

# Complex Diseases, Success and Failure

Finding the „right“ target → valid targets

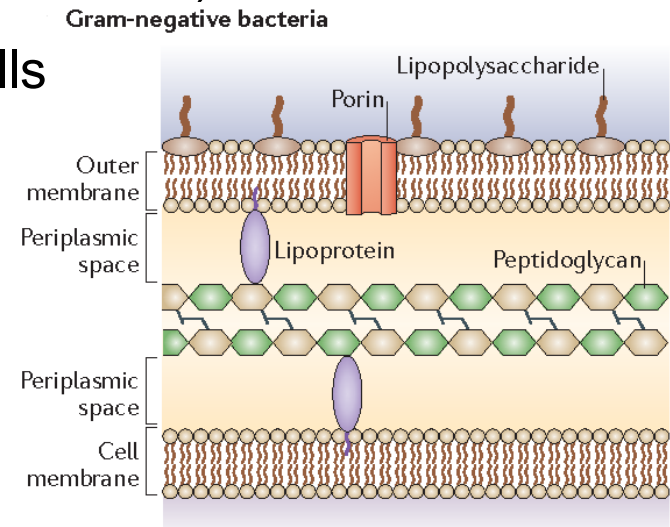
which constraints are limiting factors?

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)

Biological barriers: e.g. bacterial cell walls

Picture source: N.L.Brown et al.  
Nature Rev. Biology (2015)  
DOI:10.1038/nrmicro3480

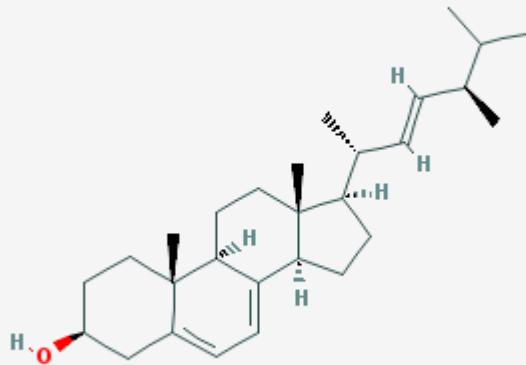


# Success

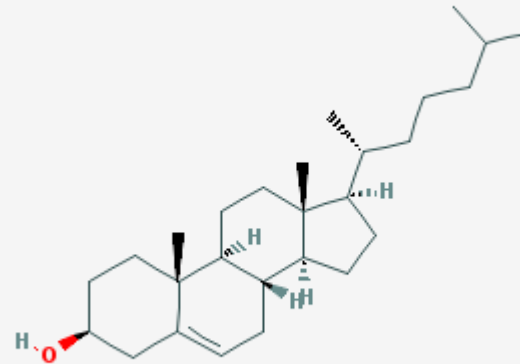
## Antifungals

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).



ergosterol

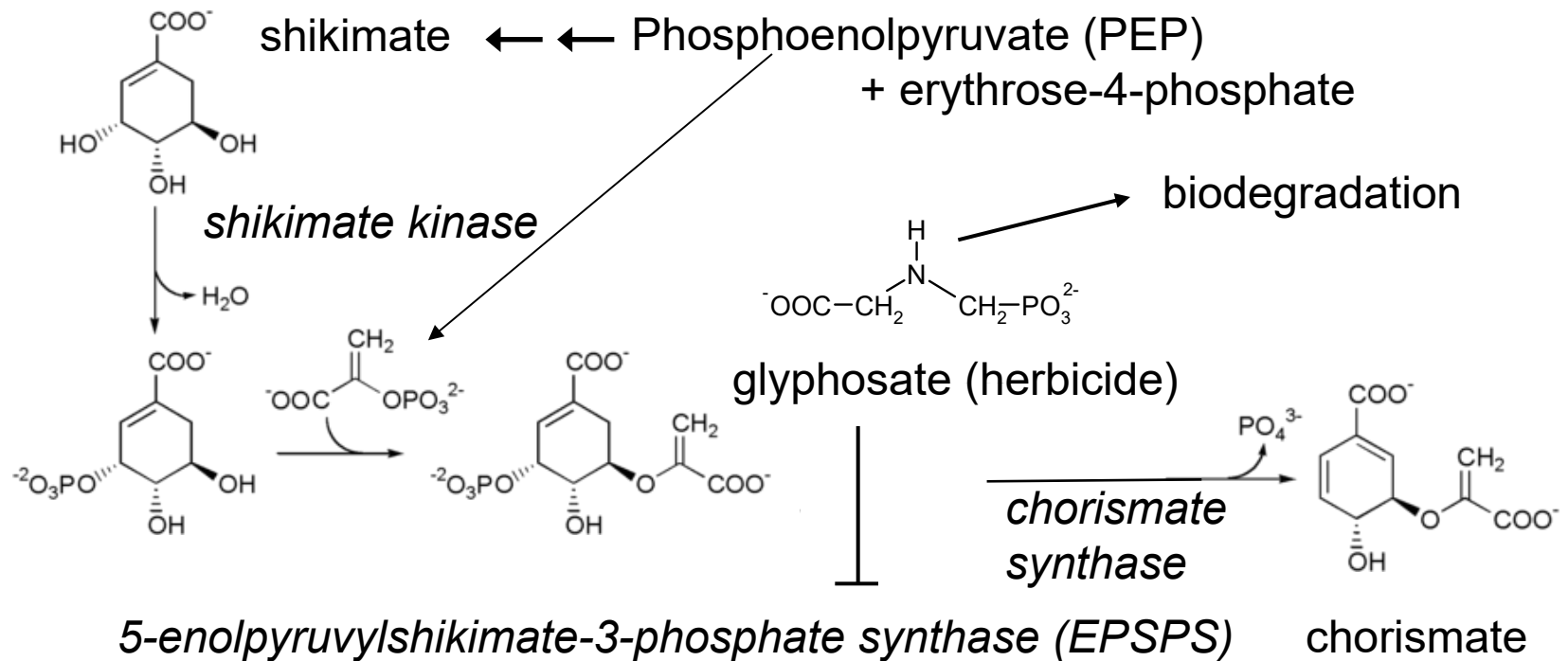


cholesterol

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

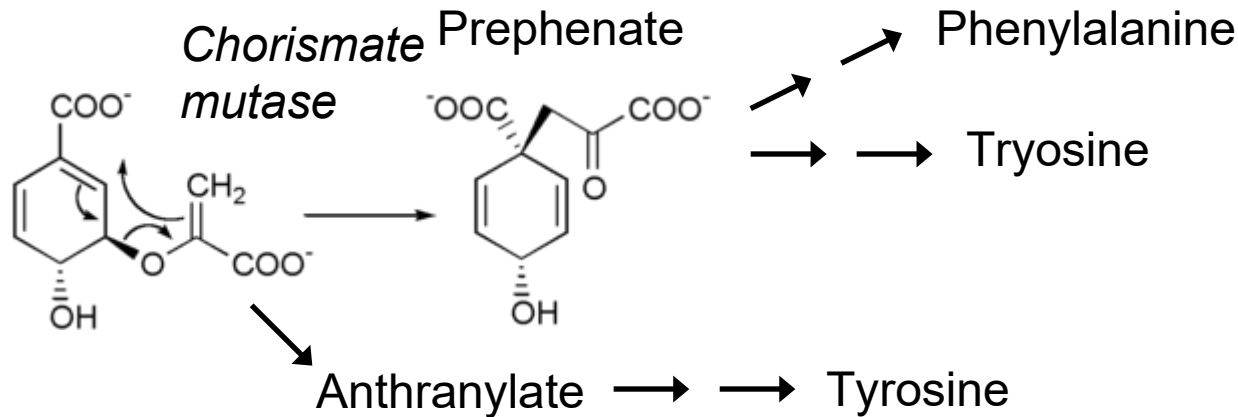
# Failure (so far) (I)

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:

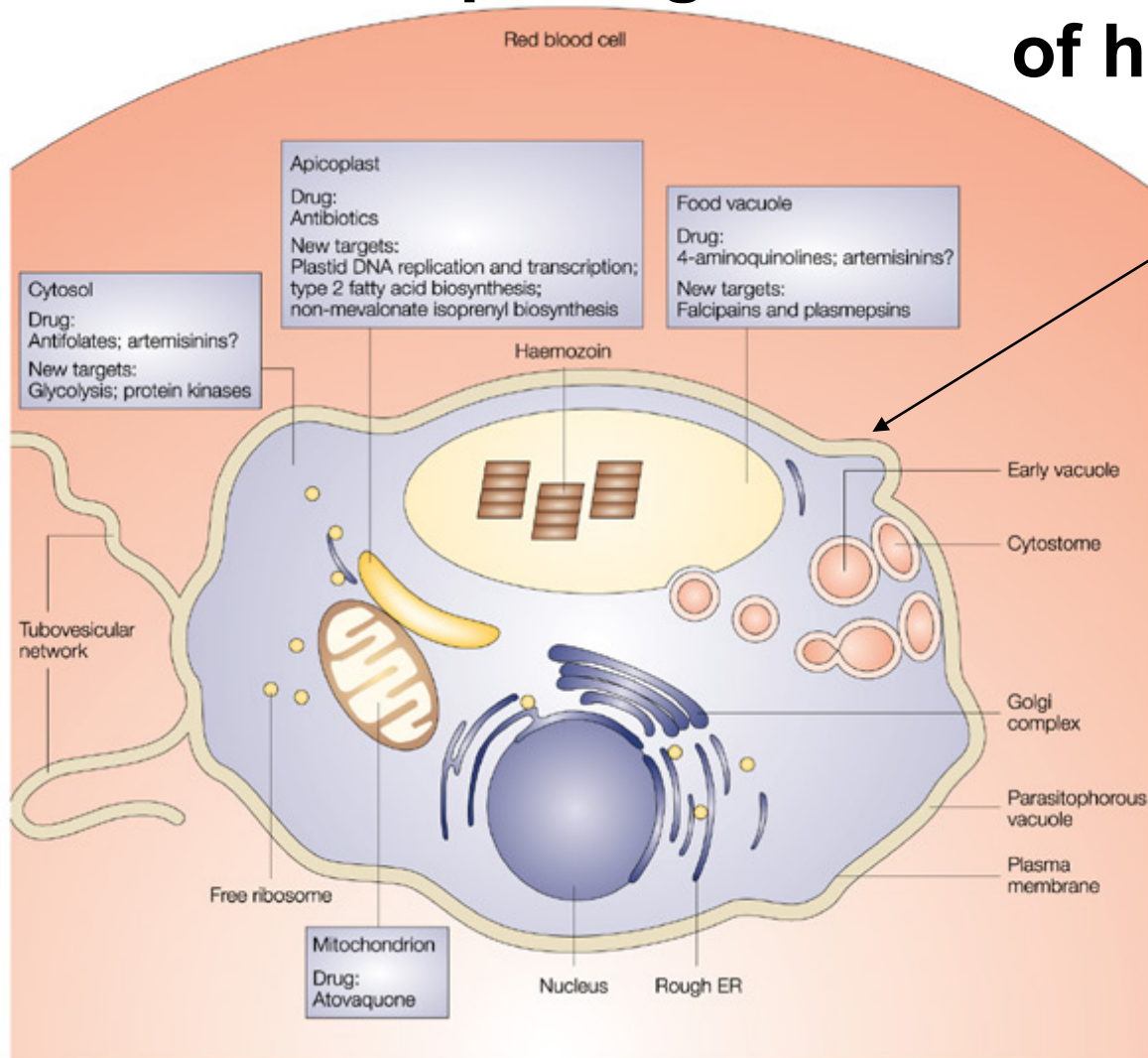
Strong fever, anemia, acidosis,  
multiple failure of organs



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:

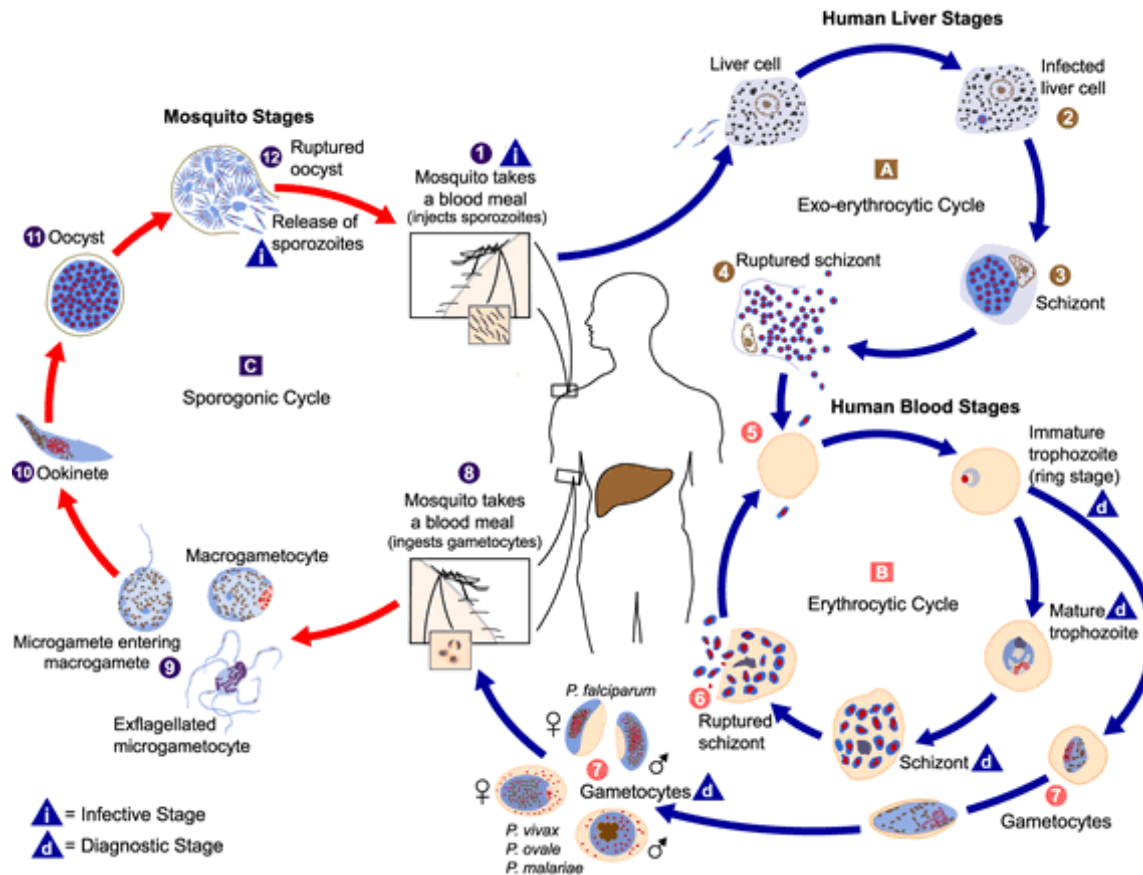
*P. vivax*

*P. malariae*

*P. ovale*

and about 56 more species of *Plasmodium*

# Lifecylce of the malaria pathogens



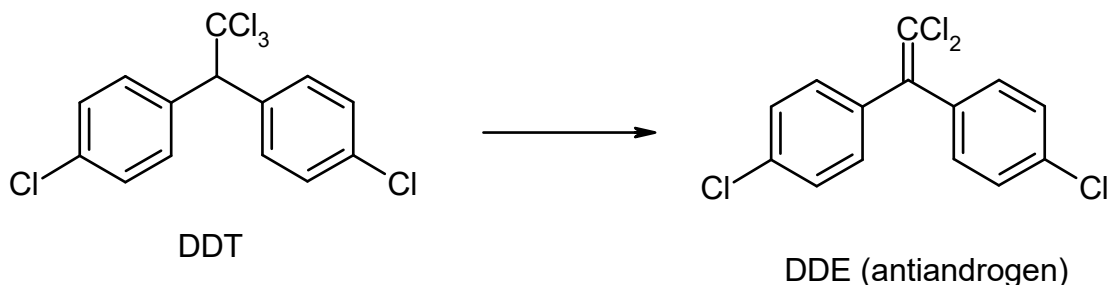
source: [http://www.dpd.cdc.gov/.../body\\_Malaria\\_page1.htm](http://www.dpd.cdc.gov/.../body_Malaria_page1.htm)

# 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:

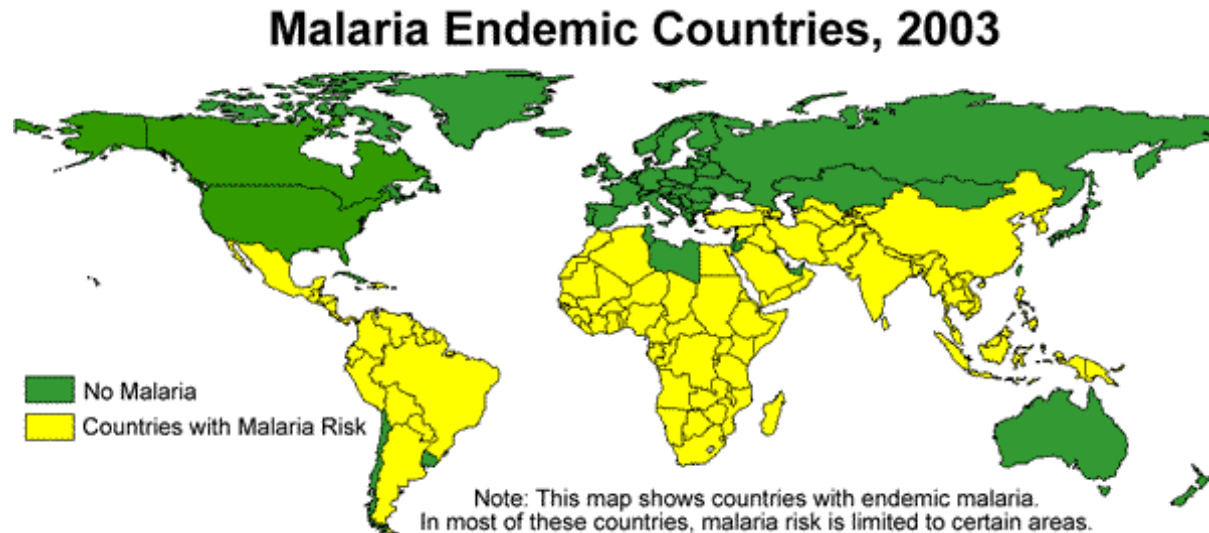
- Accumulation of DDT in the adipose tissue [Fettgewebe] of all creatures (mammals, birds, fish)
- DDT is biologically (almost) undegradable
- Metabolismus leads to a neurotransmitter-like substance (acts as contact insecticide !)



- Increasing resistance to DDT has been observed



# Distribution of Malaria (I)



Areas with risk of malaria

# Distribution of malaria (II)

Distribution of actual and potential malaria transmission stability

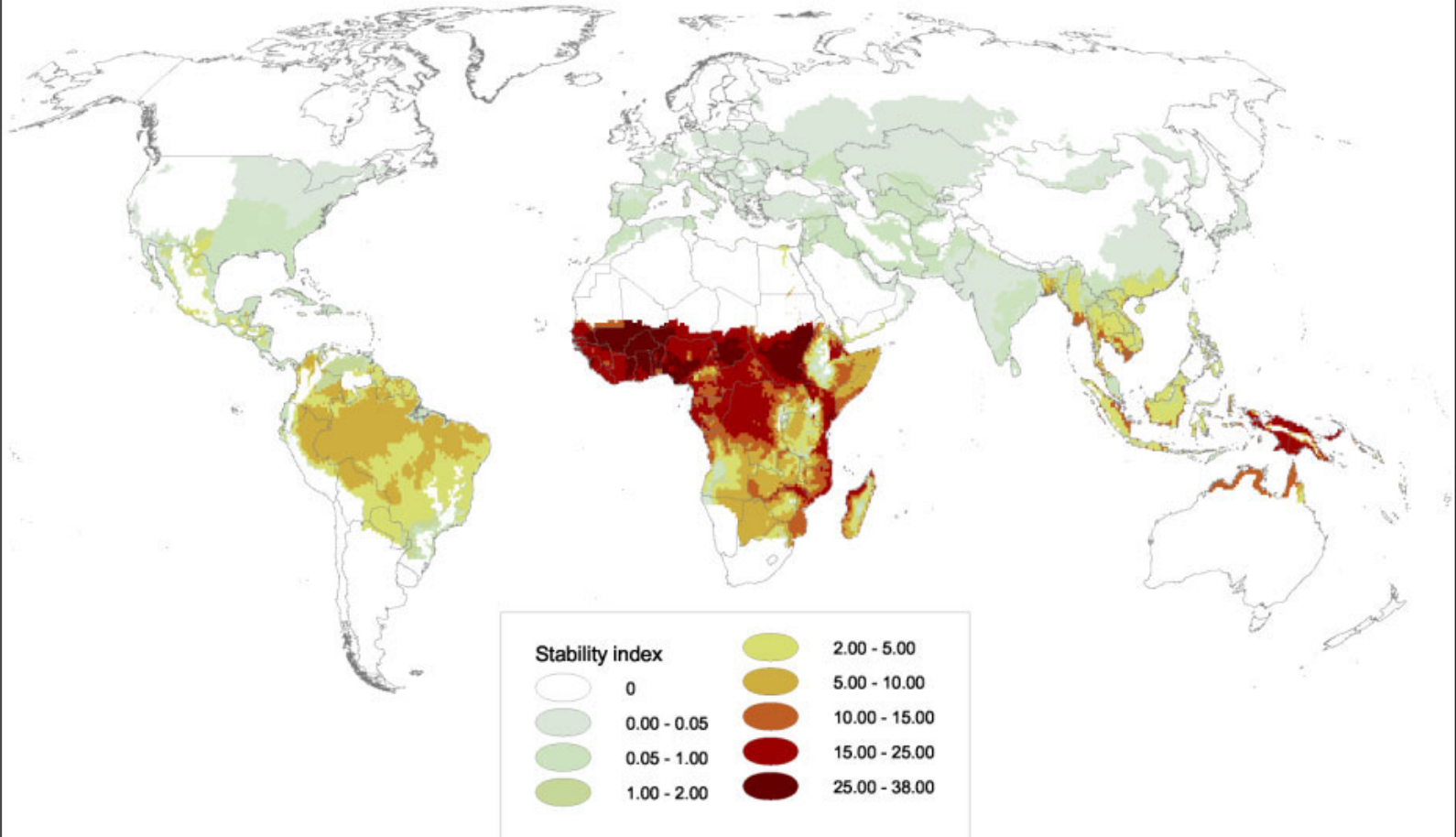
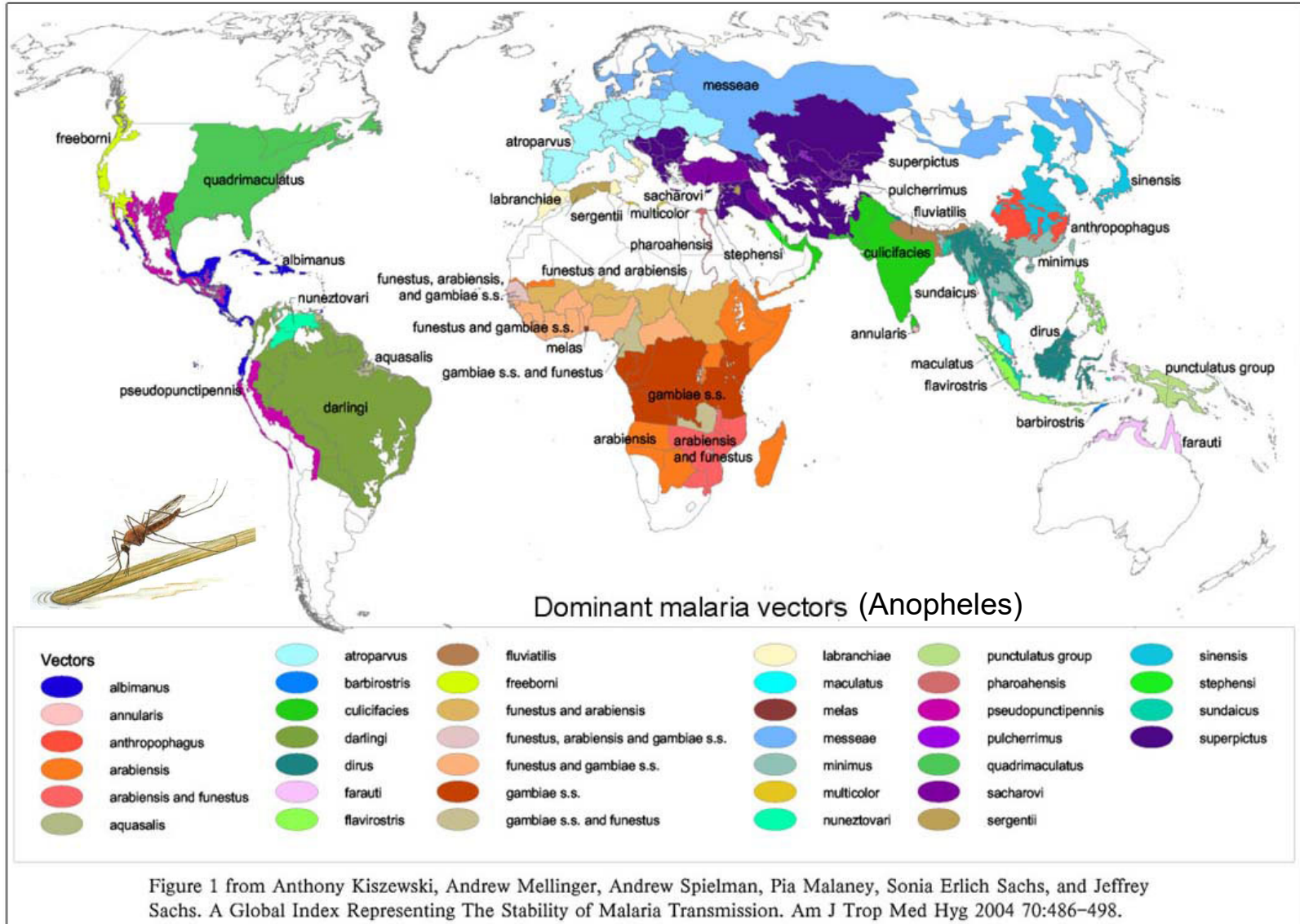


Figure 1 from Anthony Kiszewski, Andrew Mellinger, Andrew Spielman, Pia Malaney, Sonia Erlich Sachs, and Jeffrey Sachs. **A Global Index Representing The Stability of Malaria Transmission.** *Am J Trop Med Hyg* 2004 70:486-498.

# Distribution of the Anopheles fly



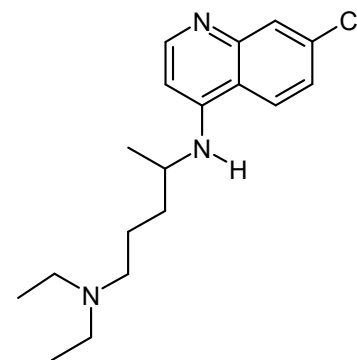
# Approaches to controlling (II)

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

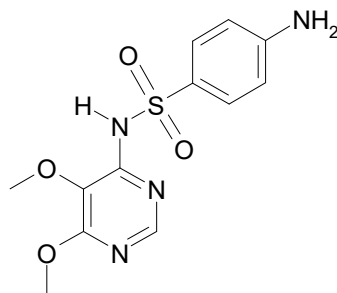
mode of action (still partly unclear):

binds to HEM groups

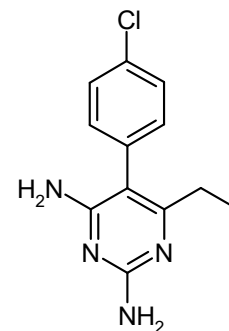
inhibition of the glutathion-S-transferase



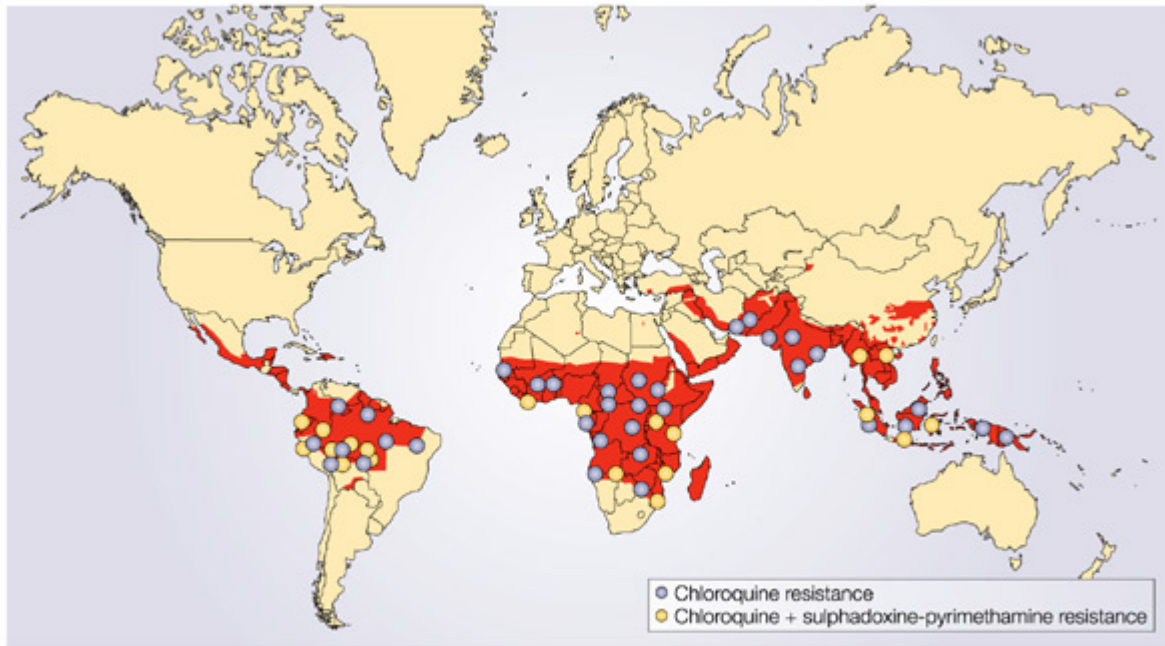
sulfadoxine  
antibacterial



pyrimethamine  
blocks the dihydrofolate reductase  
respectively the dihydropterate synthetase



# Resistance of the Anopheles fly

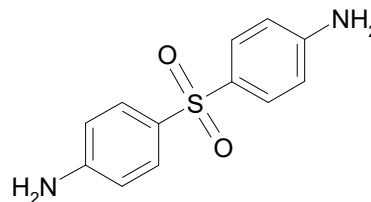
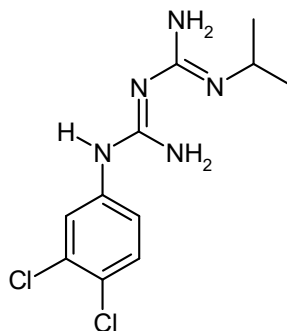
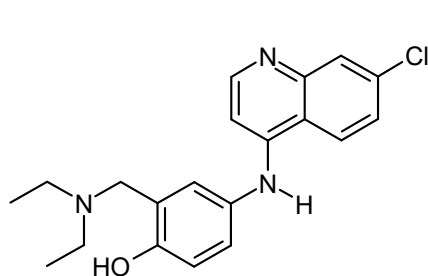


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red: areas with malaria

# Approaches to controlling (III)

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



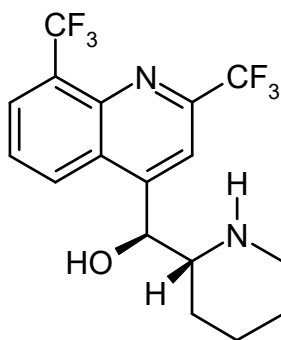
Disadvantage: expected build up of resistances due to identical targets

# 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®)

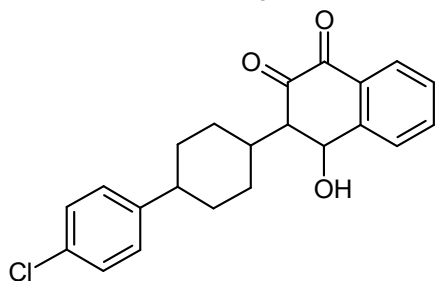


Mode of action due to interaction with phospholipids (cell membrane, fatty acid synthesis)

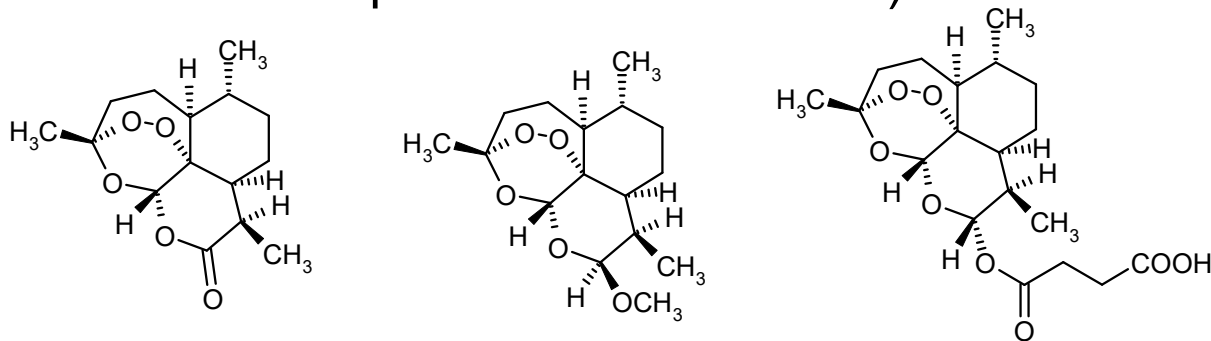
Only very few adverse effects

# 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: rapid metabolism 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	Pyrimethamine, proguanil	Chlorproguanil	82,83
	Glycolysis	Dihydropteroate synthase	Sulphadoxine, dapsone		
		Thymidylate synthase		5-fluoroorotate	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	Plasmepsins		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 biosynthesis	FabH		Thiolactomycin	29
		FabI/PfENR		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; PfENR, *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

The first malaria vaccine Mosquirix (recombinant protein viruslike particle) has been endorsed by the WHO in October 2021.

# **(New) malaria drugs and targets (as of 2018)**

## **Target**

Fe(II)protoporphyrin IX

Fe(II)protoporphyrin IX

Ferredoxin-NADPH reductase

Dehydroorotate dehydrogenase

Dehydroorotate dehydrogenase

Phosphatidylinositol-4 kinase

Glutathione S-transferase

Glutathione S-transferase

Mitochondrial Elongation Factor G

Ca<sup>2+</sup> transporting P-ATPase 4

## **Drug**

mefloquine

primaquine

tafenoquine (approved)

atovaquone

DSM265 (phase II)

MMV390048 (phase II)

artesunate

artefenomel (phase II)

M7517 (phase I)

cipargamin (phase II)

# 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*.

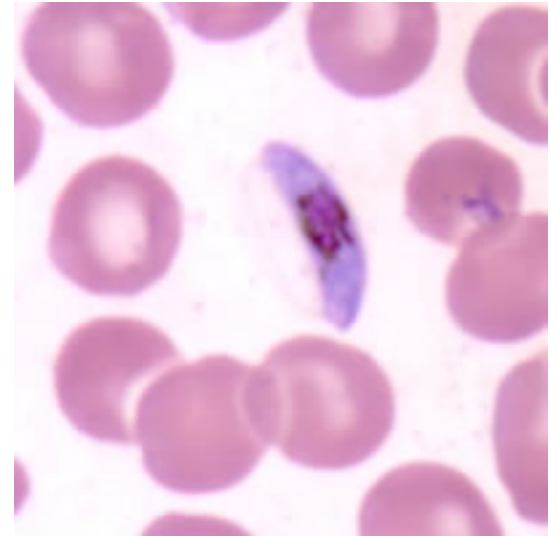
<http://www.ncbi.nlm.nih.gov/Malaria/>

<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, ancylostomiasis  
infection by soil transmitted helminths (worms)

Schistosomiasis (snail fever, bilharzia)

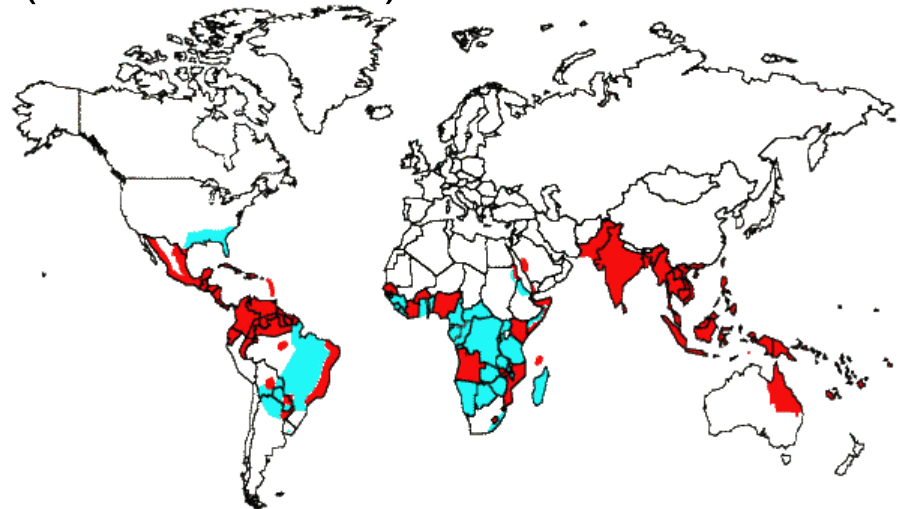
Trachoma and onchocerciasis (river blindness)

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)

Rabis [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]



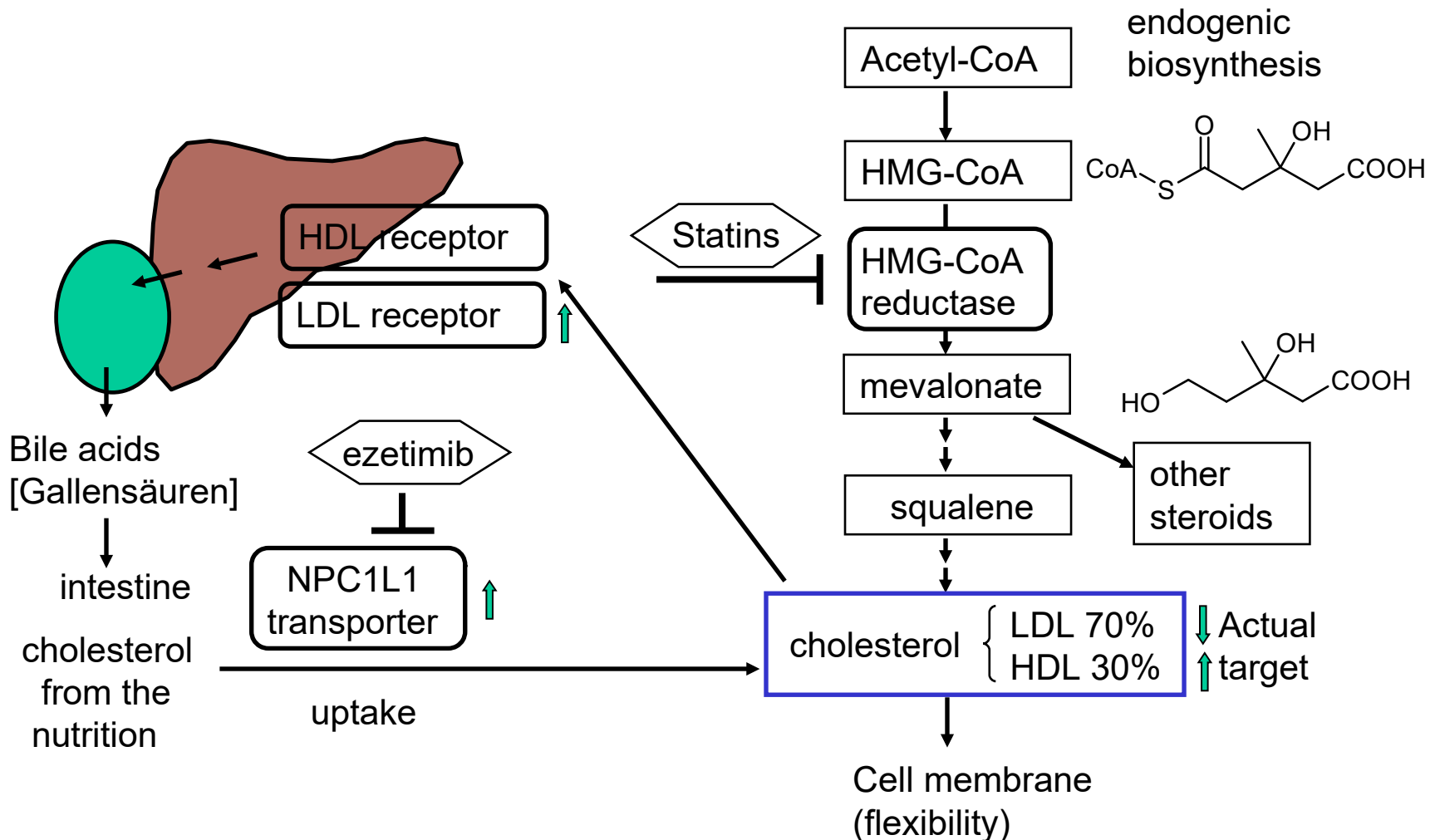
typical symptoms:

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

} increased  
cardiovascular  
risk

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

# Regulation of the cholesterol pool



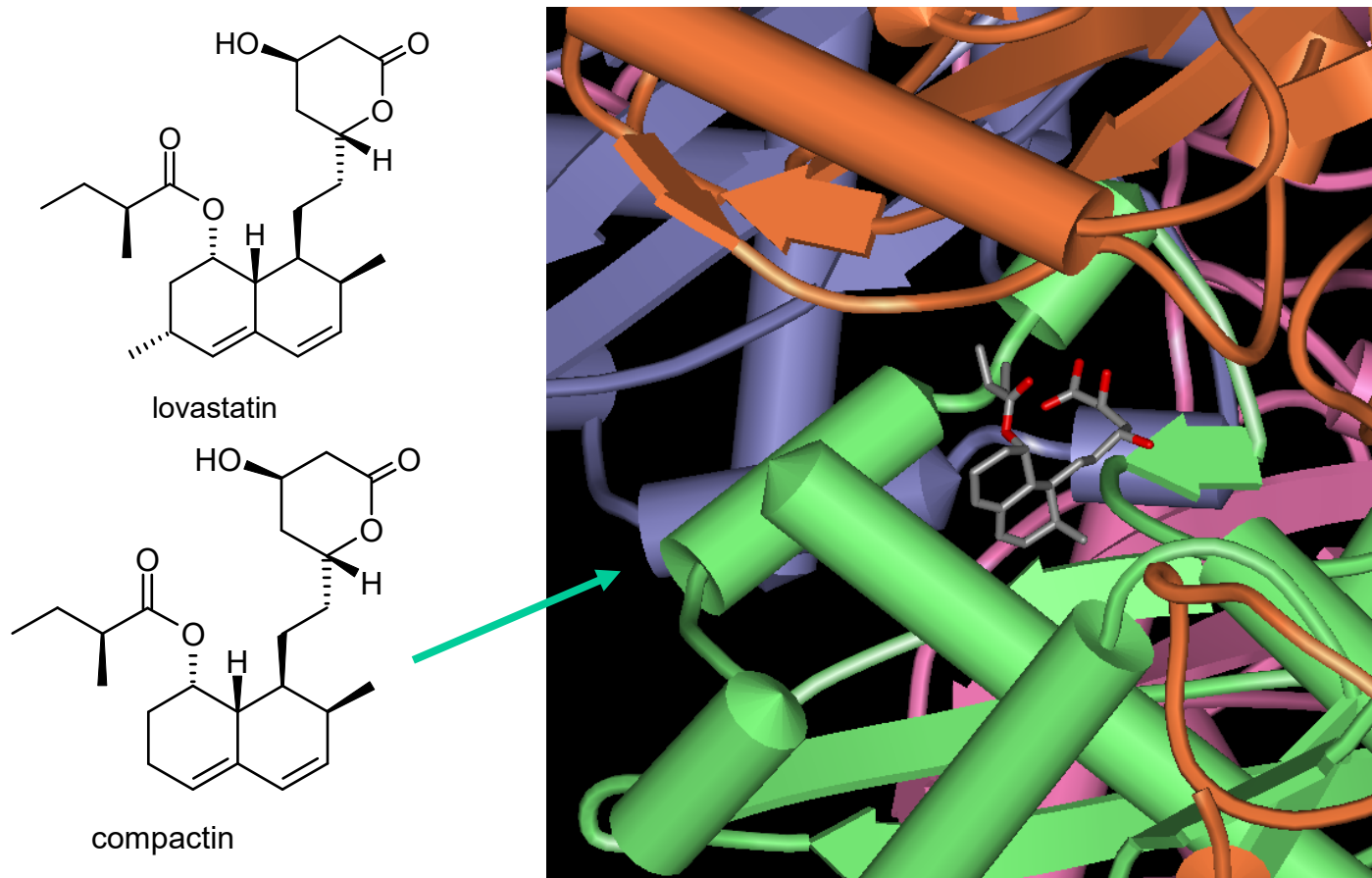
Lit. F.Rinninger & H.Greten *Dtsch. Ärztebl.* **102** (2005) A516

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



# Inhibition of HMG-CoA reductase (I)

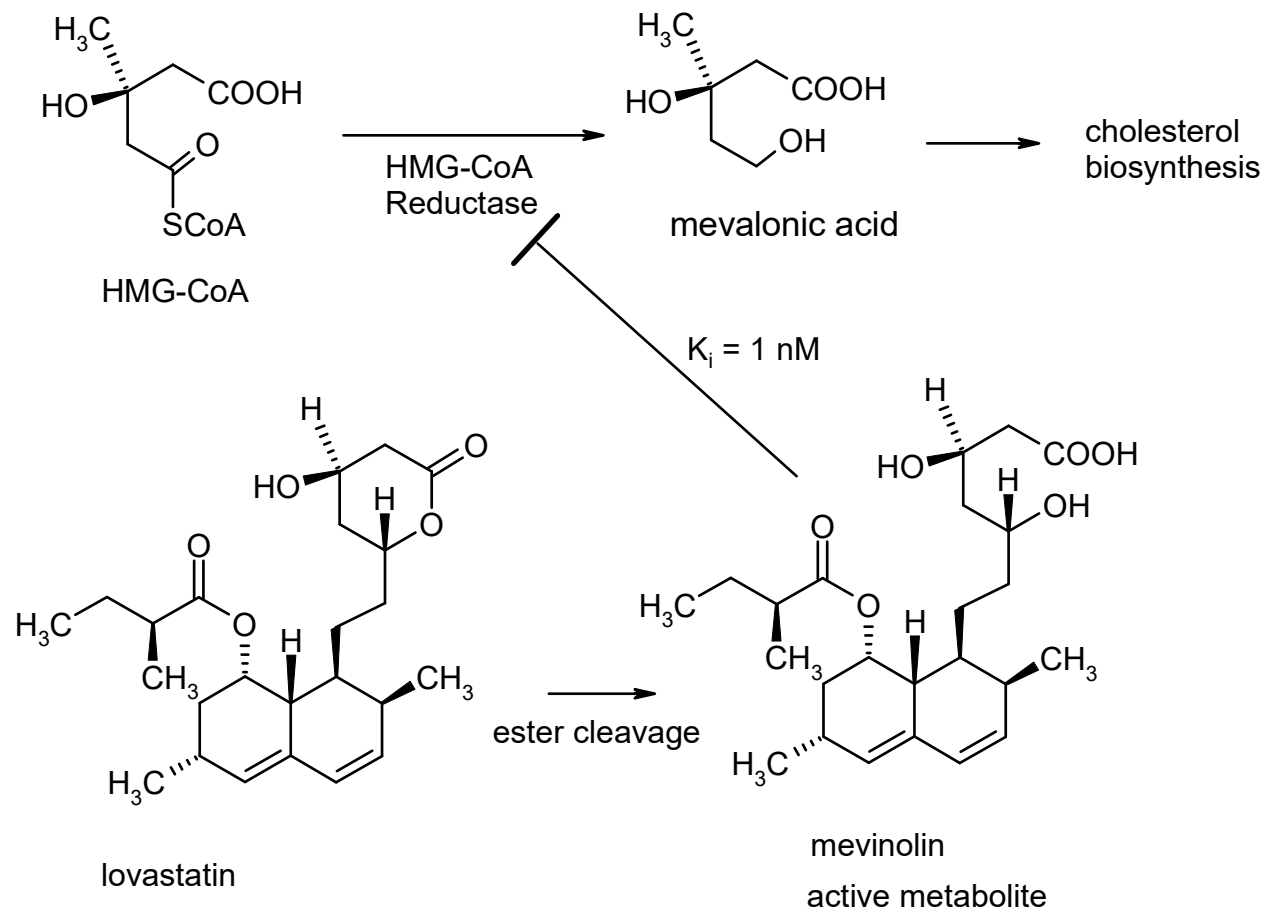
compactin (from *Penicillium citrinum*) and mevinolin (=lovastatin) (from *Aspergillus terreus*) were first found as inhibitors.



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

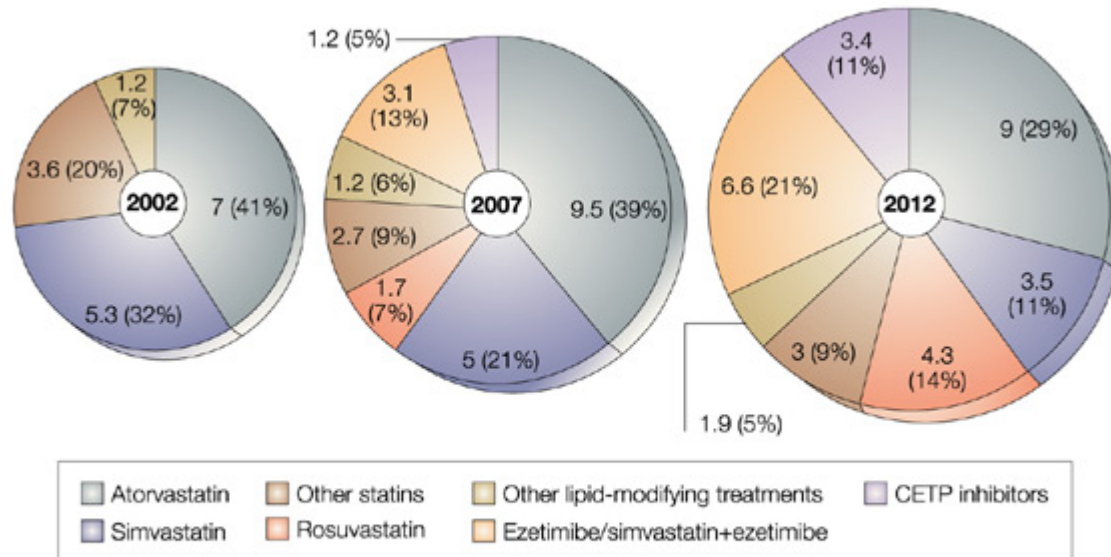
# Inhibition of HMG-CoA reductase (II)

The actually effective substance is the metabolite



# Sales potential of Statins

Market volume of cholesterol reducing agents



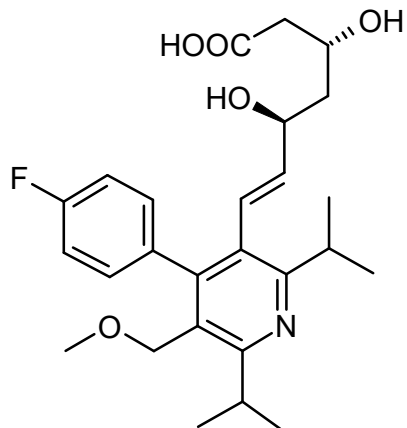
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Turnover in billion US\$ for USA, France, Germany, Italy, Spain, England and Japan, (market volume in %)

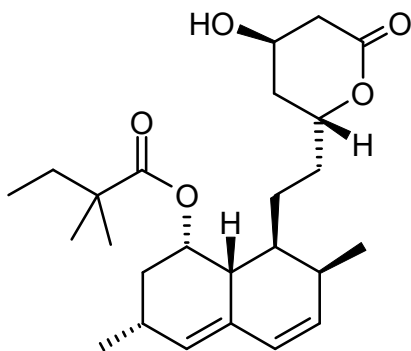
CEPT= cholesteryl ester transferase protein

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

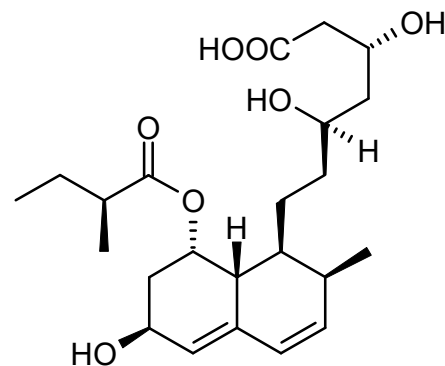
# Further statins



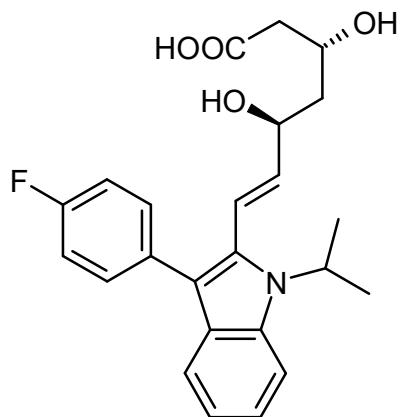
cerivastatin (Bayer)



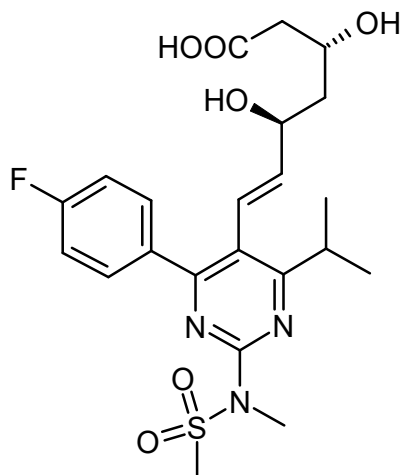
simvastatin (Merck & Co)



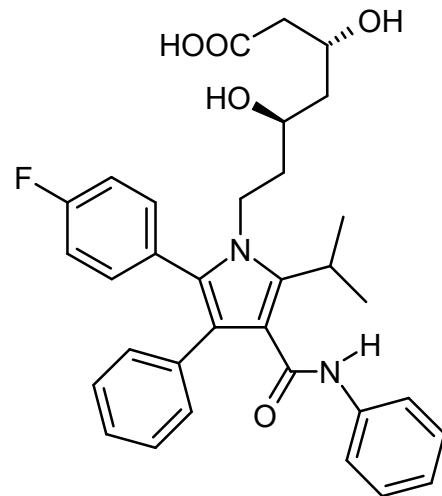
pravastatin (Sankyo)



fluvastatin (Sandoz)



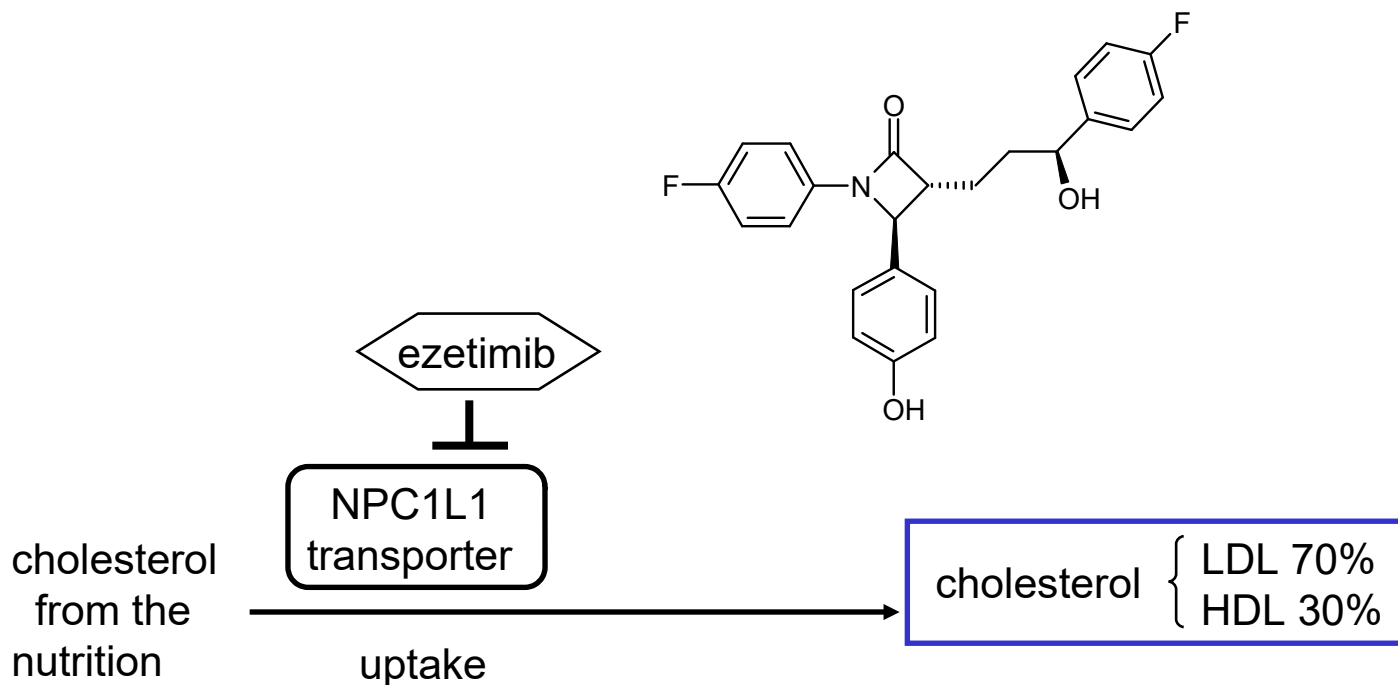
rosuvastatin (Astra-Zeneca)



atorvastatin (Warner-Lambert)

# Further lipid lowering agents (I)

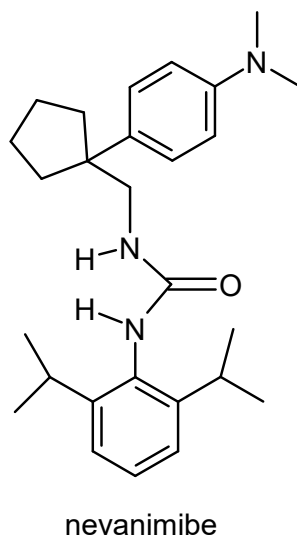
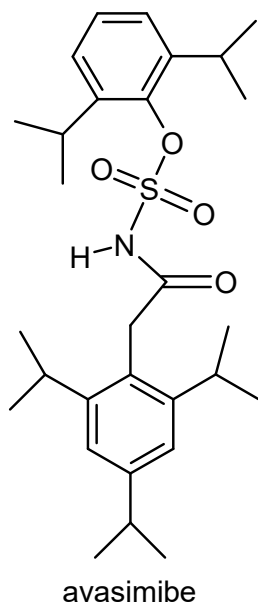
ezetimib inhibits the cholesterol transporter



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

## Further lipid lowering agents (II)

avasimibe and nevanimibe inhibit the acetyl-coenzyme-A-cholesterol- acetyltransferase (ACAT), aiming to prevent cholesterol deposition in the arterial wall.

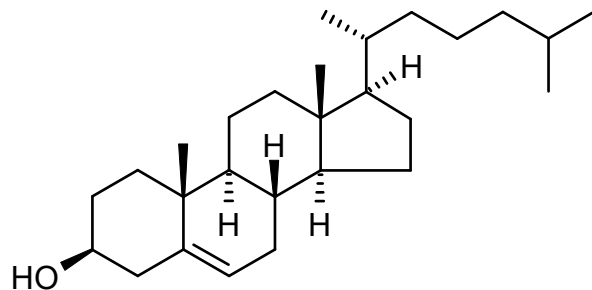


Further inhibitors: canosimibe, eflusimibe, eldacimibe, lecimibe, pactimibe

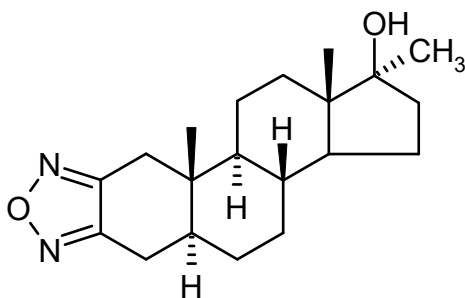
Clinical development of avasimibe was discontinued in 2003, however research for repurposing it as antitumor and antibacterial drug is ongoing.

# Further lipid lowering agents (III)

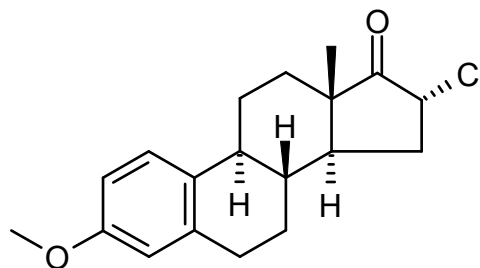
competitive cholesterol analogs



cholesterol



furazabol

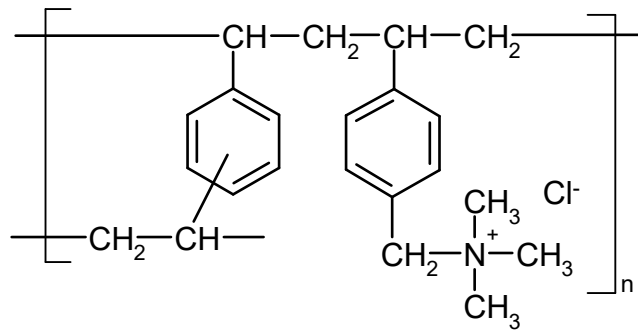
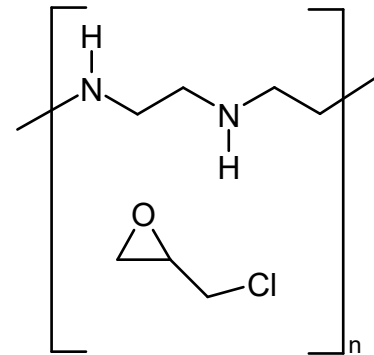


clomestrone

## Further lipid lowering agents (IV)

## Bile acid sequestrants

## Polymers that are not absorbed from the intestine

cholestyramine MW>10<sup>6</sup>

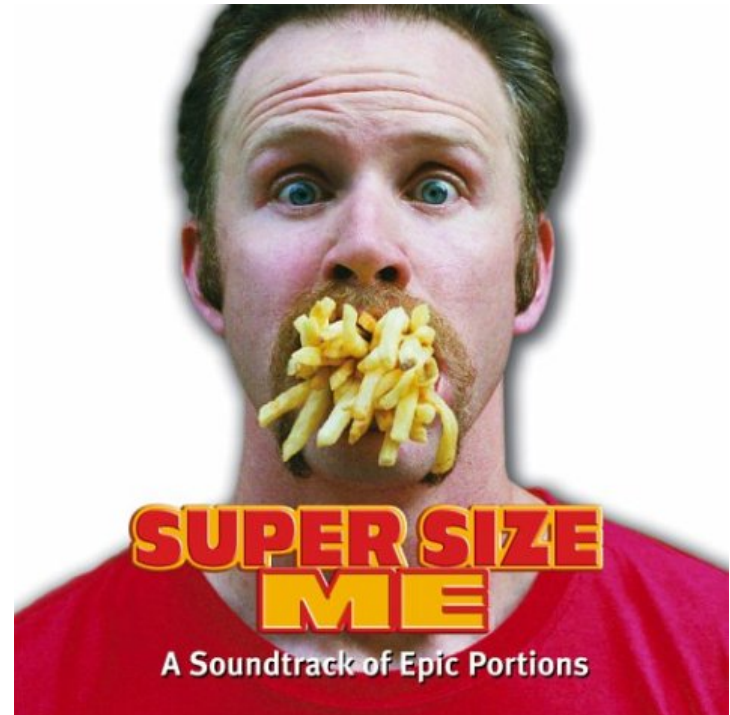
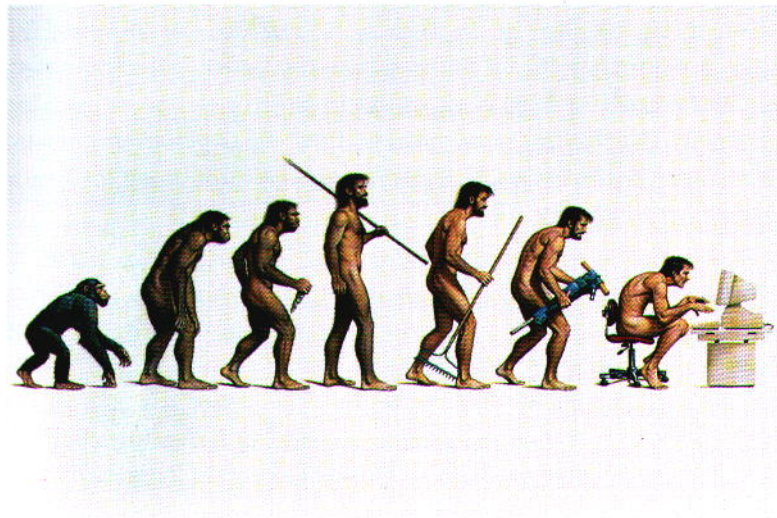
colestipol

absorb cholesterol and bile acid and therefore prevent uptake of cholesterol



# Opinion drugs vs. life style modification

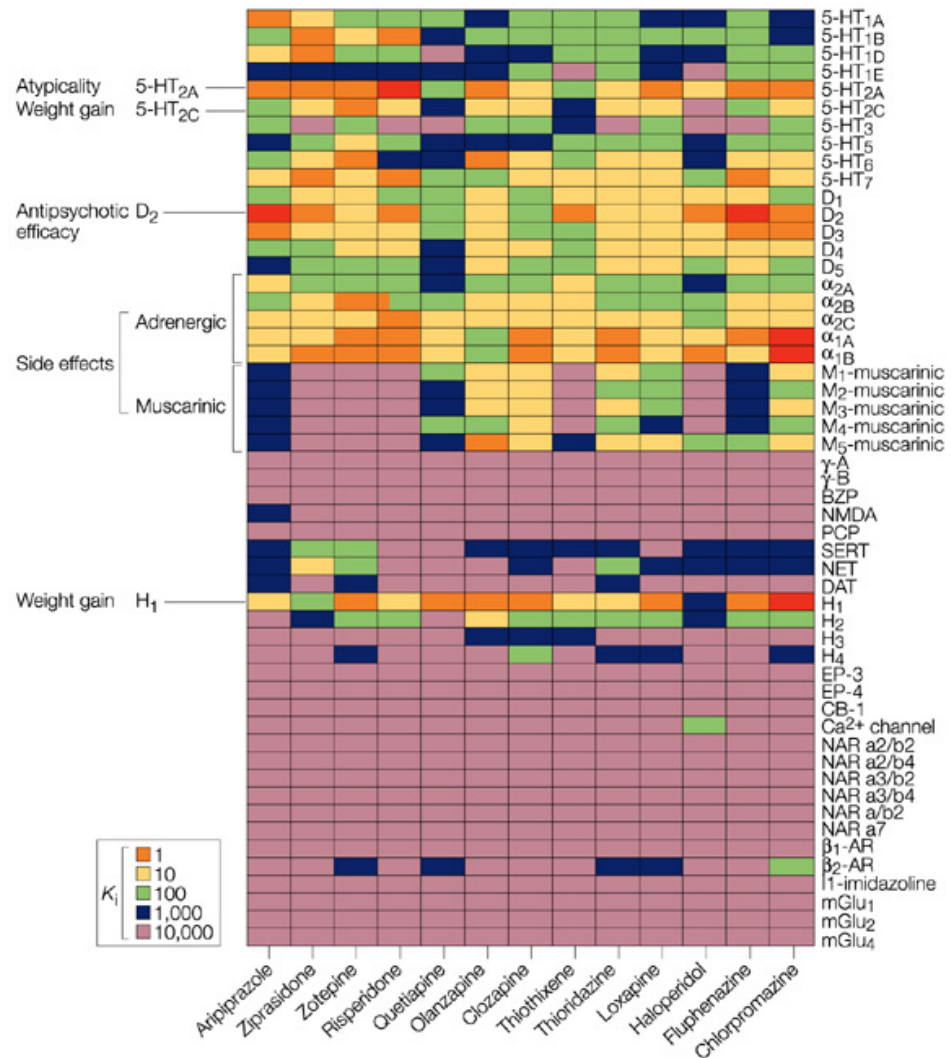
„obesity is a form of depression in which the eating is an antidepressant“



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.

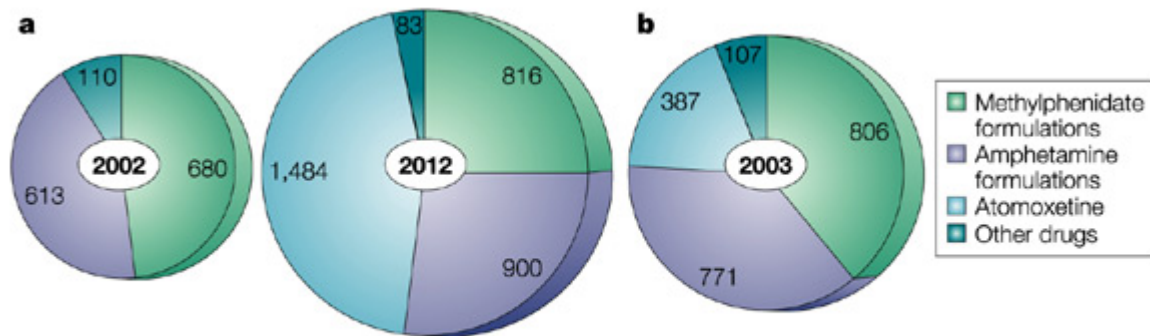
# Anorexic drugs (II)

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

methylphenidate (Ritalin®)      ADHD

atomoxetine (Strattera®)      [Aufmerksamkeitsdefizitsyndrome]

fluoxetine (Prozac®)



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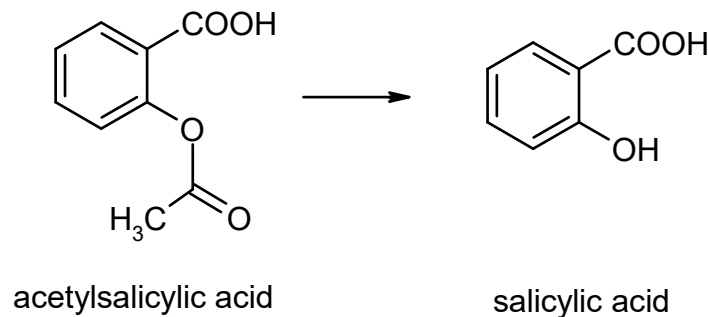
Market volume of ADHD pharmacological formulations in million US \$

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

# Prodrugs

Actually effective substance is the main metabolite of the drug

Example: ester cleavage

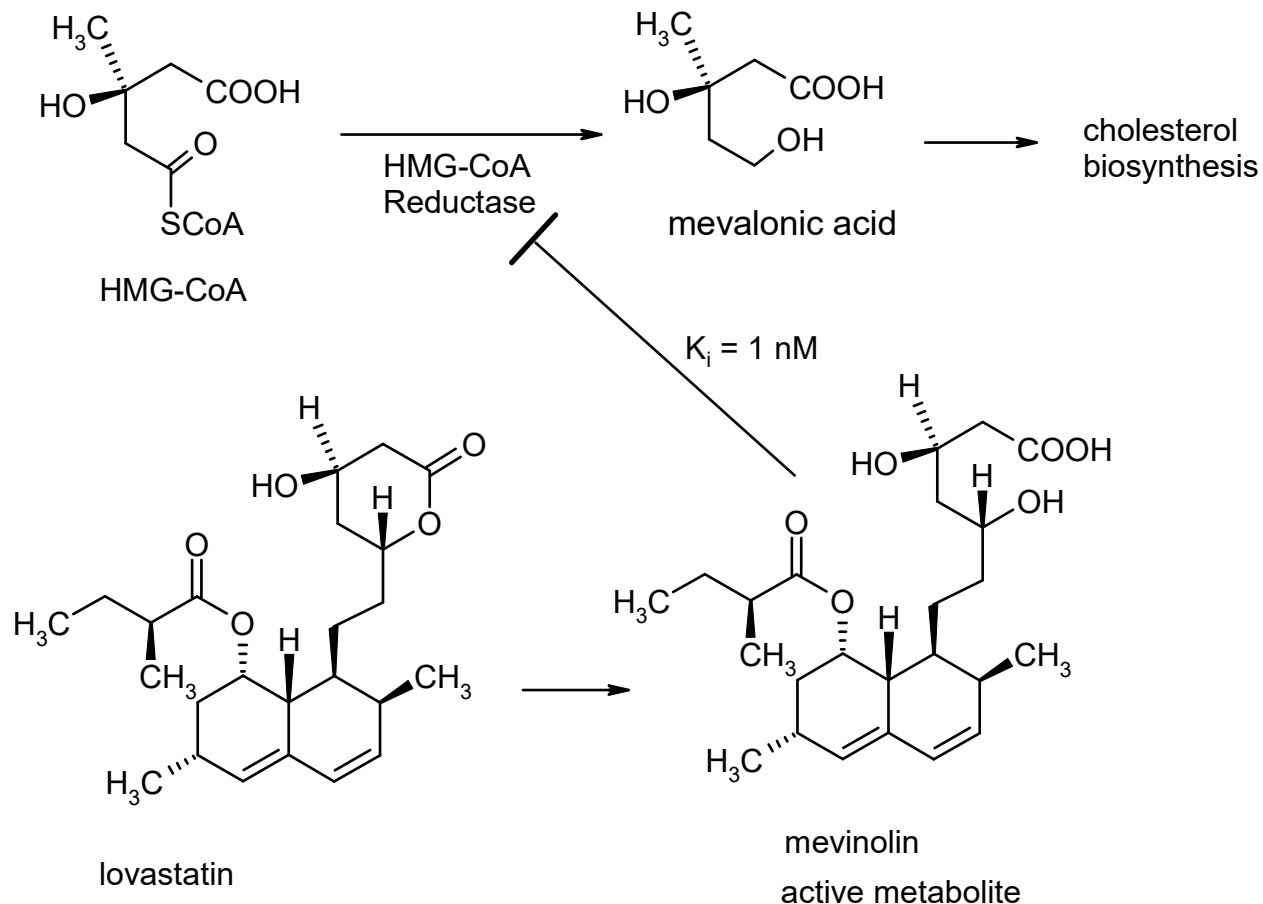


Irreversible inhibitor of cyclooxygenase (COX)

Esters are better absorbed than carbonic acids  
(which are negatively charged at pH 7)

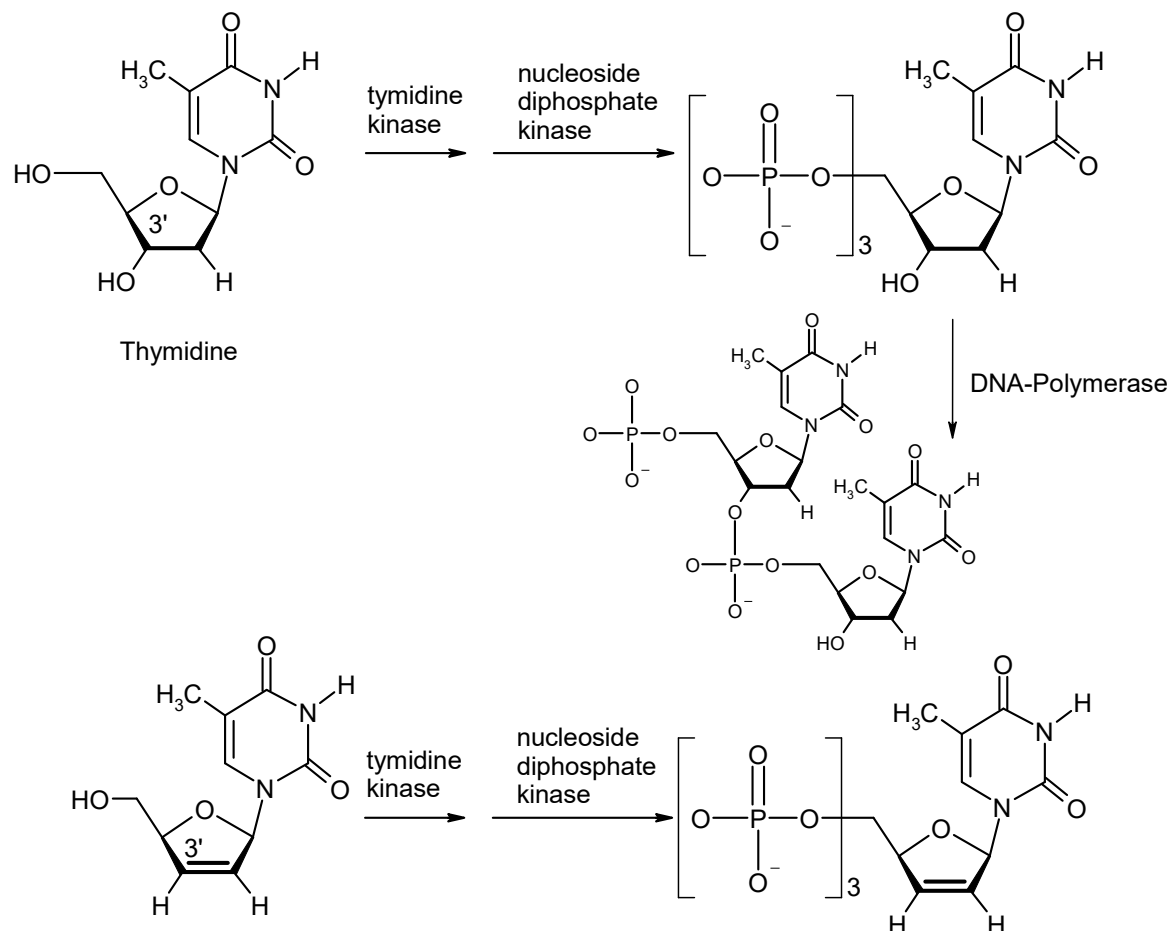
# Statins as HMG-CoA Reductase Inhibitors

The prodrug is a lactone (cyclic ester) whereas its metabolite is the actual inhibitor.



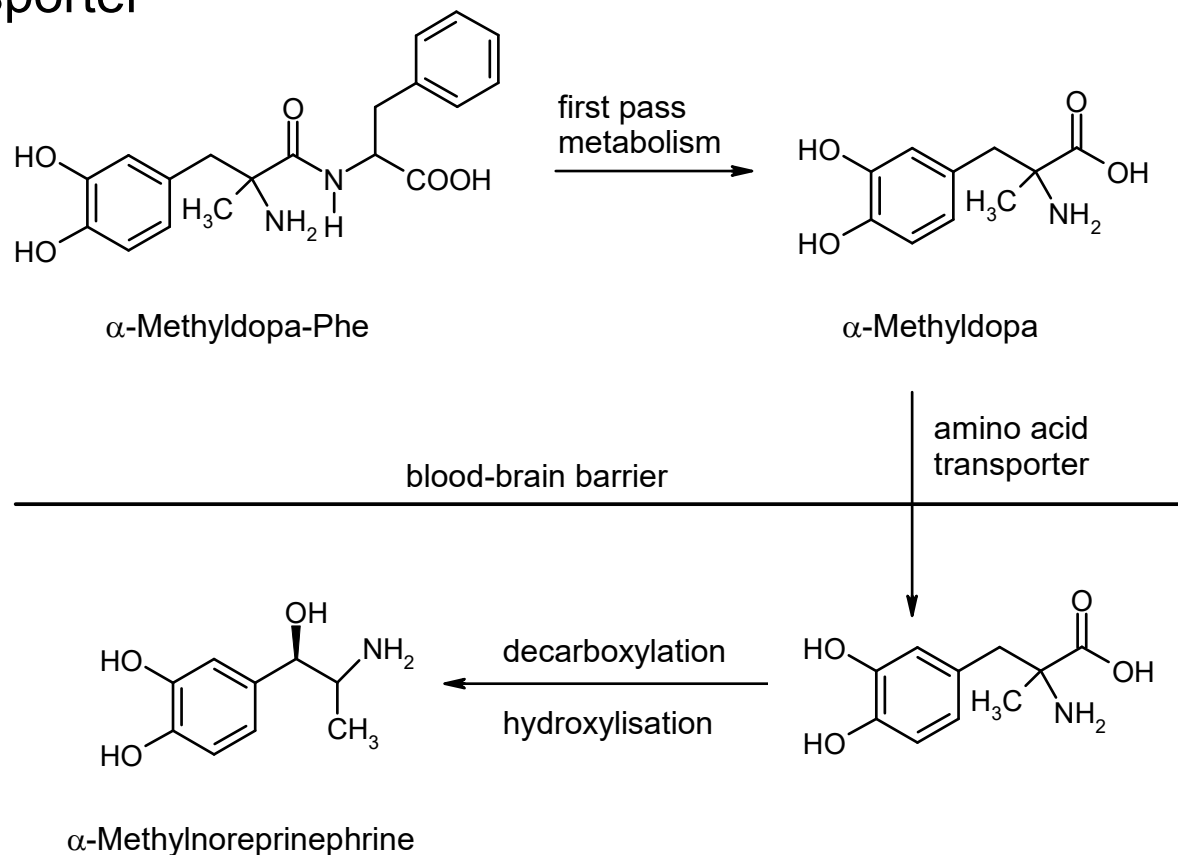
# Antiviral Nucleoside Analogs

Nucleosides missing the 3'-OH group cause disruption of the synthesis of a new DNA strain



# Multi level prodrugs

Active uptake of  $\alpha$ -Methyldopa-Phe by the dipeptide transporter

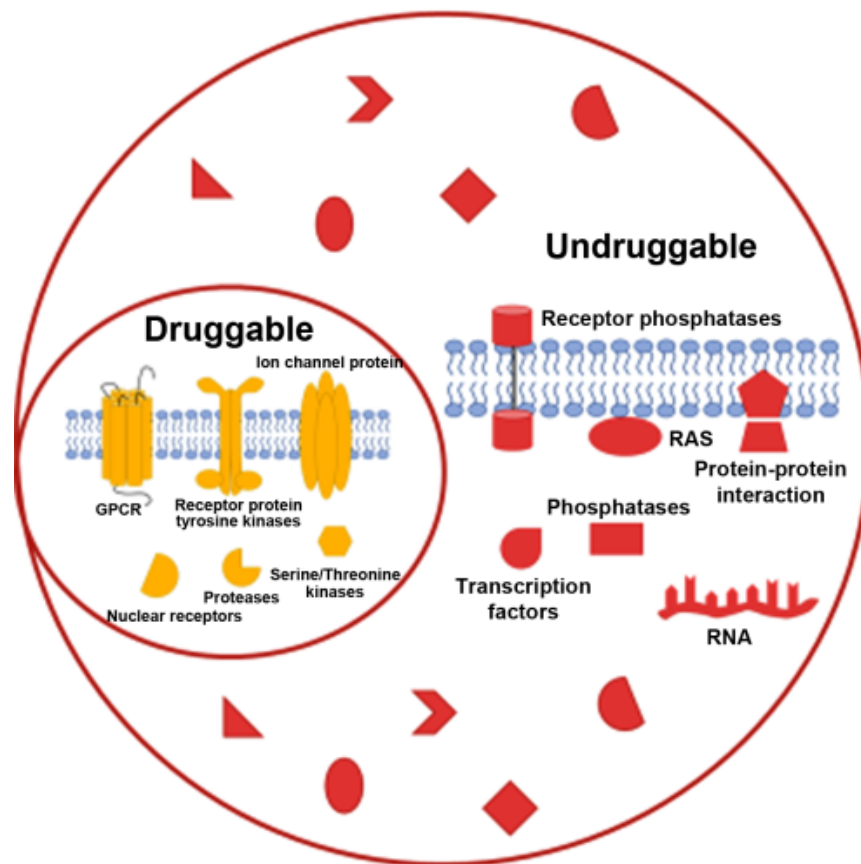


$\alpha$ -Methylnorepinephrine is an  $\alpha_2$  agonist  
(false neurotransmitter)

# Difficult and Undruggable Targets (I)

If there is no distinct binding pocket for typical small molecules, such targets are hard to inhibit:

Transcription Factors  
Receptor Phosphatases  
(soluble) Phosphatases  
(K)RAS  
RNA  
Protein-Protein-Interaction

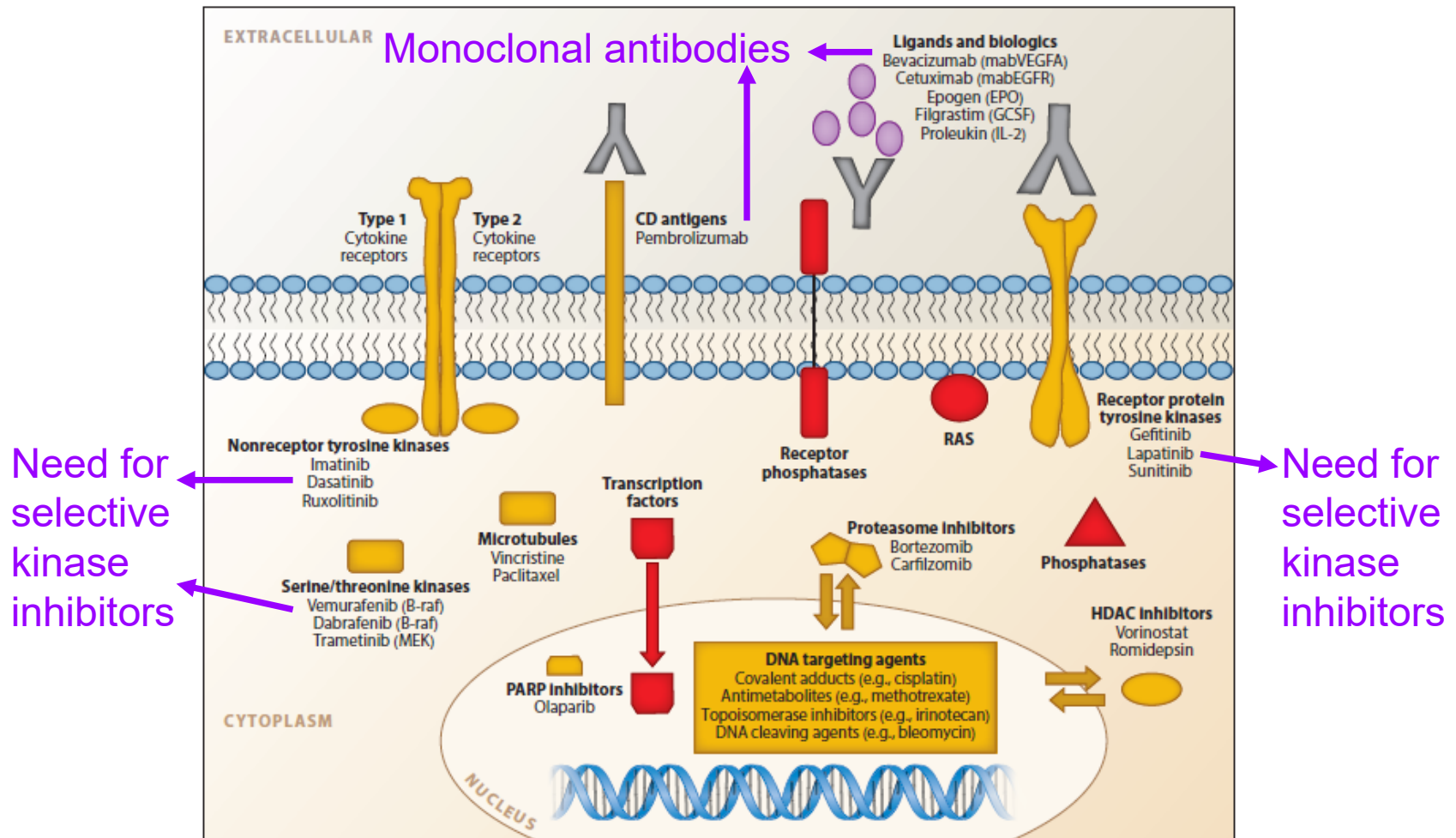


Lit: J.Wang et al. *Chin. J. Chem.* **37** (2019) 501.



# Difficult and Undruggable Targets (II)

Many of those targets are, however, crucial in cancer therapy

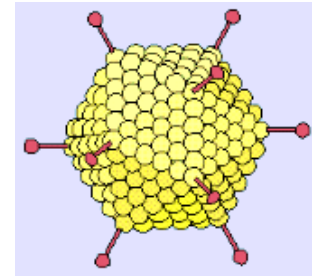


Lit: J.S.Lazo & E.R.Sharlow *Annu.Rev.Pharmacol.Toxicol. Chem.* **56** (2016) 23.

# Lifestyle vs. Disease

The great challenges

- Virostatics
- Antibiotics (Zn- $\beta$ -lactamases, malaria)
- Anticancer drugs
- Neurodegenerative diseases (Antidementia, Alzheimer)
- Diabetes type 2
- civilization diseases (obesity, ADHD)?



# Lifestyle vs. Disease (II)

The top selling drugs during recent times (selection):

adalimumab*	(arthritis)
apixaban	factor Xa-inhibitor (anti-coagulant)
pregabalin	calcium channels (epilepsy)
lenalidomide	antitumor/apoptosis
nivolumab*	(oncology, various cancers)
pembrolizumab*	(cancer immunotherapy)
etanercept*	(rheumatoid arthritis)
trastuzumab*	(breast cancer)
bevacizumab*	(colon cancer)
rituximab*	(autoimmune diseases, cancer)
sofosbuvir	antiviral nucleoside
fluticasone	anti-inflammatory/corticosteroid
rosuvastatin	HMG-CoA reductase

\* monoclonal antibody

Source: wikipedia (2019)

# Lifestyle vs. Disease (III)

Most „blockbuster“ drugs were not predicted by analysts of the marketing departments:

tamoxifen  
captopril  
cimetidine

indication

breast cancer  
hypertension

gastric ulcers [Geschwulstbildung  
im Magen]

fluoxetine (Prozac™)      depression

atorvastatin (Lipitor™)      hyperlipidaemia, obesity



Lit: J.Knowles & G.Gromo *Nat.Rev.Drug.Discov.* **2** (2003) 63.

Picture source: [www.investorschronicle.co.uk](http://www.investorschronicle.co.uk)

# Lifestyle vs. Disease (IV)

Innovative new drugs that have emerged (source until 2006: Hugo Kubinyi)

2020	Tozinameran	mRNA vaccine (COVID-19)
2010	Dabigatran	anticoagulant (thrombosis)
2006	Deferasirox	iron chelator (thalassemia)
2003	Roflumilast	PDE-4 inhibitor (asthma)
2002	Ezetimib	cholesterol uptake inhibitor
2001	Imatinib	leucemia (tyrosine kinase inhibitor)
2001	Fondaparinux	thrombosis (antagonist)
1999	Zanamivir	influenza (viral neuraminase inhibitor)
1999	Amprenavir	HIV (protease inhibitor)
1999	Celecoxib	arthritis (COX-2 inhibitor)
1998	Sildenafil	erectile dysfunction (PDE-5 inhibitor)
1998	Orlistat	obesity (pancreas lipase inhibitor)
1997	Sibutramine	obesity (GPCR inhibitor)
1997	Finasteride	prostate (steroid reductase inhibitor)
1997	Nelfinavir	HIV (protease inhibitor)
1996	Indinavir	HIV (protease inhibitor)
1996	Nevirapin	HIV (reverse transcriptase inhibitor)

# Lifestyle vs. Disease (V)

Innovative new drugs from 1982-1996:

(source: Hugo Kubinyi)

1996	Meloxicam	arthritis (COX-2 inhibitor)
1995	Dorzolamine	glaucoma (carboanhydrase inhibitor)
1995	Losartan	hypertension (GPCR antagonist)
1994	Famciclovir	herpes (DNA polymerase inhibitor)
1993	Risperidon	psychose ( $D_2$ / $5HT_2$ antagonist)
1991	Sumatriptan	migraine ( $5HT_1$ rezeptor antagonist)
1990	Ondansetron	antiemetic ( $5HT_3$ antagonist)
1988	Omeprazole	gastric ulcers (proton pump inhibitor)
1987	Lovastatin	cholesterol (biosynthesis inhibitor)
1986	Artemisinin	anti-malarial (natural compound)
1985	Fluoxetine	depression (5HT inhibitor)
1985	Mefloquine	anti-malarial
1984	Enalapril	hypertension (ACE inhibitor)
1983	Cyclosporin A	immunosuppressant
1982	Ranitidine	gastric ulcers ( $H_2$ antagonist)

# Lifestyle vs. Disease (VI)

How are innovative drugs defined?

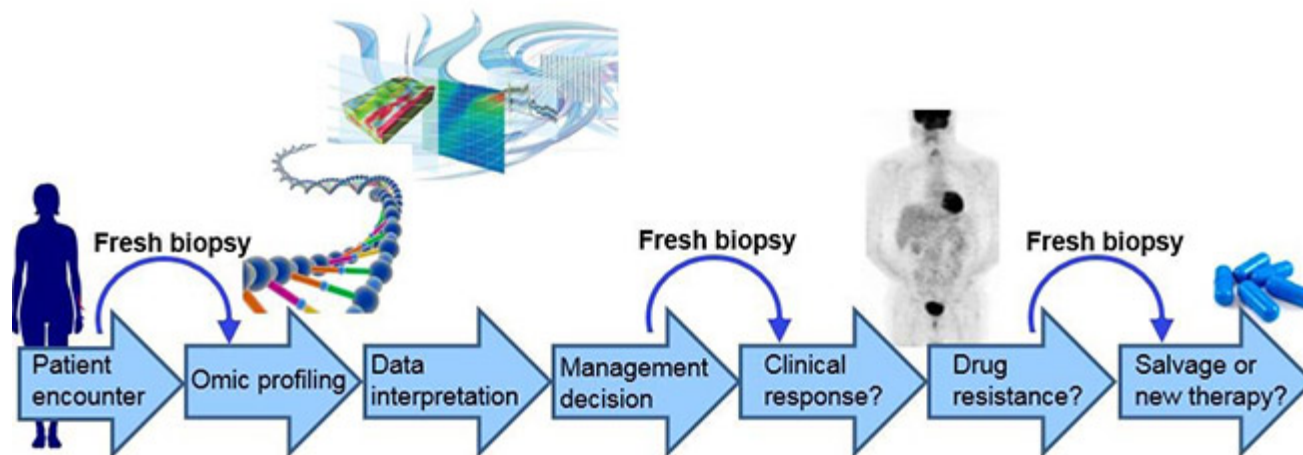
- improved mode of action (selectivity)
- improved ADMET profile
- Improved administration (e.g. oral instead of intravenous)
- pro-drugs
- new targets

# personalized medicine

Variable metabolic content and predisposition  
(Genotyping)

Avoiding rare, complicated adverse effects  
(in part already used in the clinic)

Will the necessary financial effort of screening and of clinical studies limit the genetic pool to inhabitants of wealthy nations ?



picture source: [www.dana-farber.org](http://www.dana-farber.org)



# Resume

The available knowledge on the human genome and the present SNPs in it allow two approaches:

1. Finding new targets (either on the genome, the mRNA, or the protein level)
2. pharmacogenomic methods will lead to personalized medicine (which drug and at what dosage), esp. for long term application of certain drugs (hypertension, analgesics, anti-psychotics) and those that possess a narrow therapeutic band width (cardiotonics, antineoplastics)

Personalized oncology (choice of treatment options) has become routine.