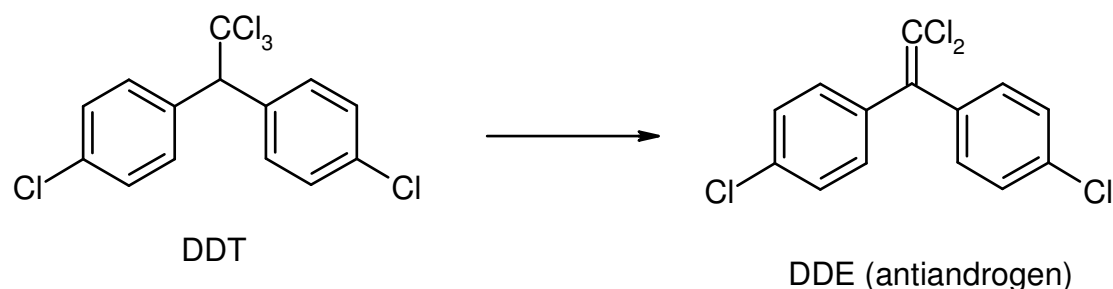
source: [http://www.dpd.cdc.gov/.../body\\_Malaria\\_page1.htm](http://www.dpd.cdc.gov/.../body_Malaria_page1.htm)

# Approaches to controlling (I)

1960-1980 exhaustive use of insecticides against the Anopheles fly with very good results by the use of DDT (dichloro-diphenyl-trichloroethane)

Disadvantages:

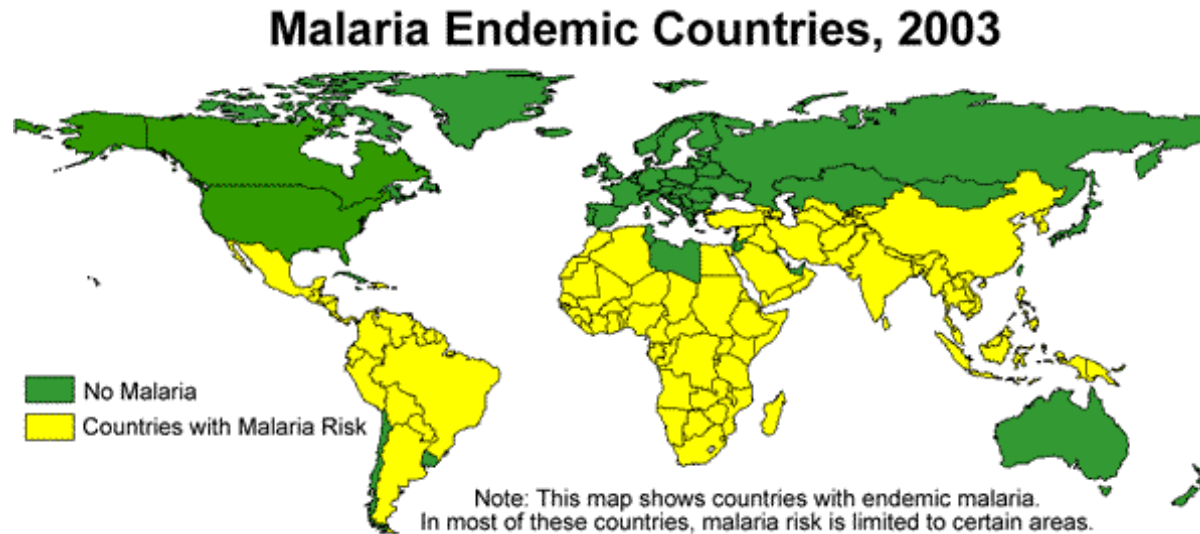
- Accumulation of DDT in the adipose tissue [Fettgewebe] of all creatures (mammals, birds, fish)
- DDT is biologically (almost) undegradable
- Metabolismus leads to a neurotransmitter-like substance (acts as contact insecticide !)



- Increasing resistance to DDT has been observed

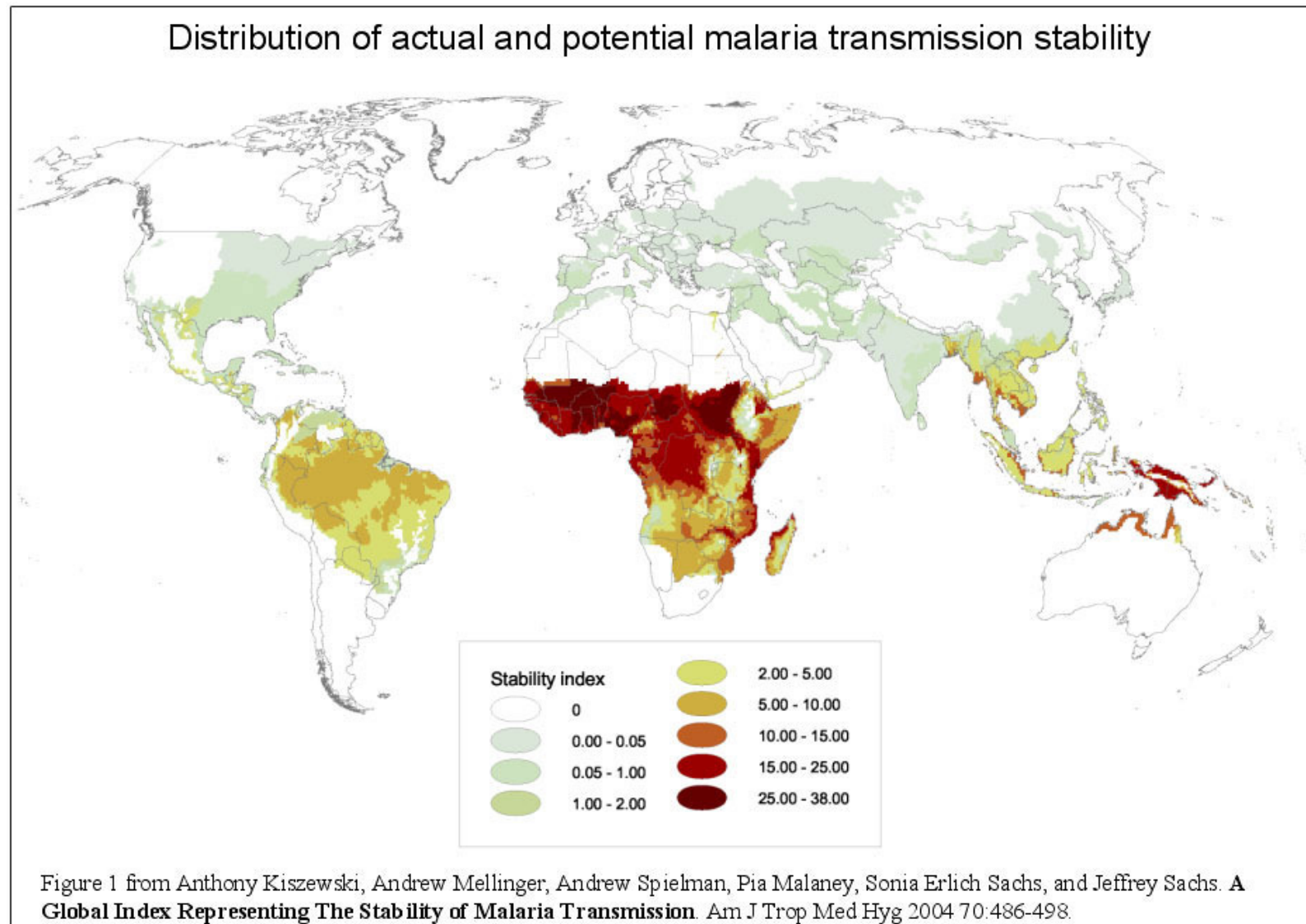


# Distribution of Malaria (I)

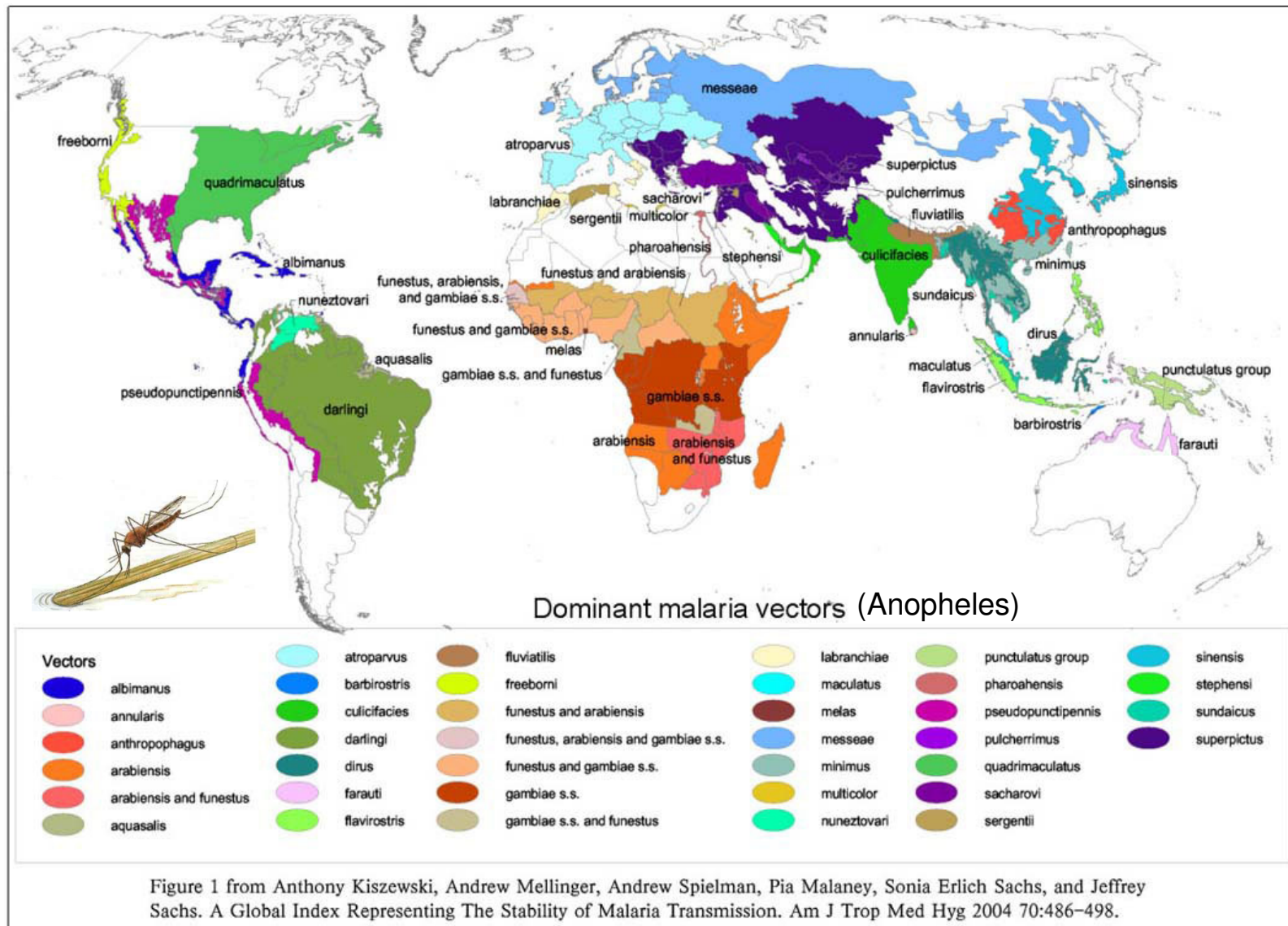


Areas with risk of malaria

# Distribution of malaria (II)



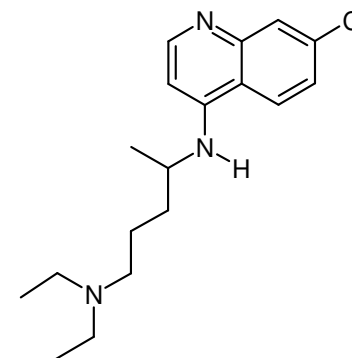
# Distribution of the Anopheles fly



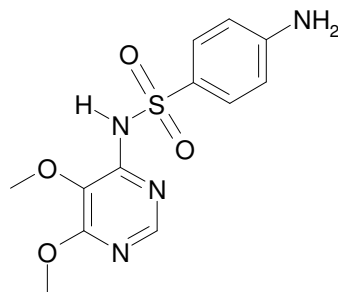
## Approaches to controlling (II)

chloroquine: since the late 1940's worldwide application at very low costs (0.2 US\$ per dose)

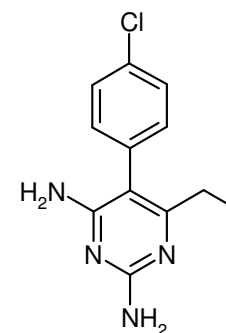
mode of action (still partly unclear):  
binds to HEM groups  
inhibition of the glutathion-S-transferase



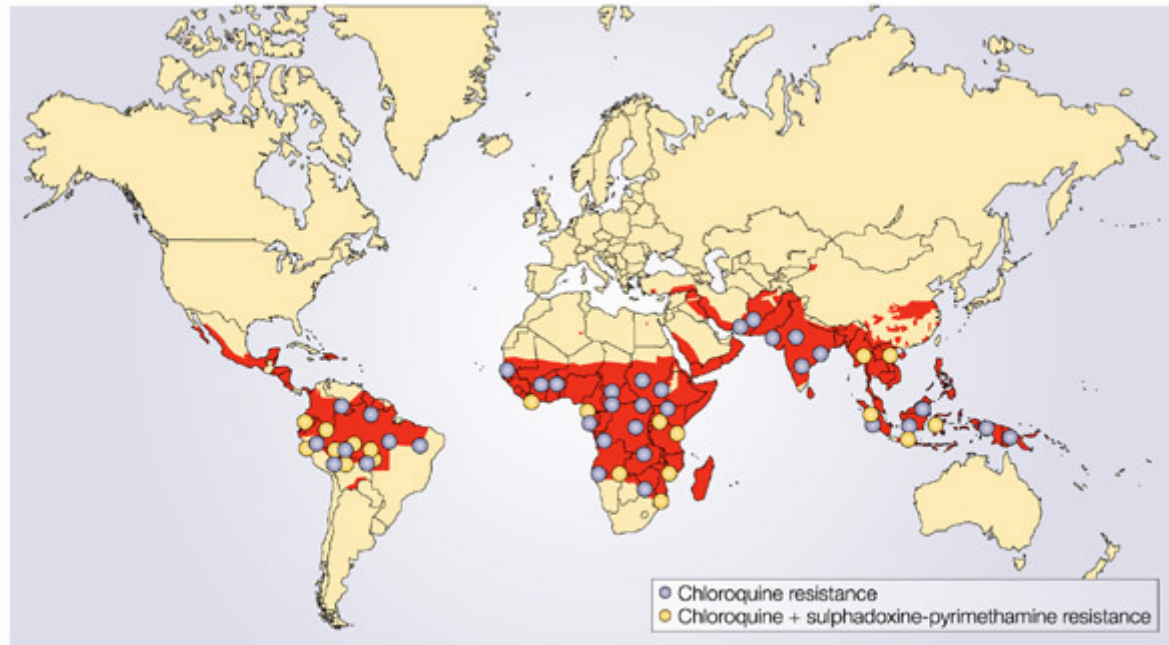
sulfadoxine  
antibacterial



pyrimethamine  
blocks the dihydrofolate reductase  
respectively the dihydropterate synthetase



# Resistance of the Anopheles fly

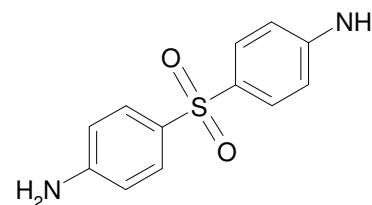
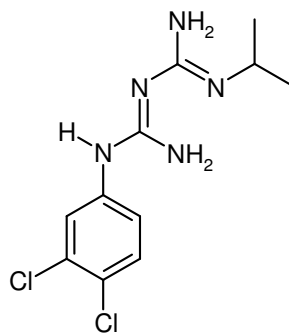
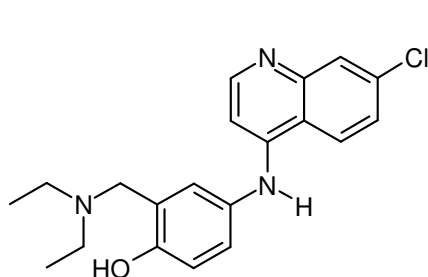


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red: areas with malaria

## Approaches to controlling (III)

Alternatives to chloroquine and sulfadoxine/pyrimethamine  
amodiaquine respectively chlorproguanil/dapsone



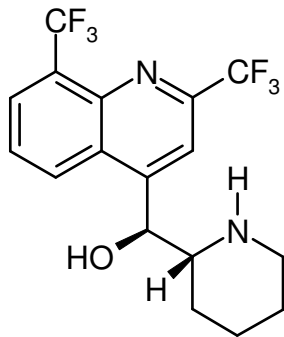
Disadvantage: expected build up of resistances due to identical targets

# Approaches to controlling (IV)

Profile for new drugs and chemoprophylaxis

- efficient, cheap
- effective against the more rare, but lethal *Plasmodium vivax*
- Avoiding of resistances by the use of combinations drugs (several targets at the same time)

Example for chemoprophylaxis: mefloquine (Lariam®)

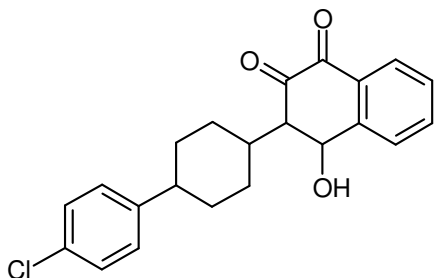


Mode of action due to interaction with phospholipids (cell membrane, fatty acid synthesis)

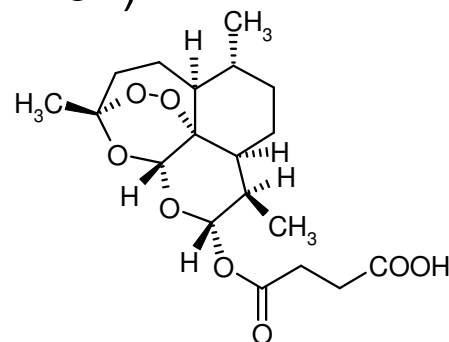
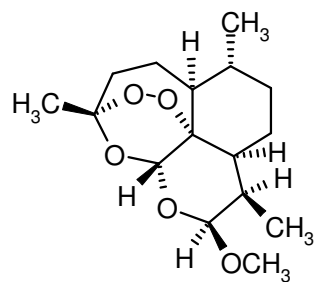
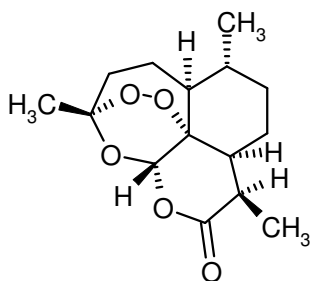
Only very few adverse effects

# Approaches to controlling (V)

Example for combination drugs:  
atovaquone (antiparasitic) together with an antibiotic



Drugs derived from natural compounds:  
artemisinin → artemether and artesunate (form cytotoxic radicals in the presence of HEM iron)



Disadvantage: metabolisms and thus short half life



# New malaria targets (I)

Table 2 | **Targets for antimalarial chemotherapy**

Target location	Pathway/mechanism	Target molecule	Examples of therapies		References
			Existing therapies	New compounds	
Cytosol	Folate metabolism	Dihydrofolate reductase Dihydropteroate synthase	Pyrimethamine, proguanil Sulphadoxine, dapsone	Chlorproguanil	82,83
	Glycolysis	Thymidylate synthase		5-fluorocrotate	84
		Lactate dehydrogenase		Gossypol derivatives	85
		Peptide deformylase		Actinonin	86
	Protein synthesis	Heat-shock protein 90		Geldanamycin	87
	Glutathione metabolism	Glutathione reductase		Enzyme inhibitors	88
	Signal transduction	Protein kinases		Oxindole derivatives	89
	Unknown	Ca <sup>2+</sup> -ATPase	Artemisinins		90
Parasite membrane	Phospholipid synthesis	Choline transporter		G25	71
	Membrane transport	Unique channels	Quinolines	Dinucleoside dimers	91
		Hexose transporter		Hexose derivatives	92
Food vacuole	Haem polymerization	Haemozoin	Chloroquine	New quinolines	93,94
	Haemoglobin hydrolysis	Plasmeprins		Protease inhibitors	95,96
		Falcipains		Protease inhibitors	97,98
	Free-radical generation	Unknown	Artemisinins	New peroxides	99,100
Mitochondrion	Electron transport	Cytochrome c oxidoreductase	Atovaquone		101
Apicoplast	Protein synthesis	Apicoplast ribosome	Tetracyclines, clindamycin		102
	DNA synthesis	DNA gyrase	Quinolones		
	Transcription	RNA polymerase	Rifampin		
	Type II fatty acid bio-synthesis	FabH		Thiolactomycin	29
		FabI/PIENR		Triclosan	32,33,103
	Isoprenoid synthesis	DOXP reductoisomerase		Fosmidomycin	30
	Protein farnesylation	Farnesyl transferase		Peptidomimetics	25,104
Extracellular	Erythrocyte invasion	Subtilisin serine proteases		Protease inhibitors	97,105

DOXP, 1-deoxy-D-xylulose 5-phosphate; PIENR, *Plasmodium falciparum* enoyl-ACP reductase.

Lit. D.A.Fidock et al. *Nature Rev. Drug Disc.* **3** (2004) 509

## New malaria targets (II)

- Target identification on the gene level  
homolog enzymes of known diseases
- Improvement of drugs that are already in use against other  
(infective) diseases:

dihydrofolate reductase	→ cancer
cysteine protease	→ osteoporosis
protein farnesyl transferase	→ cancer
protein synthesis	→ other parasites

vaccines: proteins that are expressed on the cell surface  
→ sequencing of the *Plasmodium falciparum* genome

## New malaria targets (III)

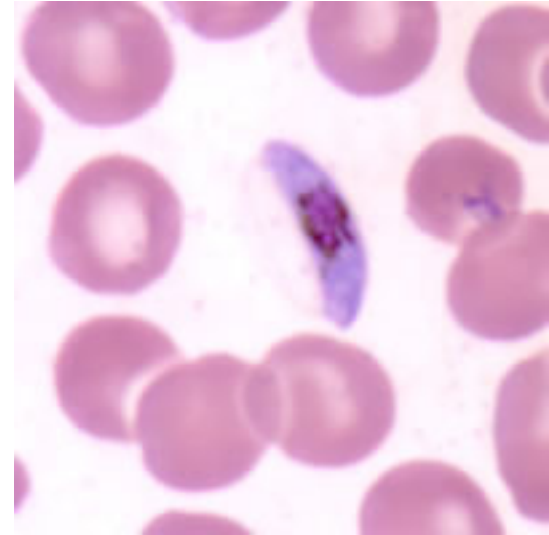
Sequencing of *Plasmodium falciparum*

25 Mb on 14 chromosomes, ca. 5000 genes

6 Kb genome of the mitochondrion

35 Kb circular DNA of the Apicoplast

Similar dimensions are also to be expected for *P. yoelii* and *P. vivax*.



<http://www.ncbi.nlm.nih.gov/Malaria/>

<http://plasmodb.org> (annotated Plasmodium genome)

Metabolic paths of *P. falciparum*:

<http://sites.huji.ac.il/malaria/> (contains EC numbers)

Lit. S.L.Hoffman et al. *Nature* **415** (2002) 702

# Neglected Tropical Diseases (I)

Infections with pathogens prevalent in developing regions around the tropical belt of Africa, Asia, and America.

ascariasis, trichuriasis, necatoriasis, ancyclostomiasis  
infection by soil transmitted helminths (worms)

Schistosomiasis (snail fever, bilharzia)

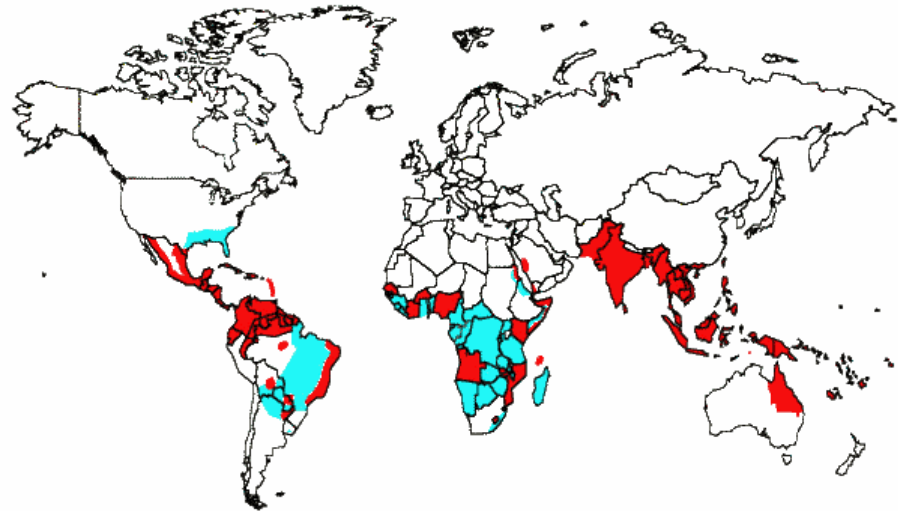
Trachoma and onchocerciasis (river blindness)

Leishmaniasis

Chagas disease

Leprosy

African Trypanosomiasis  
(sleeping sickness)



The impact of these diseases in numbers is similar to that of malaria and tuberculosis

# Neglected Tropical Diseases (II)

The World Health Organisation lists further diseases, such as  
Cysticercosis (infection by the pork tapeworm)

Dengue / dengue haemorrhagic fever  
(virus transmitted by mosquitos)

Rabies [Tollwut] (viral)

Yaws (bacterial) a similar treponemal disease is syphilis

Snake bites

Tropical diseases with outbreaks in other areas due to  
transmission by mosquitos:

West Nile virus

Ross River fever

# Complex diseases

**obesity** [Fettleibigkeit]



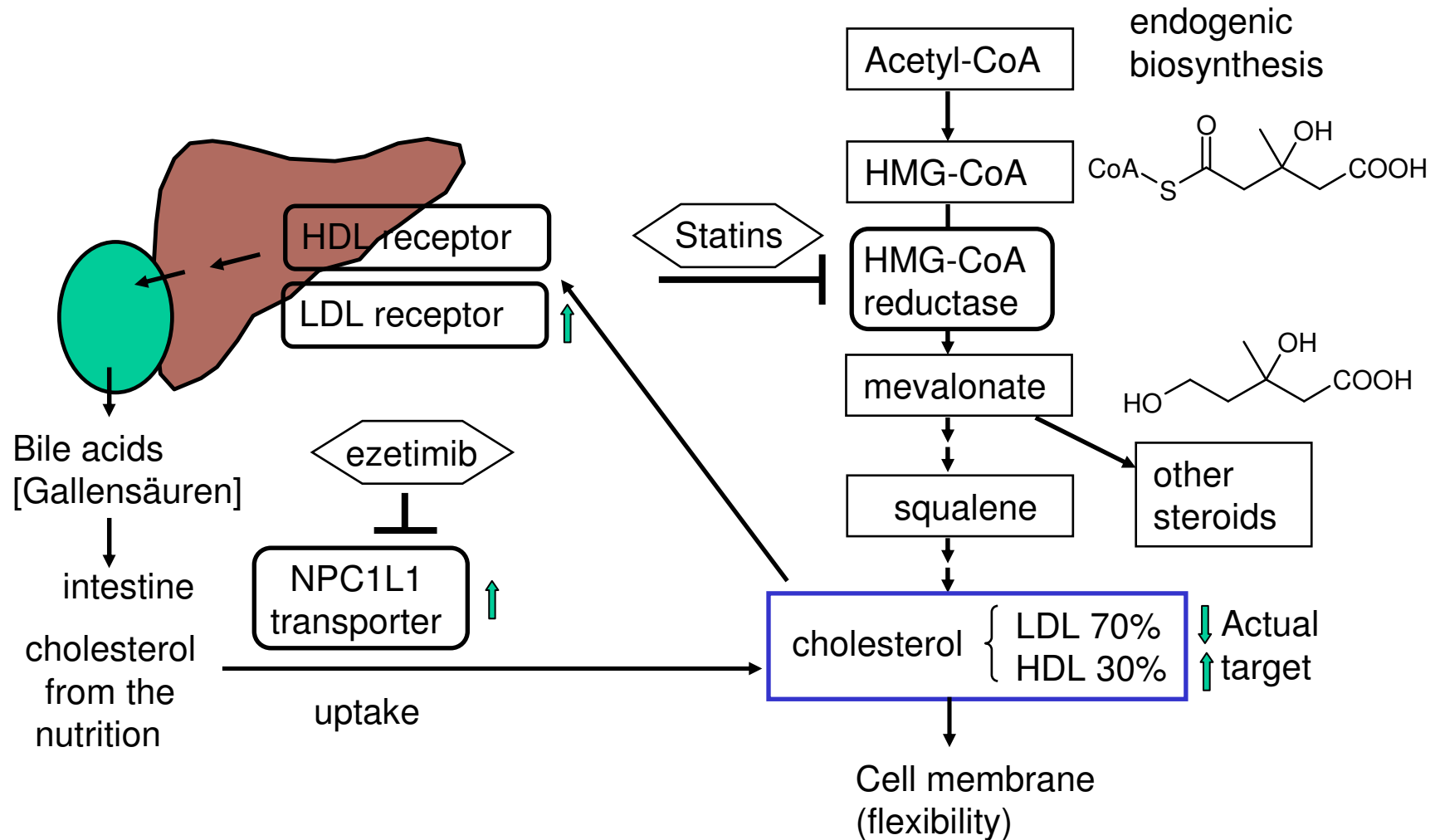
typical symptoms:

- excess weight
- increased levels of cholesterol  
→ arteriosclerosis
- hypertension

} increased  
cardiovascular  
risk

The connection to obesity was established by the genetic lack of cholesterol receptors (hypercholesterolaemia) and especially cholesterol-rich nutrition in animal studies.

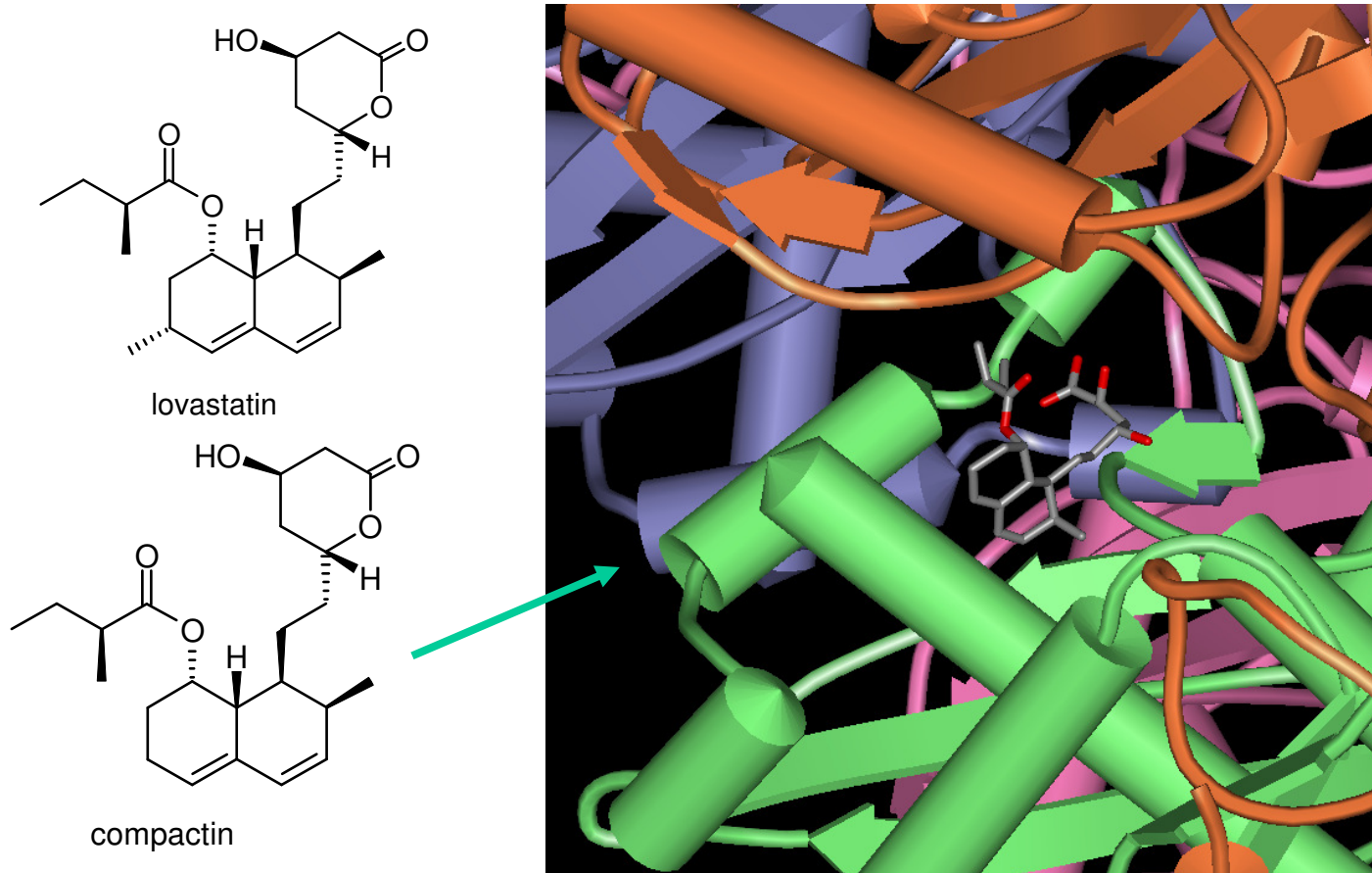
# Regulation of the cholesterol pool



Lit. F.Rinninger & H.Greten *Dtsch. Ärztebl.* **102** (2005) A516  
 J.A.Tobert *Nature Rev. Drug Disc.* **2** (2003) 517

# Inhibition of HMG-CoA reductase (I)

compactin (from *Penicillium citrinum*) and mevinolin (=lovastatin) (from *Aspergillus terreus*) were first found as inhibitors.

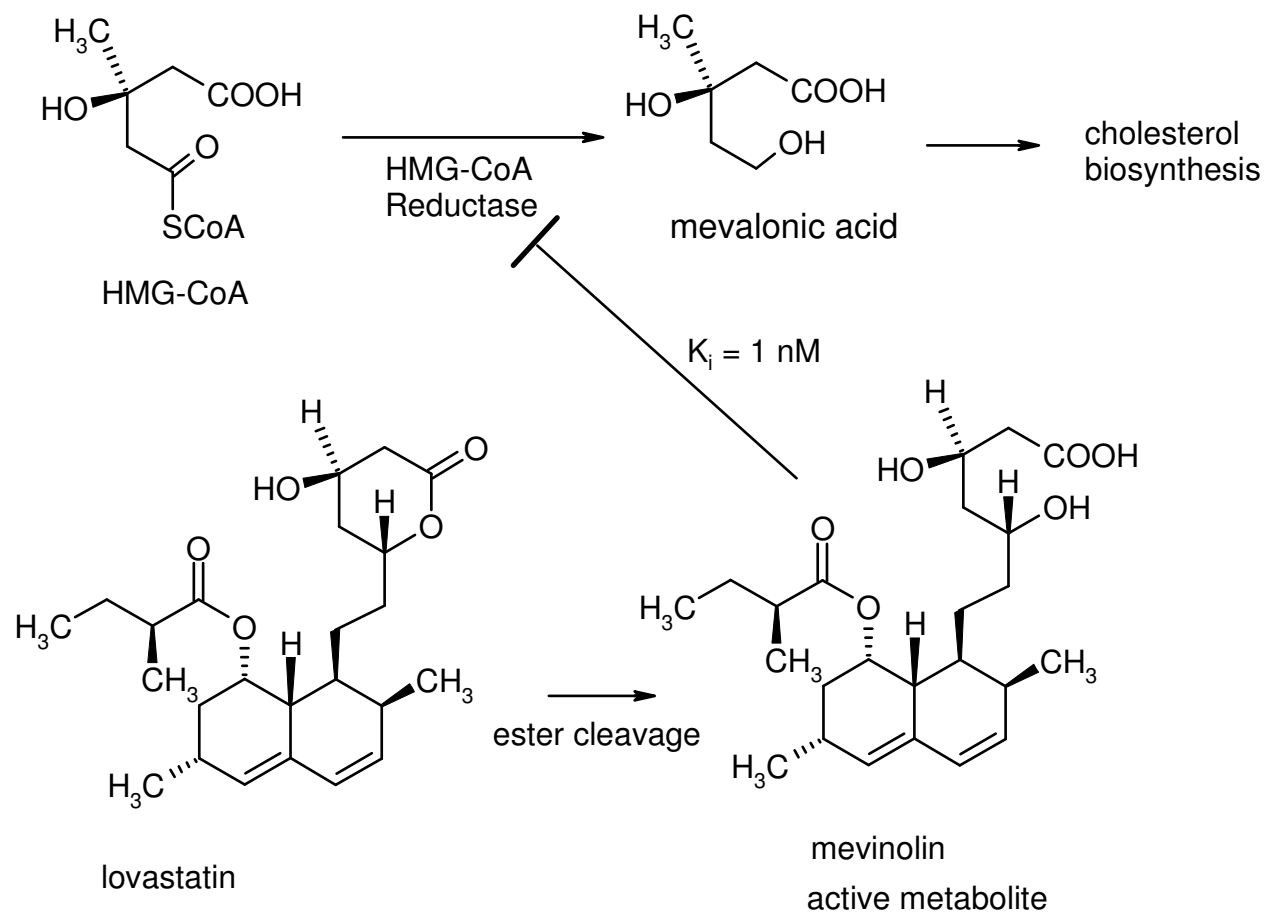


Lit. J.A.Tobert *Nature Rev. Drug Disc.* **2** (2003) 517



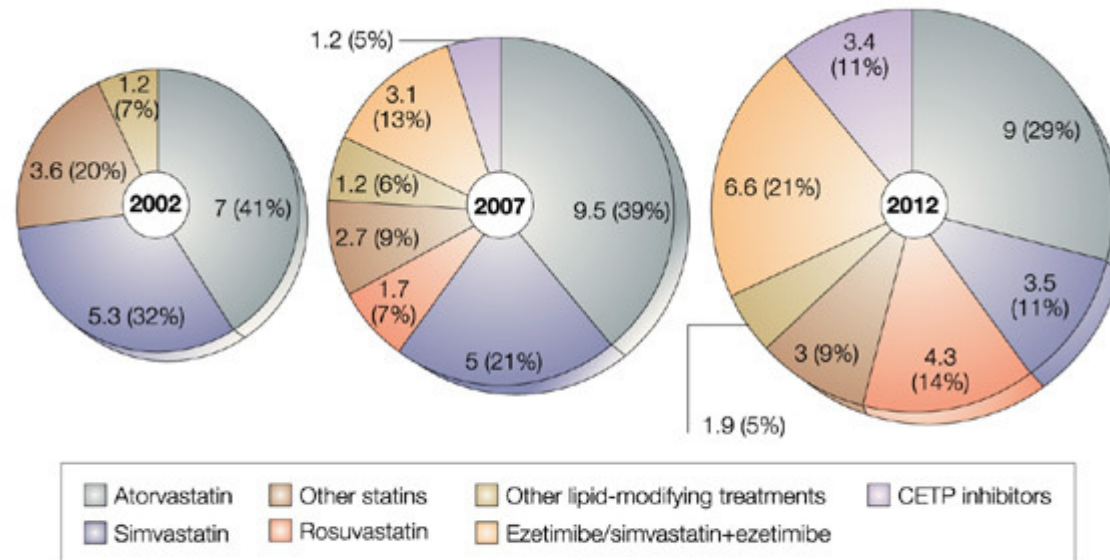
# Inhibition of HMG-CoA reductase (II)

The actually effective substance is the metabolite



# Sales potential of Statins

Market volume of cholesterol reducing agents



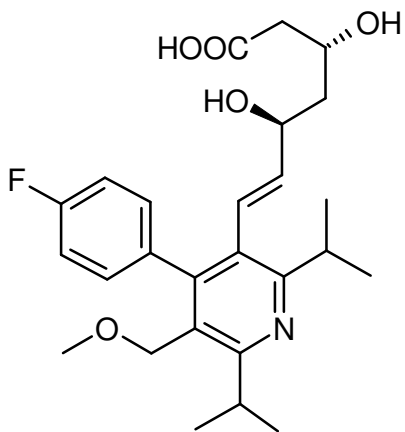
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Turnover in billion US\$ for USA, France, Germany, Italy, Spain, England and Japan, (market volume in %)

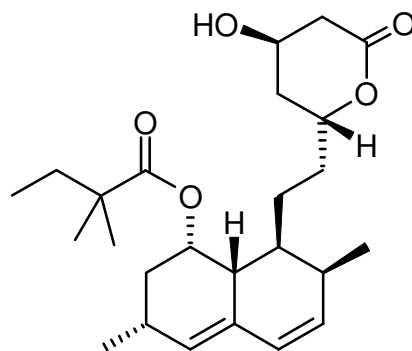
CEPT= cholesteryl ester transferase protein

Lit. J.Quirk et al. *Nature Rev. Drug Disc.* **2** (2003) 769

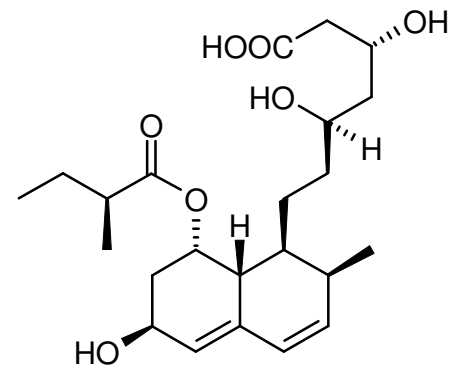
## Further statins



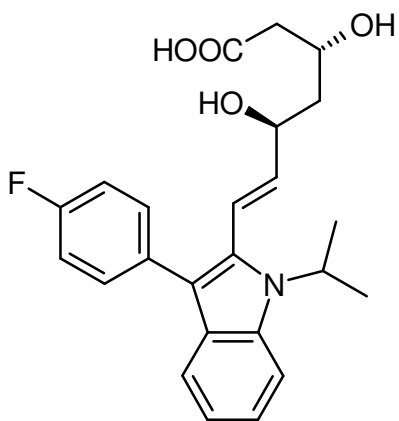
cerivastatin (Bayer)



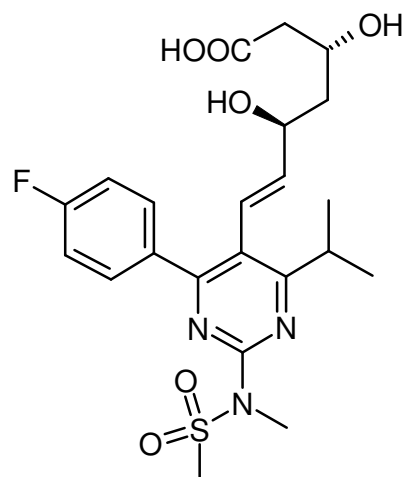
simvastatin (Merck & Co)



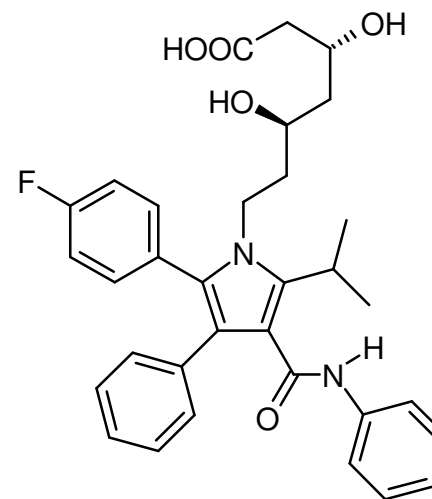
pravastatin (Sankyo)



fluvastatin (Sandoz)



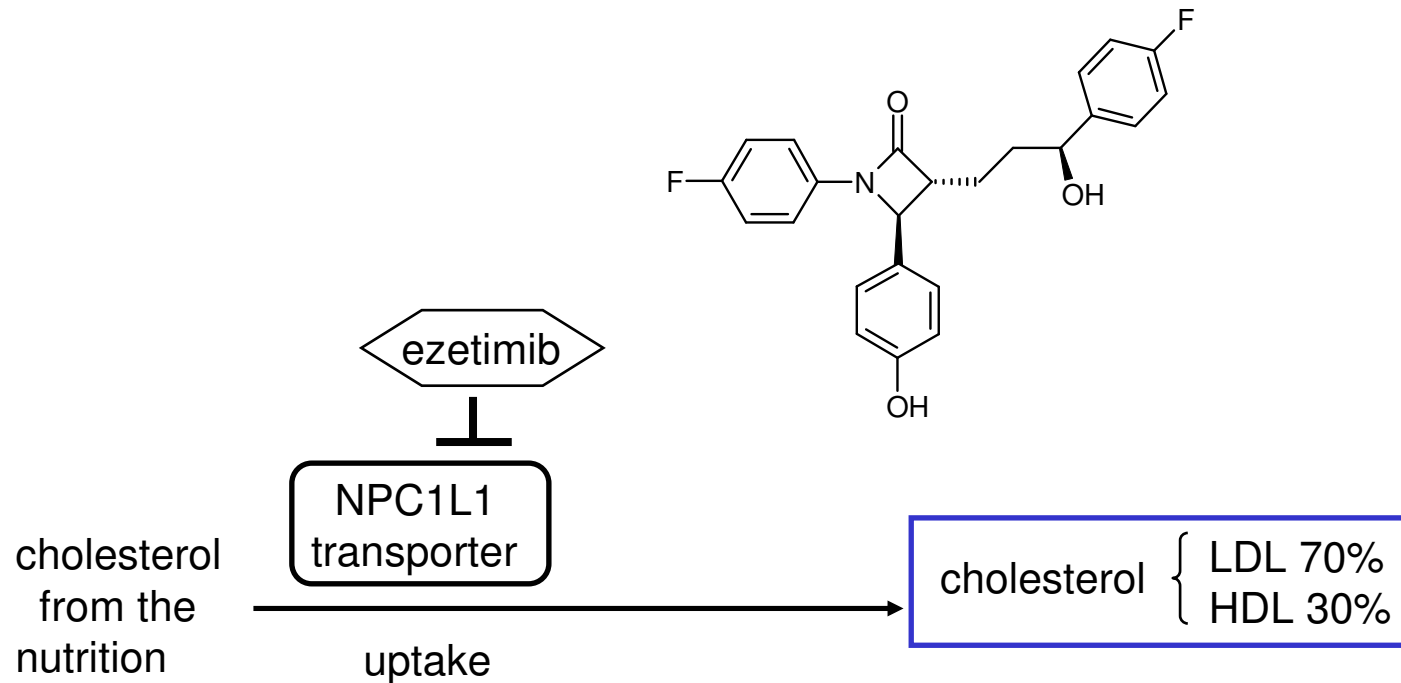
rosuvastatin (Astra-Zeneca)



atorvastatin (Warner-Lambert)

# Further lipid lowering agents (I)

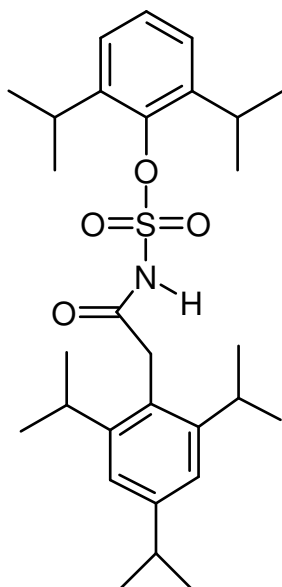
ezetimib inhibits the cholesterol transporter



Lit. Van Heek *Brit.J.Pharmacol.* **129** (2000) 1748.

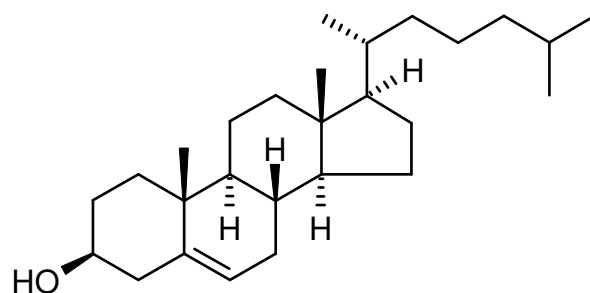
## Further lipid lowering agents (II)

avasimibe inhibits the acetyl-coenzyme-A-cholesterol-acetyltransferase (ACAT-inhibitor)

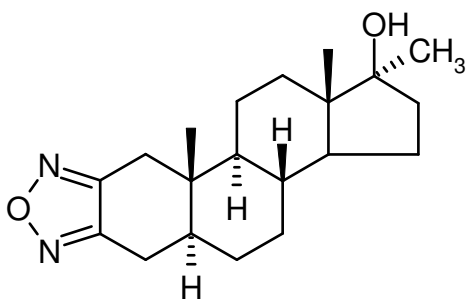


# Further lipid lowering agents (III)

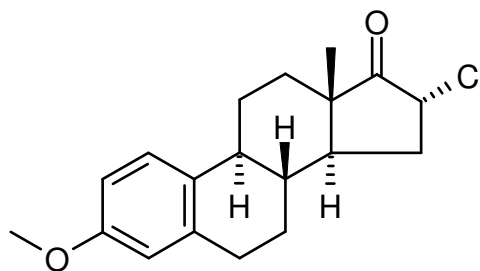
competitive cholesterol analogs



cholesterol



furazabol

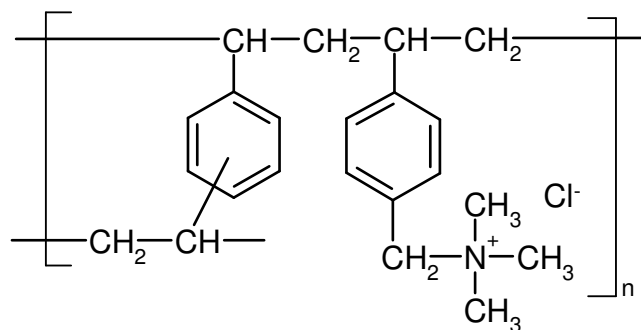


clomestrone

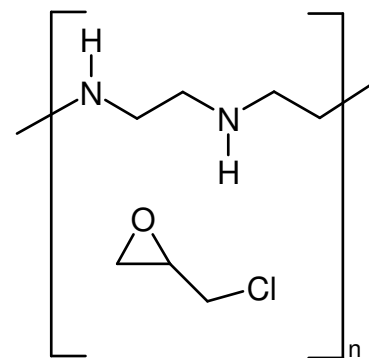
# Further lipid lowering agents (IV)

Bile acid sequestrants

Polymers that are not absorbed from the intestine



cholestyramine  $MW > 10^6$

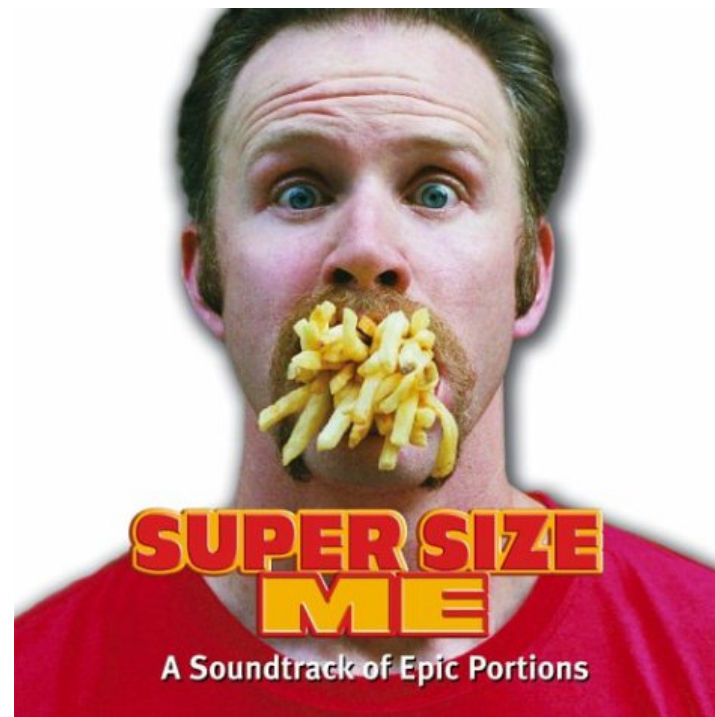
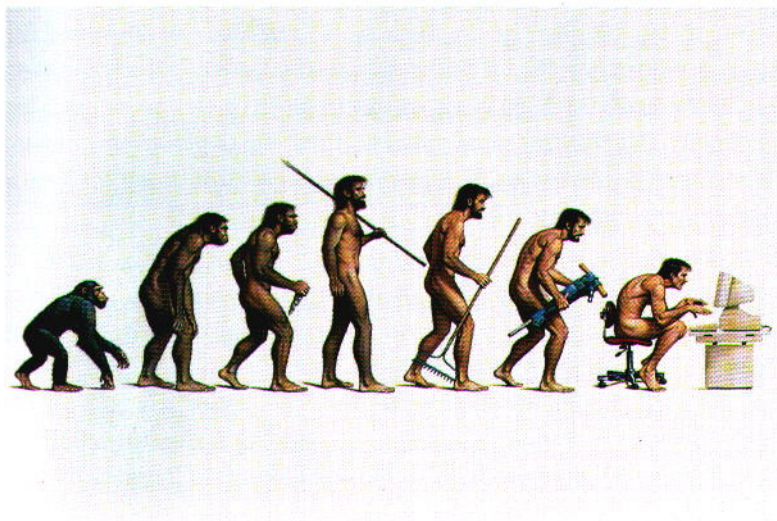


colestipol

absorb cholesterol and bile acid and therefore prevent uptake of cholesterol

# Opinion drugs vs. life style modification

„obesity is a form of depression in which the eating is an antidepressant“

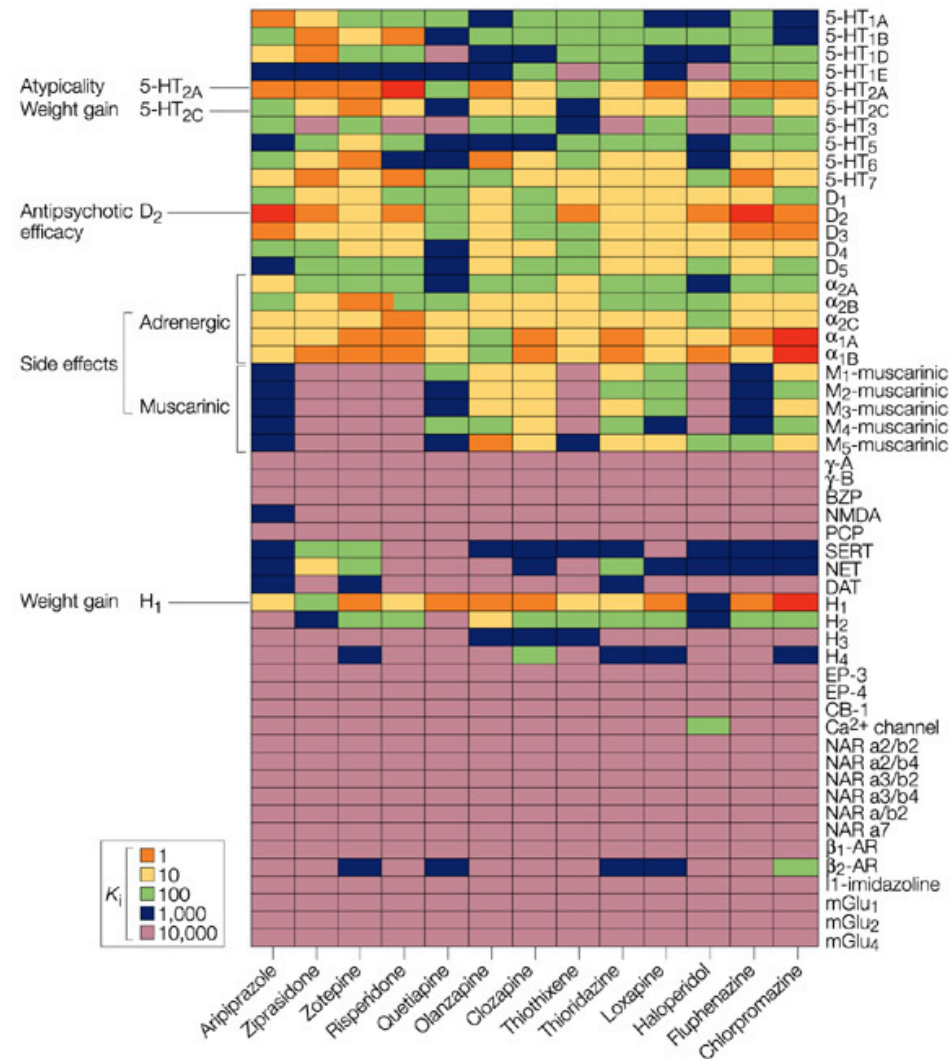


Fat storage is most efficient to preserve energy



# Anorexic drugs (I)

Due to their complex affinity profile regarding a whole series of receptors („dirty drugs“) psychoactive drugs also modify the eating behaviour



Lit. B.L.Roth et al.  
*Nature Rev. Drug Disc.* **3** (2004) 353.

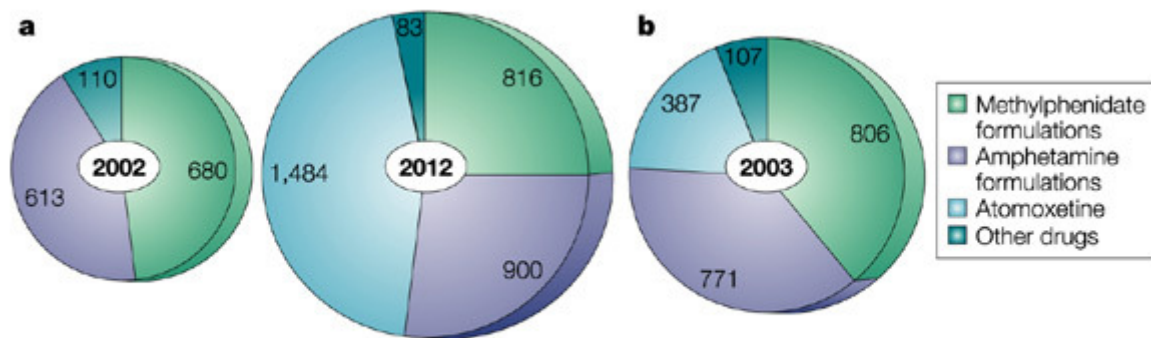
## Anorexic drugs (II)

Prominent examples of psychoactive drugs with mit appetite suppressant (side-) effect:

methylphenidate (Ritalin®)      ADHD

atomoxetine (Strattera®)      [Aufmerksamkeitsdefizitsyndrome]

fluoxetine (Prozac®)



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Market volume of ADHD pharmacological formulations in million US \$

Lit. M.Garland, P.Kirkpatrick *Nature Rev. Drug Disc.* **3** (2004) 385.