Drug Discovery - Introduction

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Basilius Besler
Hortus Eystettensis
Eichstätt, 1613

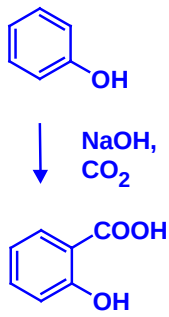
Squill
(Scilla alba = Urginea maritima)

Theriak

originally a mixture of 54 materials, as antidote against all kind of poisons (1st century B.C. till 18th century), used also as a remedy against the plague.

Public theriak preparation at a market.

The Doctrine of Signatures: „Nature helps Mankind“



Kolbe salicylic acid synthesis (1859)

The Doctrine of Signatures: „Nature helps Mankind“

Mistletoe, *Viscum album*

St. John‘s Wort,
Hypericum perforatum

Truelove, *Paris quadrifolia*

„Diß Beerlein ist von Gestalt wie ein Augapfel oder Äuglein anzusehen ... Zu den kranken und bösen Augen / ein sehr nützlich und heilsamb Kraut ist“ (Johannes Francke, 1618)

The Stone of Folley

Hieronymus Bosch (~1450 - 1516)

„Master snyt die keye ras. Myne name is Lubbert das“

A quack docter, assisted by a priest and a nun, extracts the „stone of folley“ from the brain of a patient.

Development of Drug Research

Time	Materials	Test systems
- ancient time	plants, venoms minerals ...	humans
- 1806	morphine	
- 1850	chemicals	
- 1890	synthetics, dyes	animals
- 1920		animals, isolated organs
- 1970		enzymes, membranes
- 1990	combinatorial libraries	human proteins, HTS
- 2000	focused libraries	uHTS, virtual screening

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Important Results in Drug Research, 1806-1981

1806	Morphine	Hypnotic agent
1875	Salicylic acid	Antiinflammatory agent
1884	Cocaine	Stimulant, local anesthetic agent
1888	Phenacetin	Analgesic and antipyretic agent
1899	Acetylsalicylic acid	Analgesic and antipyretic agent
1903	Barbiturates	Sedatives
1909	Arsphenamine	Antisyphilitic agent
1921	Procaine	Local anesthetic agent
1922	Insulin	Antidiabetic agent
1928	Estrone	Female sex hormone
1928	Penicillin	Antibiotic agent
1935	Sulphachrysoidine	Bacteriostatic agent
1944	Streptomycin	Antibiotic agent
1945	Chloroquine	Antimalarial agent
1952	Chlorpromazine	Neuroleptic agent
1956	Tolbutamide	Oral antidiabetic agent
1960	Chlordiazepoxide	Tranquillizer
1962	Verapamil	Calcium channel blocker
1963	Propranolol	Antihypertensive agent (beta-blocker)
1964	Furosemide	Diuretic agent
1971	L-Dopa	Anti-Parkinson agent
1975	Nifedipine	Calcium channel blocker
1976	Cimetidine	Anti-ulcus agent (H_2 blocker)
1981	Captopril	Antihypertensive agent (ACE inhibitor)
1981	Ranitidine	Anti-ulcus agent (H_2 blocker)

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Important Results in Drug Research, 1983-2001

1983	Cyclosporin A	Immunosuppressant
1984	Enalapril	Antihypertensive agent (ACE inhibitor)
1985	Mefloquine	Antimalaria agent
1986	Fluoxetine	Antidepressant (5-HT transporter)
1987	Artemisinin	Antimalaria agent
1987	Lovastatin	Cholesterol biosynthesis inhibitor
1988	Omeprazole	Anti-ulcus agent (H/K-ATPase inhibitor)
1990	Ondansetron	Antiemetic agent (5-HT_3 blocker)
1991	Sumatriptan	Anti-migraine agent (5-HT_1 blocker)
1993	Risperidon	Antipsychotic agent ($D_{2/5}$-HT_2 blocker)
1994	Famciclovir	Anti-herpes (DNA polymerase inhibitor)
1995	Losartan	Antihypertensive agent (A II antagonist)
1995	Dorzolamide	Glaucoma (Carbonic anhydrase inhib.)
1996	Meloxicam	Anti-arthritis agent (COX 2 inhibitor)
1996	Nevirapin	HIV reverse transcriptase inhibitor
1996	Indinavir, Ritonavir, Saquinavir	HIV protease inhibitors
1997	Nelfinavir	HIV protease inhibitor
1997	Finasteride	Hair loss
1997	Sibutramine	Adipositas (uptake blocker)
1998	Orlistat	Adipositas (lipase inhibitor)
1998	Sildenafil	Erectile dysfunction (PDE inhibition)
1999	Celecoxib, Rofecoxib	Anti-arthritis agents (COX-2 inhibitors)
1999	Amprenavir	HIV protease inhibitor
1999	Zanamivir, Oseltamivir	Influenza (neuraminidase inhibitors)
2001	Fondaparinux	Thrombosis (synthetic LMWH)
2001	Imatinib	CML (specific abl-TK inhibitor)

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Top 20 Drugs, Sales in mio $,

		year	2000	2004est.
Losec / omeprazole	ion transporter	1988	6,260	2,575
Zocor / simvastatin	enzyme	1988	5,280	9,653
Lipitor / atorvastatin	enzyme	1997	5,031	11,304
Norvasc / amlodipine	ion channel	1990	3,362	4,260
Takepron / lansoprazole	ion transporter	1992	3,046	4,877
Claritin / loratadine	GPCR	1988	3,011	1,900
Procrit / erythropoetin	agonist	1988	2,709	2,875
Celebrex / celecoxib	enzyme	1999	2,614	3,411
Prozac / fluoxetine	transporter	1986	2,574	525
Zyprexa / olanzapine	GPCR	1996	2,350	4,445
Seroxat / paroxetine	transporter	1991	2,348	3,409
Vioxx / rofecoxib	enzyme	1999	2,160	3,800
Zoloft / sertraline	transporter	1990	2,140	2,750
Epogen / erythropoetin	agonist	1988	1,963	2,155
Glucophage / metformin	unknown		1,892	1,400
Premarin / oestrogens	nucl. receptor		1,870	2,300
Augmentin / amox.+clav.acid	enzyme		1,847	2,603
Pravachol / pravastatin	enzyme	1989	1,817	2,581
Vasotec / enalapril	enzyme	1984	1,790	575
Cozaar / losartan	GPCR	1994	1,715	2,764

Source: Nature Rev. Drug Discov. 1, 176 (2002)

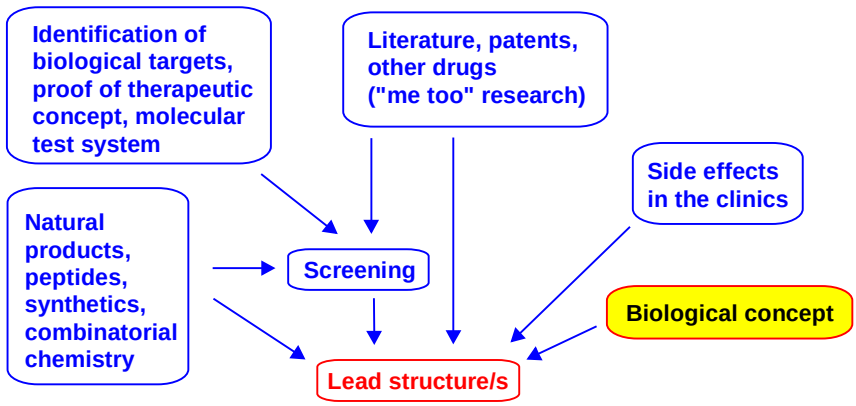
Lead Structure Search

- Identification of a pathophysiologically relevant molecular target, e.g. an enzyme, receptor, ion channel, or transporter
- Determination of the DNA and protein sequence
- Elucidation of the function and mechanism of the protein
- Proof of the therapeutic concept in animals
- High-throughput molecular test system
- Synthesis program and/or mass screening
- Selection of one or several lead structures

Lead Optimization and Drug Development

- 3D structure determination of the molecular target and its complexes with low-molecular weight ligands
- Molecular modelling and design of new ligands
- Further syntheses and biological tests of selected candidates
- Optimization of selectivity, bioavailability and pharmacokinetics
- Pharmaceutical formulation
- Preclinical and clinical development
- Drug approval and market introduction

The Design Cycle: Lead Structure Search



The design cycle describes the optimization of a lead structure to one or several development candidates. It is an iterative process with evolutionary character.

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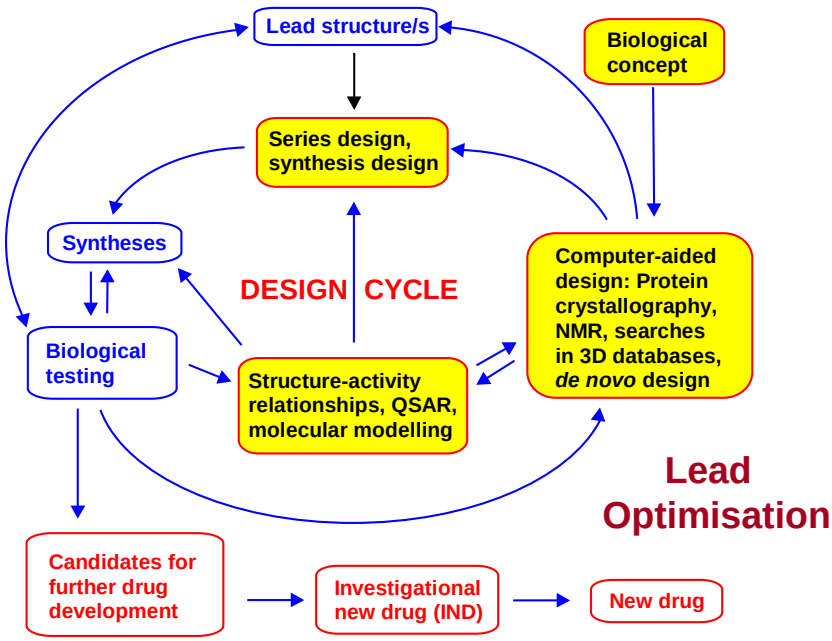


Drug Targets and Mechanisms of Drug Action

Enzymes - reversible and irreversible inhibitors

Receptors - agonists and antagonists

Ion Channels - blocker and opener

Transporters - uptake inhibitors

DNA - alkylating agents, „minor groove binders“, intercalating agents, wrong substrates (trojan horses)

Mechanisms of Drug Action, Definitions

Ligand: Any molecule that binds to a biological macromolecule.

Enzyme: Endogeneous biocatalyst; converts one or several substrate/s into one or several product/s.

Substrate: Any educt of an enzymatic reaction.

Inhibitor: Ligand that prevents the binding of a substrate to its enzyme, either in a direct (competitive) or indirect (allosteric) manner, reversibly or irreversibly.

Receptor: A membrane-bound or soluble protein or protein complex, which exerts a physiological effect (intrinsic effect), after binding of an agonist, via several steps.

Agonist: A receptor ligand that mediates a receptor response (intrinsic effect).

Antagonist: A receptor ligand, which prevents the action of an agonist, in a direct (competitive) or indirect (allosteric) manner.

Partial Agonist: A (high affinity) antagonist, which itself has more or less pronounced intrinsic activity.

Mechanisms of Drug Action, Definitions

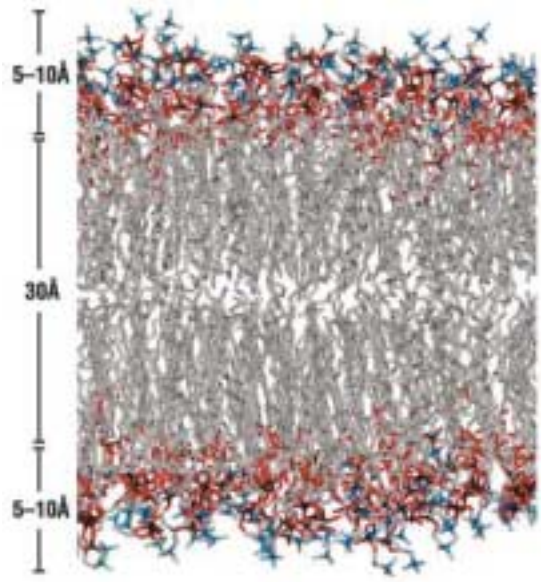
Functional antagonist: A compound, which prevents a receptor response by any other mechanism (e.g. ß-adrenolytic activity of calcium channel blockers).

Allosteric effector molecule: A ligand, which modifies the function of a protein by a change of its 3D-structure (2,3-DPG / Hb) or modulates the affinity of a receptor for a certain ligand (benzodiazepin / GABA-receptor system).

Ion channel: A pore, formed by proteins, that allows the diffusion of certain ions through the cell membrane along a concentration gradient; the channel opening is either ligand- or voltage-controlled.

Transporter: A protein, which transports molecules or ions through the cell membrane, against a concentration gradient, under energy consumption.

Antimetabolite: A compound that participates in the biosynthesis of an essential intermediary metabolite, either as a false substrate or as an inhibitor.

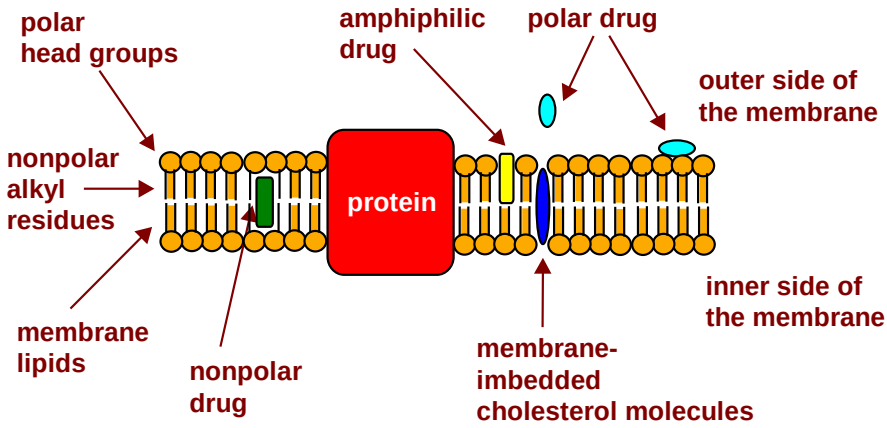


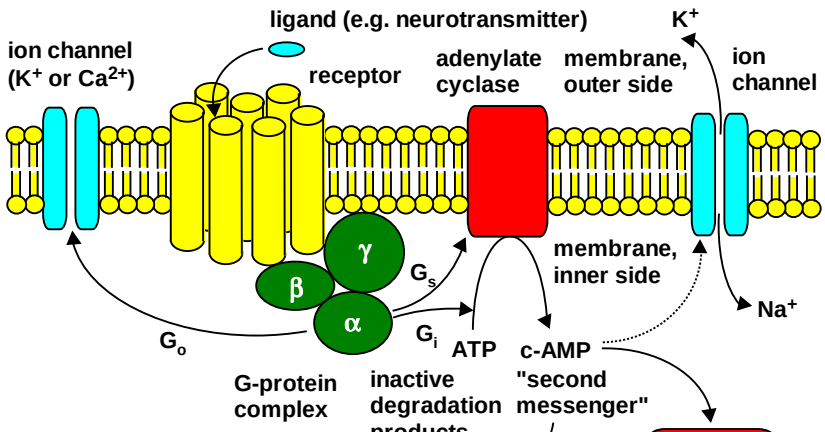
Membranes

Computer Simulation of a Bilayer Cell Membrane

www.new-science-press.com/browse/protein/1/11

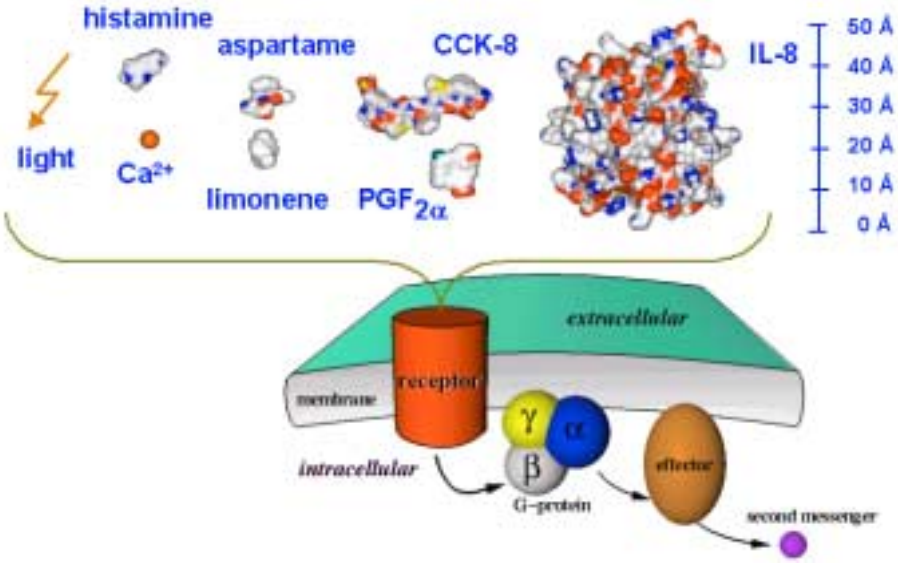
Membranes and the Action of Drugs



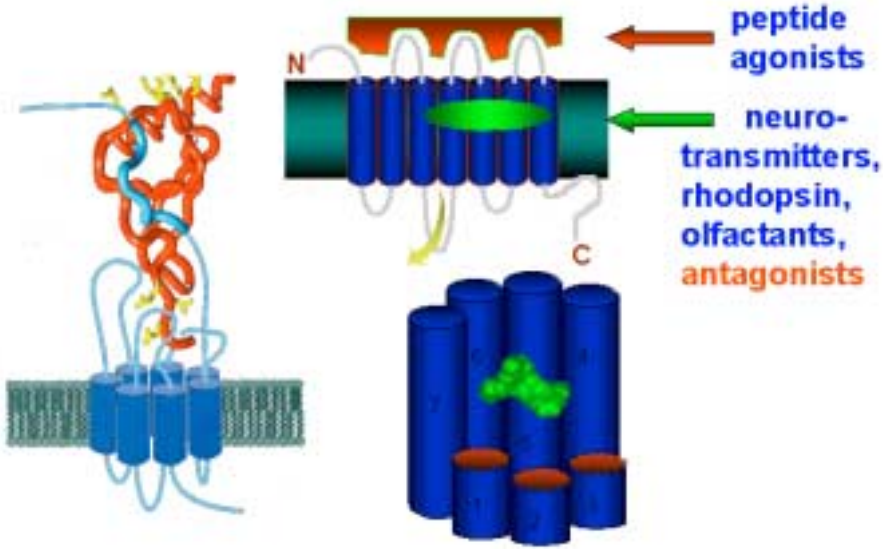


The Function of G Protein-Coupled Receptors

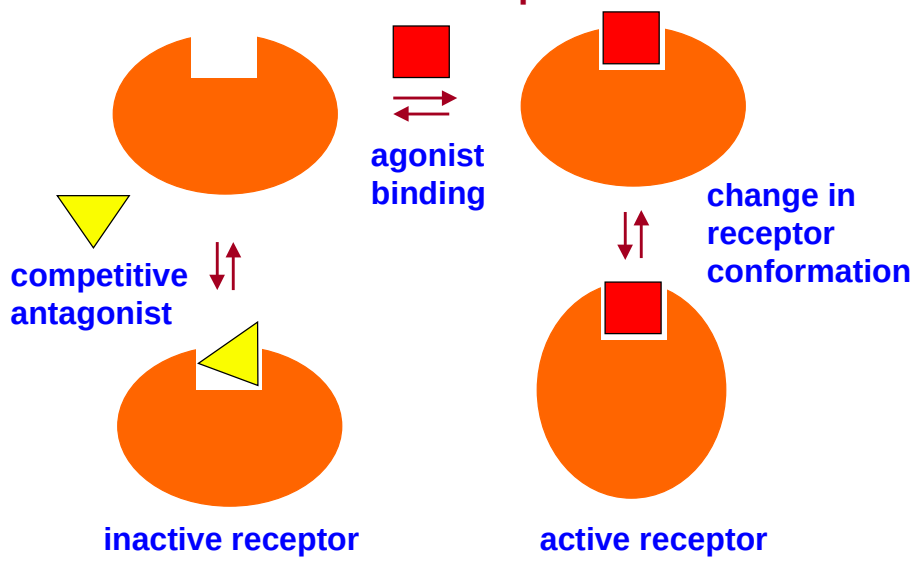
Ligands of G Protein-Coupled Receptors



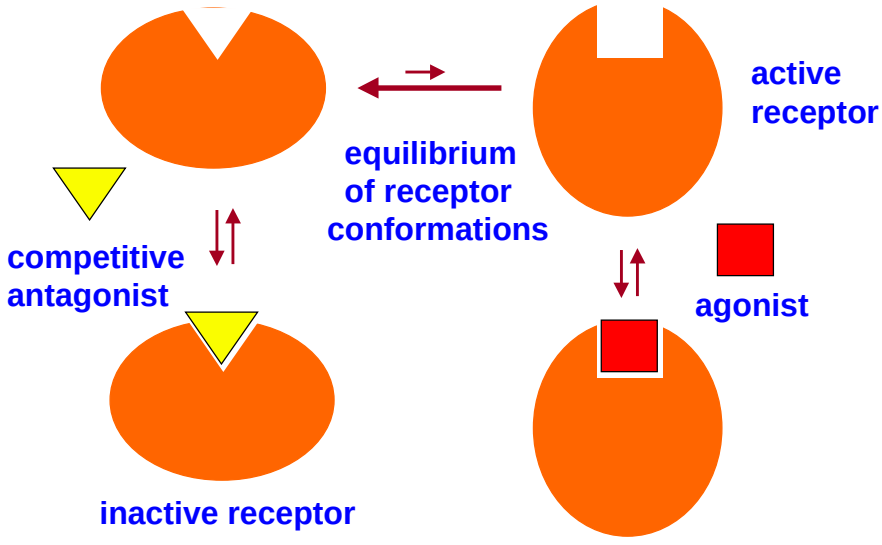
Binding Sites of Agonists and Antagonists



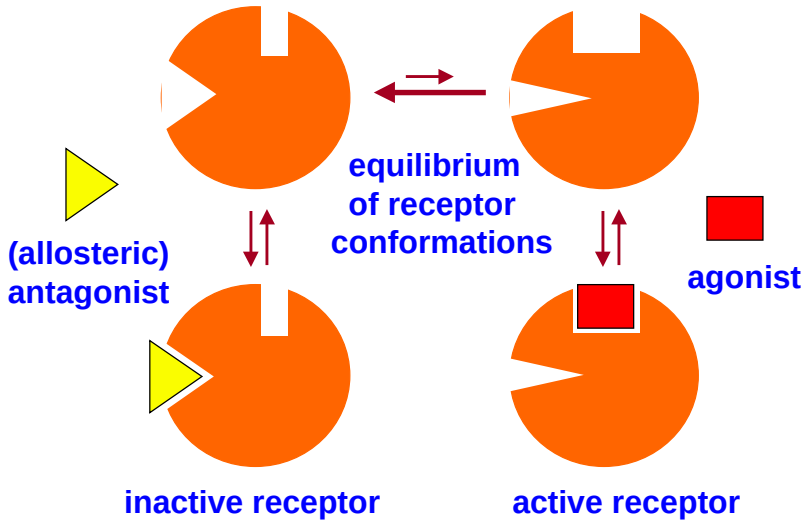
Classical Model of Receptor Activation



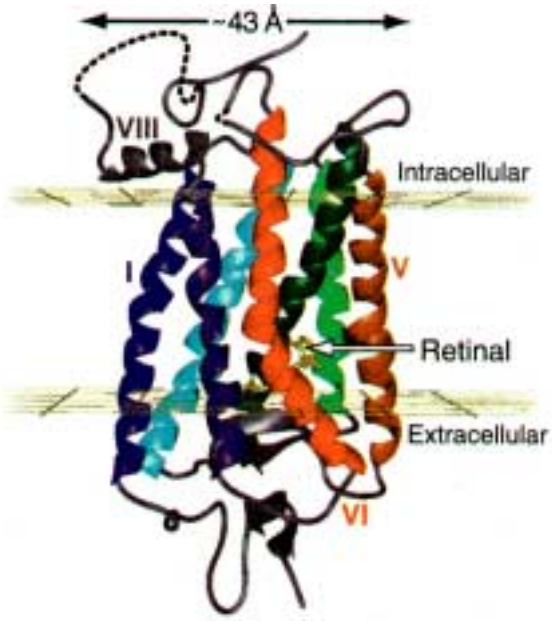
Two-State Model of Receptor Activation



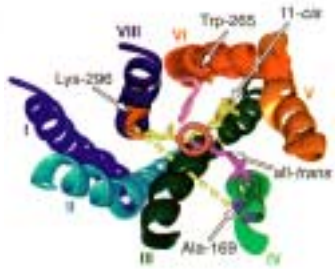
Two-State Model of Peptide Receptors



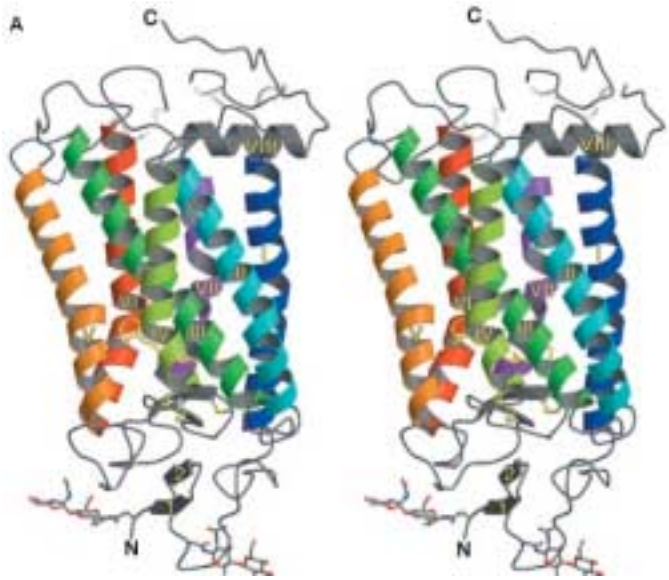
3D Structure of Rhodopsin at 2.8 Å Resolution



Palczewski et al., Science 289, 733, 739 (2000)

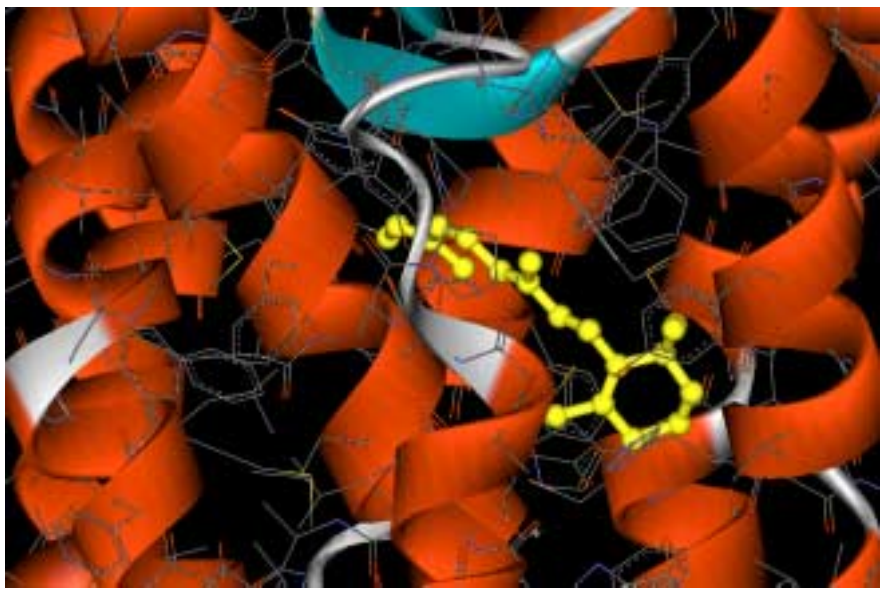


Bovine Rhodopsin (1f88) - Stereo Presentation



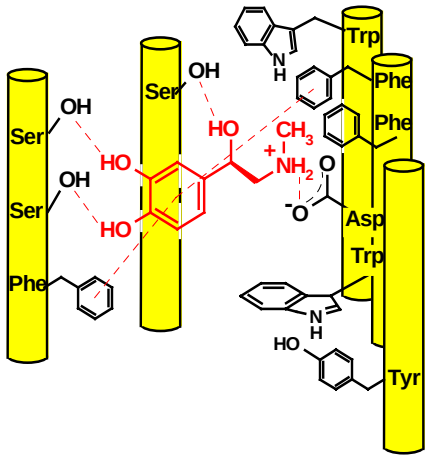
Palczewski et al., Science 289, 733, 739 (2000)

Binding Mode of Retinal in Bovine Rhodopsin (1f88)

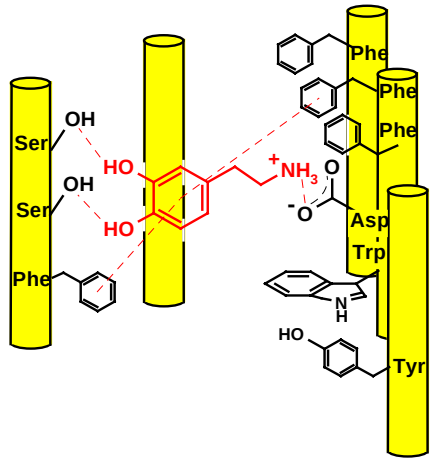


Models of GPCR Ligand Binding

Adrenergic ß$_2$ receptor



Dopaminergic D$_2$ receptor



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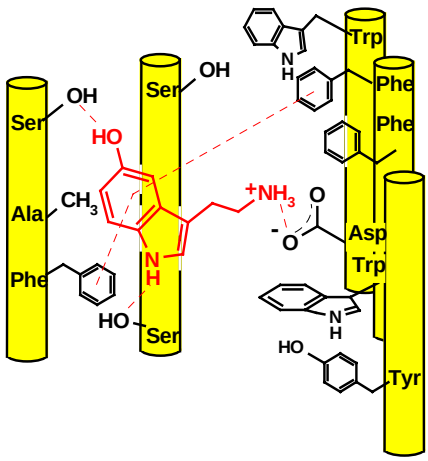
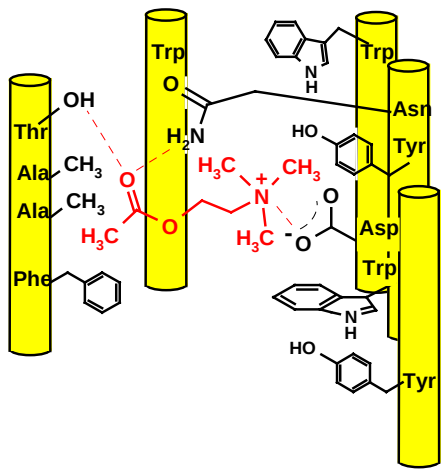
Models of GPCR Ligand Binding

Serotonin 5HT$_2$ receptor



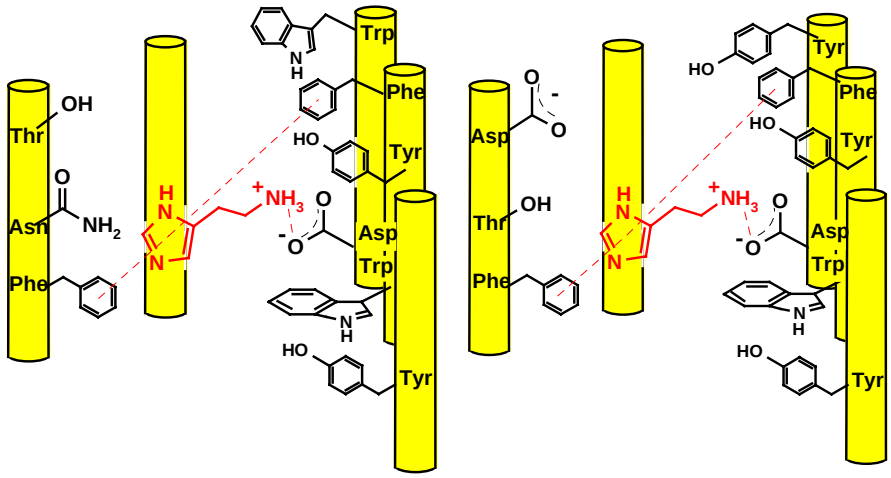
Muscarinic M$_2$ receptor

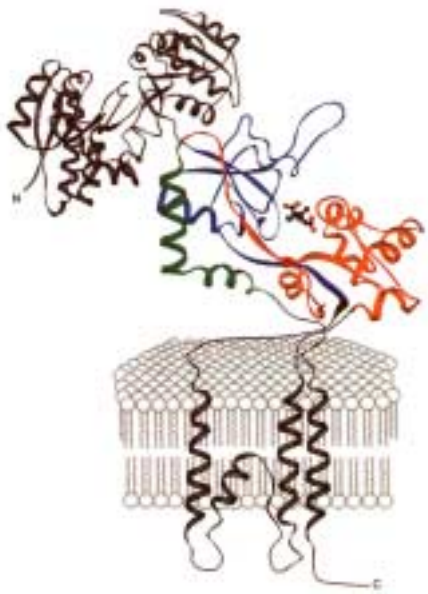


Models of GPCR Ligand Binding

Histaminic H_1 receptor

Histaminic H_2 receptor





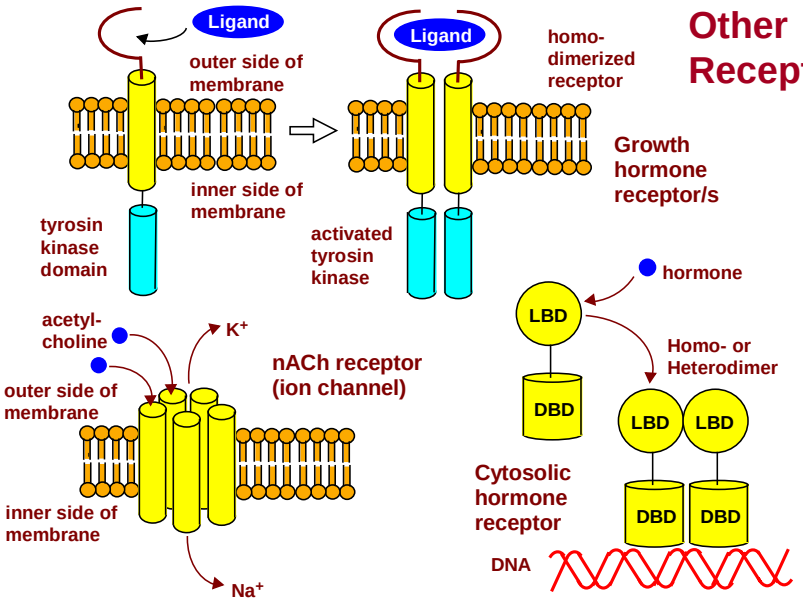
Glutamate Receptor Subunit

Extracellular part: Binding domain (experimental 3D-structure)

Ligand: GABA

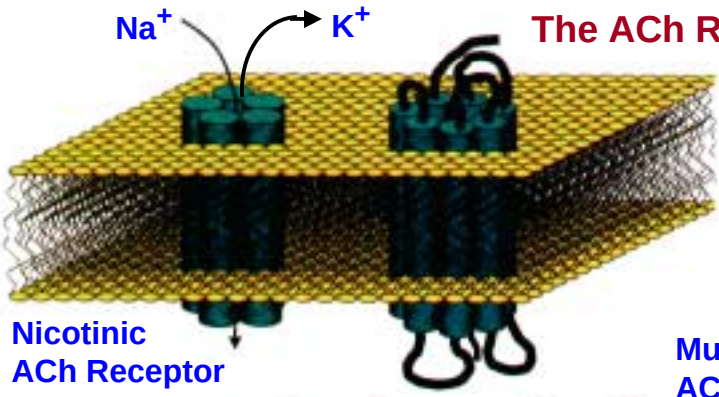
Membrane helices (from molecular modelling)

Other Receptors



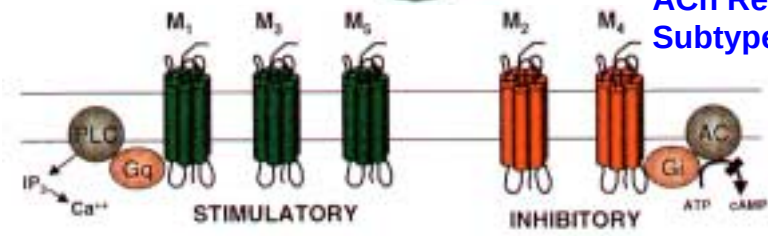
The ACh Receptor Family

C. C. Felder et al., J. Med. Chem. 43, 4333-4353 (2000)

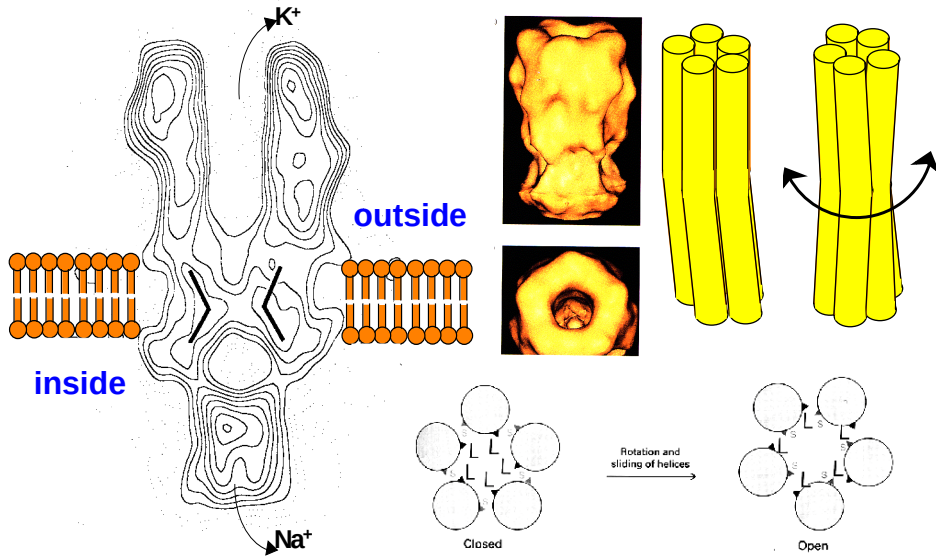


Nicotinic ACh Receptor

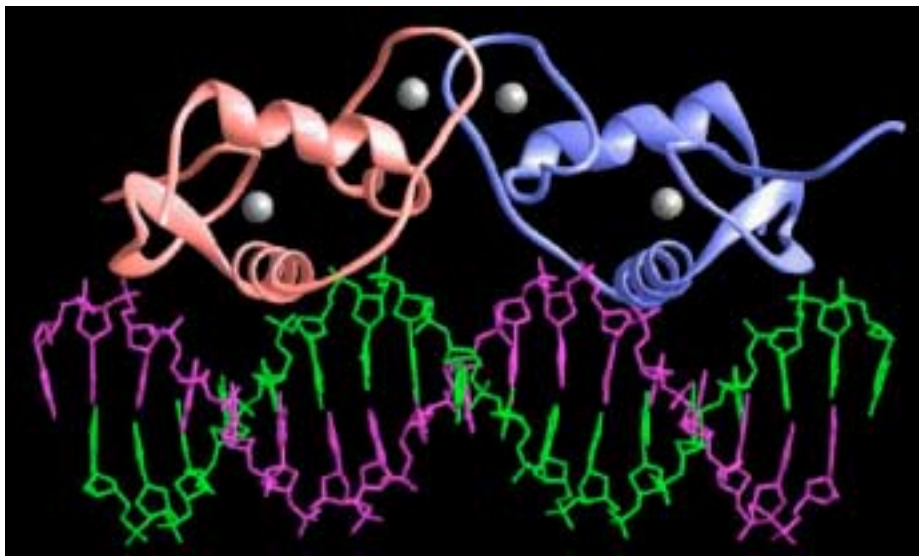
Muscarinic ACh Receptor Subtypes



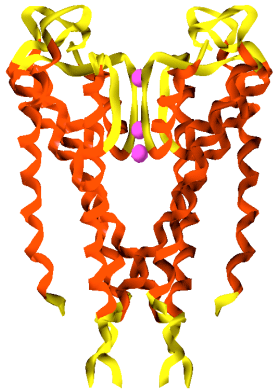
Nicotinic Acetylcholine Receptor

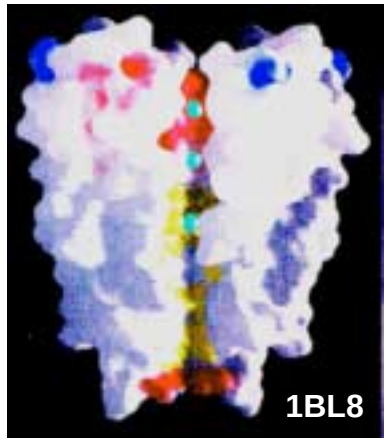


DNA-Binding Domain of the Oestrogen Receptor



KcsA Potassium Channel (Roderick McKinnon, Nobel Price 2003)





Four subunits with two TM helices each, entrance: 18 Å diameter, selectivity filter for potassium ions

D. A. Doyle et al., Science 280, 69-77 (1998)

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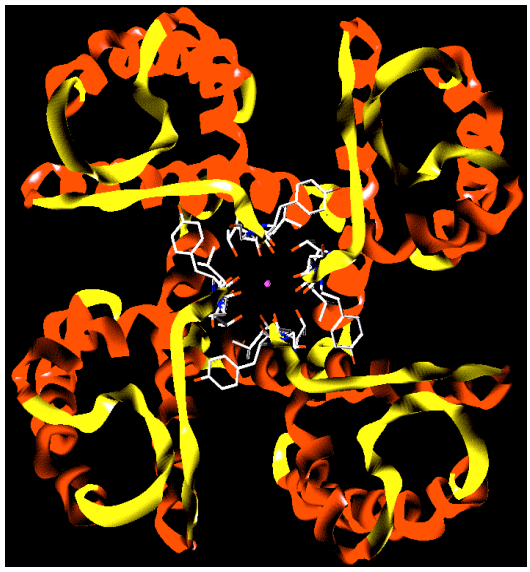
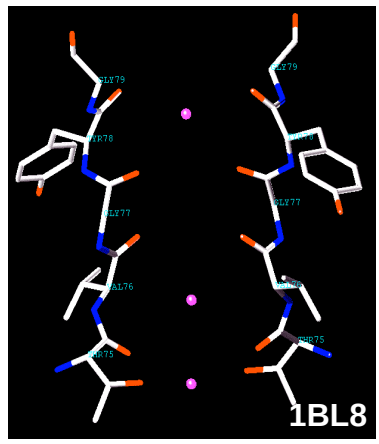


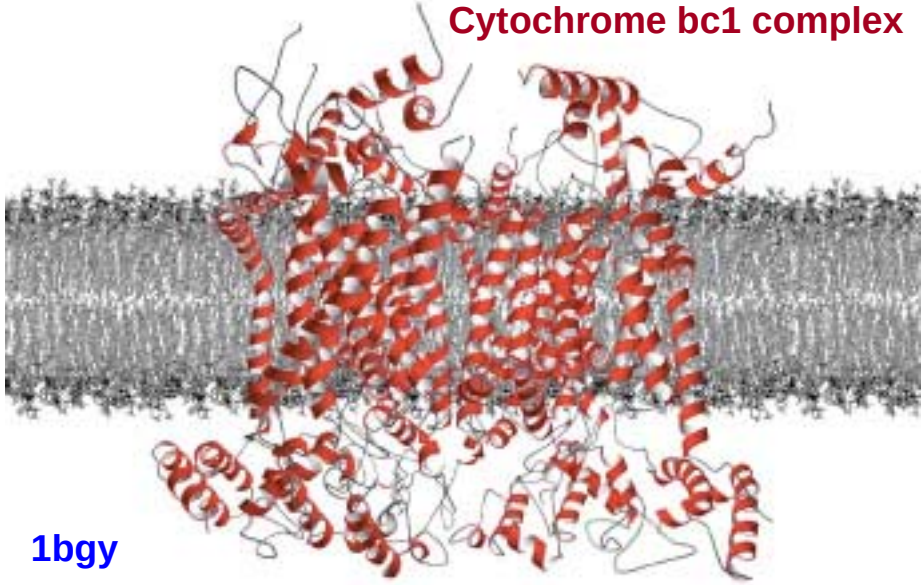
KcsA Channel

coordination geometry does not fit Li^+ or Na^+



D. A. Doyle et al., Science 280, 69-77 (1998)

Cytochrome bc1 complex



1bgy

www.new-science-press.com/browse/protein/1/11

Mechanisms of Drug Action

1) Enzymes

- Reversible noncovalent inhibitors
- Transition state inhibitors (e.g. cytidine deaminase)
- biproduct inhibitors
  - $HOOC\text{-}CH_2CH_2\text{-}CH(CH_2Phe)COOH$, enalaprilate
- Reversible covalent inhibitors (e.g. serine proteases)
  - -CHO + HO-Ser → -CH(OH)-O-Ser
- Irreversible covalent inhibitors
  - $-CH_2Cl$ + HO-Ser → $-CH_2$-O-Ser
  - ASS + HO-Ser → CH_3CO-O-Ser + salicylic acid
  - Penicillines (acylation of transpeptidase)
  - Omeprazole (disulfide formation with H^+/K^+-ATPase)
  - "Suicide" inhibitors
    - Eflornithine, α-(CHF_2)-ornithine, as ornithine decarboxylase inhibitor
- "Non-substrate binding pocket" inhibitors
  - e.g. hirudin$^{56\text{-}65}$

Proteases and Other Enzymes as Drug Targets

Enzyme	Function	Disease	Development Status
ACE	Renin-Ang-System	hypertension	launched
HMG-CoA-Red	biosynthesis	hypercholesterinemia	launched
cyclooxygenase	biosynthesis	inflammation	launched
HIV Protease	replication	AIDS	launched
thrombin	coagulation	stroke, infarct	launched
kinases	signal transduction	cancer	launched
MMP's	cell matrix	inflammation, cancer	clinical phase III
tryptase	phagocytosis	inflammation, asthma	clinical phase II
elastase	connective tissue degradation	pulmonary diseases, inflammation, ...	clinical phase I
renin	RAS	hypertension	preclinical
factor Xa	coagulation	stroke, infarct	preclinical
cathepsin B	cellular metabolism	cancer, inflammation	preclinical
cathepsin K	bone resorption	osteoporosis	preclinical
cathepsin L	bone resorption	osteoporosis	preclinical
cathepsin S	MHC-II maturation	inflammation, asthma	preclinical
β,γ secretase	βAPP processing	Alzheimer's disease	preclinical
calpains	protein turnover, ...	stroke, Alzheimer's	preclinical
caspases	apoptosis	broad spectrum	preclinical

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Mechanisms of Drug Action

2) Receptors

- Receptor agonists: dopamine, epinephrine, morphine
- Competitive receptor antagonists: most neurotransmitter antagonists
- Noncompetitive receptor antagonists: most peptide receptor antagonists

3) Ion channels (ligand- or voltage-controlled passive diffusion)

- e.g. calcium, sodium and potassium channel blockers, potassium channel openers

4) Transporter (against a concentration gradient)

- Na^+/K^+-ATPase (cardiac glycosides), H^+/K^+-ATPase (proton pump; omeprazole)
- Inhibition of neutrotransmitter uptake („re-uptake", uptake blocker): cocaine, imipramine, fluoxetine

5) DNA as target

- Alkylating and intercalating compounds, "minor groove" binders; DNA termination: aciclovir (activation by TK)

Other Mechanisms of Drug Action

Antacids
- Neutralization of gastric acid

Antibacterial and antimycotic agents
- Quinolone carboxylates (DNA-gyrase inhibitors)
- Azole antimycotics (inhibition of ergosterol biosynthesis causes defects of the cell membrane)
- Polyene antibiotics (channel formation in the fungal membrane)

Antibiotics
- Penicillins and cephalosporins (irreversible transpeptidase inhibitors; inhibition of cell wall biosynthesis)
- D-Cycloserine (cell membrane permeation by the D-alanine transporter; inhibition of cell wall biosynthesis)
- Streptomycin, tetracyclin, chloramphenicol and other antibiotics: Inhibition of protein biosynthesis

Nucleoside analogs
- Modification in the base and/or in the sugar; metabolic activation in the virus-infected cell

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Antimetabolites
- Sulfamidochrysoidine (prodrug of sulfanilamide, an antimetabolite of DHF biosynthesis)
- Isoniazide (prodrug of isonicotinic acid, an antimetabolite of nicotinic acid)

Antituberculostatics
- Ethambutol (inhibition of RNA biosynthesis)

Antitumor therapeutics
- Alkylating agents (cause reading errors in DNA duplication)
- Intercalating agents (DNA reading frame errors)
- Minor groove binders (e.g. distamycin A, neotropsin)
- Taxol (tubulin microaggregation equlibria)

Immunosuppressives
- Cyclosporin A (complex formation with cyclophilin inhibits the activation of immunocompetent helper cells)

Integrin antagonists
- Fibrinogen receptor (mediates platelet aggregation)
- Vitronectin receptor (mediates neoangiogenesis)

Therapeutically Valuable Drug Combinations

Parkinson therapy

Antihypertensive therapy: ACE blockers, calcium antagonists, β–blockers, diuretics, α-blockers

Antibacterial and antiviral therapy: sulfonamides + dihydrofolate reductase inhibitors; HIV-protease, reverse transcriptase and integrase inhibitors for AIDS therapy

Hormonal contraceptives: gestagen + estrogen

Meaningless Combinations

Most “minor” **analgesics** (e.g. with caffeine)

Most symptomatic **flu remedies**

Some **antitussives** (e.g. codein + N-acetylcystein)

Complex **mixtures of plant extracts**, and, of course, (mixed) **homeopathics** !

Recommended Literature

F. J. Clarke, How Modern Medicines are Discovered, Futura Publishing Company, Mount Kisco, 1973.

●● **A. Burger, A Guide to the Chemical Basis of Drug Design, John Wiley & Sons, New York, 1983.**

● **W. Sneader, Drug Discovery: The Evolution of Modern Medicines, John Wiley & Sons, Chichester, 1985**

● **E. Bäumler, Die großen Medikamente. Forscher und ihre Entdeckungen schenken uns Leben, Gustav Lübbe Verlag, Bergisch Gladbach, 1992.**

●● **W. Sneader, Drug Prototypes and their exploitation, John Wiley & Sons, Chichester, 1996.**

●● **H.-J. Böhm, G. Klebe and H. Kubinyi, Wirkstoffdesign, Spektrum Akademischer Verlag, Heidelberg, 1996.**

● **J. Ryan, A. Newman, and M. Jacobs, Editors, The Pharmaceutical Century. Ten Decades of Drug Discovery, Supplement to ACS Publications, American Chemical Society, Washington, 2000.**

R. Silverman, The Organic Chemistry of Drug Design and Drug Action, 2nd Edition, Elsevier Academic Press, Burlington, 2004.