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List of abbreviations

AC:	Adenylyl Cyclase
ACE:	Angiotensin-Converting Enzyme
ADME:	Absorption, Distribution, Metabolism, and Excretion
ADMET:	Absorption, Distribution, Metabolism, Excretion, and Toxicity
AF-1:	Activation Function-1
AF-2:	Activation Function-2
AMP / 5'-AMP:	Adenosine 5'-Monophosphate
ANF:	Atrial Natriuretic Factor
API:	Active Pharmaceutical Ingredient
AR:	Aromatic Ring
AS:	Active Site
ATP:	Adenosine Triphosphate
Bmax:	Maximum Binding Capacity
Ca ²⁺ :	Calcium Ion
CaM:	Calmodulin
CaMKs:	Ca ²⁺ /Calmodulin-dependent Protein Kinases
cAMP:	Cyclic Adenosine Monophosphate
CFTR:	Cystic Fibrosis Transmembrane Conductance Regulator
CICR:	Calcium-Induced Calcium Release
CNS:	Central Nervous System
COX / COX-1 /	Cyclooxygenase Enzymes
COX-2:	
CRE:	cAMP Response Element
CREB:	cAMP Response Element-Binding Protein
DAG:	Diacylglycerol
DBD:	DNA-Binding Domain
DPP-4:	Dipeptidyl Peptidase-4
EGF:	Epidermal Growth Factor
ER:	Endoplasmic Reticulum
ERK:	Extracellular signal-Regulated Kinase
4E-BP1:	Eukaryotic Translation Initiation Factor 4E-Binding Protein 1
FOXO:	Forkhead Box O
GABA / GABAA:	Gamma-Aminobutyric Acid
GAP:	GTPase-Activating Protein
GDP:	Guanosine Diphosphate
GEF:	Guanine Nucleotide Exchange Factor
GLUT4:	Glucose Transporter Type 4
GMC:	Gi/o-mediated cascade (Arachidonic Acid precursor pathway)
GPCR:	G Protein-Coupled Receptor
Grb2:	Growth Factor Receptor-Bound Protein 2

GTP:	Guanosine Triphosphate
H1 / H2 / H3 / H4:	Histamine Receptor subtypes
HBA:	Hydrogen Bond Acceptor
HBD:	Hydrogen Bond Donor
HMG-CoA:	3-Hydroxy-3-Methylglutaryl-Coenzyme A
HREs:	Hormone Response Elements
HSP:	Heat Shock Protein
HTS:	High-Throughput Screening
HY:	Hydrophobic feature
IC ₅₀ :	Half-maximal Inhibitory Concentration
INN:	International Nonproprietary Name
IP ₃ :	Inositol Trisphosphate
IP ₃ R:	Inositol Trisphosphate Receptor
JAK:	Janus Kinase
K _d :	Equilibrium Dissociation Constant
K _i :	Inhibitory Constant
k _{off} :	Dissociation Rate Constant
k _{on} :	Association Rate Constant
LBD:	Ligand-Binding Domain
LD ₅₀ :	Median Lethal Dose
LogP:	Partition Coefficient
MA:	Marketing Authorization
MAPK:	Mitogen-Activated Protein Kinase
MAPKK:	MAPK Kinase
MAPKKK:	MAPK Kinase Kinase
MEK:	MAPK/ERK Kinase
MKPs:	MAPK Phosphatases
mRNA:	Messenger Ribonucleic Acid
mTOR:	Mammalian Target of Rapamycin
mTORC1:	mTOR Complex 1
mTORC2:	mTOR Complex 2
nAChR:	Nicotinic Acetylcholine Receptor
NCX:	Sodium-Calcium Exchanger
NFAT:	Nuclear Factor of Activated T-cells
NF-κB:	Nuclear Factor Kappa B
NI:	Negative Ionizable
NMDA:	N-methyl-D-aspartate
NMR:	Nuclear Magnetic Resonance
NSAID:	Nonsteroidal Anti-inflammatory Drug
Orai1:	Pore-forming subunit of store-operated calcium channels
PBPs:	Penicillin-Binding Proteins
PDK1:	Phosphoinositide-Dependent Kinase 1
PDEs:	Phosphodiesterases
PGH ₂ :	Prostaglandin H ₂

PHLPP:	Pleckstrin Homology Domain Leucine-Rich Repeat Protein Phosphatase
Pi / P _i :	Inorganic Phosphate
PI:	Positive Ionizable
PI3K:	Phosphoinositide 3-Kinase
PIP ₂ :	Phosphatidylinositol 4,5-Bisphosphate
PIP ₃ :	Phosphatidylinositol 3,4,5-Trisphosphate
PKA:	Protein Kinase A
PKB:	Protein Kinase B
PKC:	Protein Kinase C
PLC / PLCβ /	Phospholipase C (Beta/Gamma isoforms)
PLCγ:	
PMCA:	Plasma Membrane Ca ²⁺ ATPase
pK _a :	Acid Dissociation Constant
PTEN:	Phosphatase and Tensin Homolog
PTPs:	Protein Tyrosine Phosphatases
Raf:	Rapidly Accelerated Fibrosarcoma
Ras:	Rat Sarcoma
RGS:	Regulators of G-protein Signaling
RL:	Receptor-Ligand Complex
RT / R _t :	Total Concentration of Receptors
RTK:	Receptor Tyrosine Kinase
RyR:	Ryanodine Receptors
S6K:	Ribosomal Protein S6 Kinase
SAR:	Structure-Activity Relationship
SBDD:	Structure-Based Drug Design
SERCA:	Sarco/Endoplasmic Reticulum Calcium ATPase
SH2 Domain:	Src Homology 2 Domain
SOCS:	Suppressors of Cytokine Signaling
SOS:	Son of Sevenless
SR:	Sarcoplasmic Reticulum
STAT:	Signal Transducer and Activator of Transcription
STIM1:	Stromal Interaction Molecule 1
TXA ₂ :	Thromboxane A ₂
V _{off} :	Dissociation Rate
V _{on} :	Association Rate
VGCCs:	Voltage-Gated Calcium Channels
WHO:	World Health Organization
XO:	Xanthine Oxidase

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Preface

At this advanced level, pharmacology moves past simply looking at the final effects of a medicine on the body and focuses instead on the exact molecular and chemical changes happening inside the cell. This textbook is designed specifically for Master 2 Biochemistry students to connect what they already know about protein shapes and structures with the actual creation of new drugs. Throughout these chapters, the inside of a cell is viewed not just as a collection of separate pathways, but as a highly connected network of molecular switches that follow strict rules of chemistry and physics. By combining their background in enzyme speeds, protein folding, and membrane fats, this text will guide them through how drugs physically bind to their targets , how to calculate drug binding using math formulas , and how cells pass messages along to change their behavior. Ultimately, while biochemistry identifies and explains the basic building blocks of life , molecular pharmacology shows them exactly how to control and adjust those blocks to fight disease.

Chapter 1: General principles of Pharmacology

Introduction

Pharmacology is the science that studies drugs and their interactions with living organisms. It differs from pharmacy, which is primarily concerned with the design, preparation, quality control, storage, and distribution of medicines. Today, pharmacology is an interdisciplinary field situated at the interface of numerous biological and medical sciences, including biochemistry, physiology, molecular biology, genetics, immunology, toxicology, and therapeutics.

The remarkable advances in molecular biology and genomics have profoundly transformed this discipline. Whereas classical pharmacology mainly focused on the observable effects of drugs on the organism, modern pharmacology seeks to understand the molecular mechanisms underlying these effects. In particular, it investigates the interactions between drugs and their biological targets, such as receptors, enzymes, ion channels, membrane transporters, and nucleic acids.

Pharmacology is divided into several complementary specialties. Molecular pharmacology aims to understand drug action at the molecular and cellular levels. Pharmacodynamics studies the biological effects of drugs and their mechanisms of action. Pharmacokinetics focuses on the fate of drugs within the body, from administration to elimination. Clinical pharmacology examines the effects of drugs in humans under normal and pathological conditions. Pharmacovigilance is concerned with the detection, assessment, understanding, and prevention of adverse drug reactions after marketing authorization. Finally, pharmacogenetics and pharmacogenomics investigate the influence of genetic variations on individual responses to drug therapy (figure 1).

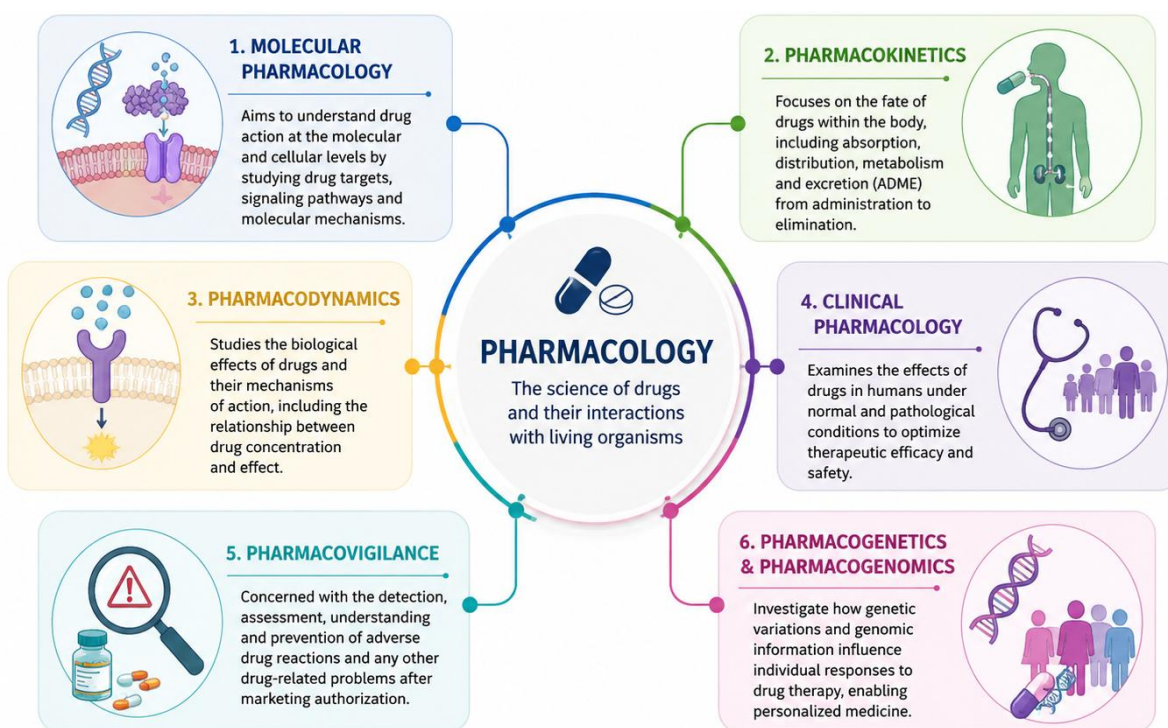


Figure 1. The major specialties of Pharmacology and their areas of investigation. Figure created by the author based on information adapted from Nefodov (2021).

1. Definition of a drug

According to Public Health legislation, a drug is defined as any substance or combination of substances presented as having curative or preventive properties with respect to human or animal diseases. This definition also includes any product administered to humans or animals for the purpose of establishing a medical diagnosis or restoring, correcting, or modifying physiological functions through pharmacological, immunological, or metabolic action.

2. Origin of drugs

The raw materials used in the production of drugs are traditionally referred to as drugs or crude drugs. These substances may originate from plant, animal, or mineral sources and have historically constituted the primary source of therapeutic agents. Natural products continue to provide numerous pharmacologically active molecules.

Medicinal plants have historically represented the most important source of active pharmaceutical ingredients. Well-known examples include morphine extracted from opium poppy (*Papaver somniferum*), quinine obtained from cinchona bark (*Cinchona spp.*), digoxin derived from purple foxglove (*Digitalis purpurea*), and paclitaxel isolated from the Pacific yew (*Taxus brevifolia*).

Some therapeutic substances originate from animals, such as heparin, while insulin was formerly extracted from porcine or bovine pancreas before the advent of biotechnology. Microorganisms also constitute a major source of drugs; notably, the discovery of penicillin by Alexander Fleming in 1928 marked the beginning of the antibiotic era.

Today, most drugs are obtained through chemical synthesis or semi-synthesis, allowing the production of compounds with optimized pharmacological properties, such as paracetamol (acetaminophen), omeprazole, and statins.

More recently, biotechnology and genetic engineering have enabled the production of complex biological medicines, including recombinant human insulin, erythropoietin, coagulation factors, and monoclonal antibodies, which are now widely used in the treatment of cancer, autoimmune diseases, and numerous chronic disorders.

3. Composition of drugs

A medicinal product consists of an active pharmaceutical ingredient (API) and excipients. The active ingredient is the substance responsible for the therapeutic effect. It is identified by its International Nonproprietary Name (INN), which allows the same substance to be recognized worldwide regardless of its commercial brand names.

Excipients are pharmacologically inactive substances added to ensure the manufacture, stability, preservation, and administration of the drug. They make it possible to obtain an appropriate dosage form (tablet, capsule, syrup, injectable solution, cream, suppository), improve taste and patient acceptability, modulate drug release, and enhance product stability.

Certain excipients, known as excipients with known effects, may have clinical implications in specific patient populations. Examples include lactose, sucrose, aspartame, and sodium salts.

For example, cellulose and starch facilitate tablet disintegration, sodium bicarbonate is used in effervescent tablets, and flavoring agents and sweeteners improve the acceptability of oral formulations.

4. Drug nomenclature

A single drug may be designated by several names corresponding to different levels of identification. This nomenclature distinguishes the chemical structure of the molecule, its active ingredient, and its commercial identity (table 1).

The **chemical name** precisely describes the molecular structure according to the rules of chemical nomenclature. It is generally complex and rarely used in clinical practice. For example, aspirin has the chemical name acetylsalicylic acid.

The **International Nonproprietary Name (INN)** is the official name assigned by the World Health Organization (WHO) to each active substance. It is internationally recognized and serves as a universal reference, avoiding confusion associated with commercial names. Thus, the INN of acetylsalicylic acid is aspirin.

The **brand name** or trade name is chosen by the pharmaceutical company marketing the drug. Several products containing the same active ingredient may therefore be marketed under different commercial names. For example, aspirin is marketed under brand names such as Aspegic®, Kardégic®, and Aspégic Nourrisson®, depending on the formulation and indication.

Knowledge of the INN is particularly important for healthcare professionals because it allows rapid identification of the active substance regardless of commercial branding.

Table 1. Examples of drug nomenclature.

Chemical Name	INN	Trade Name
Acetylsalicylic acid	Aspirin	Aspegic®, Kardégic®
N-acetyl-p-aminophenol	Paracetamol (Acetaminophen)	Doliprane®, Efferalgan®
Omeprazole	Omeprazole	Mopral®, Zoltum®

5. Types of drugs

According to their manufacturing process and regulatory status, drugs can be classified into several categories.

5.1. Pharmaceutical specialties

A pharmaceutical specialty is a medicinal product manufactured industrially in large quantities and marketed under a specific trade name after obtaining a Marketing Authorization (MA). Each production batch is subject to strict quality, safety, and efficacy controls.

Among pharmaceutical specialties, a distinction is made between the originator drug (or innovator drug) and generic drugs. The originator drug is the first product developed and marketed by a pharmaceutical company following years of research and development. This company benefits from patent protection, granting commercial exclusivity for a specified period.

After patent expiration, other manufacturers may produce generic drugs. These contain the same active ingredient, dosage, and pharmaceutical form as the originator drug. They must demonstrate bioequivalence to the reference product to ensure comparable efficacy and safety. However, excipients may differ.

Generic medicines are generally less expensive because research and development costs have already been recovered by the innovator company.

5.2. Compounded preparations

A compounded preparation is a medicinal product prepared extemporaneously by a pharmacist according to a medical prescription intended for a specific patient when no suitable industrially manufactured product is available.

5.3. Official preparations

An official preparation is prepared in a pharmacy according to a formula described in a pharmacopoeia or an officially recognized formulary. It may be prepared in advance and dispensed directly to patients.

5.4. Hospital preparations

Hospital preparations are manufactured within healthcare institutions to meet the specific needs of hospitalized patients when commercial pharmaceutical specialties are not suitable.

6. Regulatory classification of drugs

The dispensing of medicinal products is regulated according to the risks they may pose to public health.

6.1. Non-prescription drugs

Non-prescription drugs are available without a medical prescription. They are generally intended for the treatment of minor conditions and have a well-established safety profile. Depending on national regulations, some may be reimbursed, whereas others are sold over the counter without reimbursement.

6.2. Prescription drugs

Drugs included in regulatory schedules (table 2) require a medical prescription because of the potential risks associated with their use.

List I prescription medicines present the highest level of risk and require special monitoring. They include drugs with significant toxicity or a high potential for serious adverse effects.

List II prescription medicines also require medical supervision but generally pose a lower risk than Schedule I substances.

Narcotic drugs constitute a particular category that may induce physical or psychological dependence. Their prescription, dispensing, and storage are subject to strict regulations.

In addition, some drugs are subject to restricted prescribing conditions and may be reserved for specialists or hospital use because of their complexity or potential adverse effects.

Table 2. Main regulatory categories.

Category	Medical prescription required	Example
Non-prescription drugs	No	Paracetamol (certain formulations)
Schedule I	Yes	Antibiotics
Schedule II	Yes	Certain antihypertensive drugs
Narcotics	Yes (secure prescription form)	Morphine

7. Stages of drug development

Drug development is a long, complex, and costly process that may take between 10 and 15 years. Out of several thousand molecules initially investigated, only one generally reaches the market (figure 2).

7.1. Exploratory research and therapeutic target discovery

Drug development begins with the identification of a therapeutic target involved in a disease. This target is most commonly a receptor, enzyme, ion channel, or protein involved in a cellular signaling pathway.

Once the target has been identified and characterized, thousands of molecules are screened using High-Throughput Screening (HTS) techniques to identify compounds capable of modulating its activity. The most promising molecules are subsequently optimized to improve their efficacy, selectivity, and pharmacokinetic properties.

At this stage, promising compounds are generally patented, providing intellectual property protection for up to twenty years.

7.2. Preclinical studies

Preclinical studies are conducted before any administration to humans. Their objective is to evaluate the pharmacological activity, toxicity, and biological fate of the candidate drug.

Pharmacological studies are performed sequentially on isolated molecular systems, cell cultures, tissues, and animal models to confirm the efficacy of the candidate compound.

Toxicological studies aim to assess the potential risks associated with drug administration before human use. These studies are mainly conducted in animals according to standardized protocols and help determine the safety profile of the product and identify possible adverse effects.

Toxicological evaluation begins with acute toxicity studies, which assess the effects of a single administration over a period of at least seven days. These studies are conducted in at least two mammalian species using multiple routes of administration, one of which must correspond to the intended route in humans. They include determination of the median lethal dose (LD50) and analysis of dose-response relationships.

Subacute and chronic toxicity studies investigate functional, biochemical, and histopathological alterations resulting from repeated administration over prolonged periods. These studies also establish the conditions under which such effects occur according to dosage and administration schedule.

Potential effects on reproduction are evaluated through reproductive toxicity studies, which assess possible alterations in fertility in both males and females. These investigations are complemented by embryo-fetal and perinatal toxicity studies, designed to identify teratogenic or toxic effects on the embryo and fetus when the drug is administered during pregnancy.

Pharmacokinetic studies are also conducted to characterize the absorption, distribution, metabolism, and excretion (ADME) of the drug.

Safety assessment further includes mutagenicity studies, which investigate potential hereditary genetic alterations induced by the compound. When warranted by chemical structure or preliminary findings, carcinogenicity studies are performed to evaluate the drug's potential to induce cancer.

Finally, local tolerance studies assess the compatibility of the drug with tissues likely to come into contact with it during administration, such as the skin, mucous membranes, muscles, or blood vessels.

Together, these investigations establish the safety profile of the candidate drug and determine whether its benefit-risk ratio justifies progression to human clinical trials. At the end of the preclinical phase, a comprehensive dossier is submitted to regulatory authorities for authorization to initiate clinical studies.

7.3. Clinical studies

Clinical trials are conducted in humans according to protocols strictly regulated by national and international guidelines.

Phase I: Safety and tolerability studies

This phase is generally conducted in a small number of healthy volunteers. Its purpose is to evaluate safety, pharmacokinetic characteristics, and the maximum tolerated dose.

Phase II: Efficacy studies

This phase is performed in patients suffering from the target disease. It aims to demonstrate therapeutic activity and determine the optimal dose providing the best benefit-risk ratio.

Phase III: Comparative studies

This stage involves several hundred to several thousand patients. The investigational drug is compared with a reference treatment or placebo to confirm efficacy and safety. The resulting data support the application for Marketing Authorization.

Phase IV: Post-marketing surveillance

After marketing authorization and commercialization, the drug continues to be monitored under real-life conditions. This phase enables the detection of rare adverse effects, evaluation of long-term effectiveness, and study of specific patient populations.

7.4. Pharmacovigilance

Pharmacovigilance encompasses all activities aimed at detecting, assessing, understanding, and preventing adverse drug reactions and other drug-related problems after marketing.

It plays a critical role in public health by identifying rare or delayed risks that cannot be detected during clinical trials. The information collected may lead to modifications in prescribing information, additional warnings, restrictions of use, or, in some cases, withdrawal of the drug from the market.

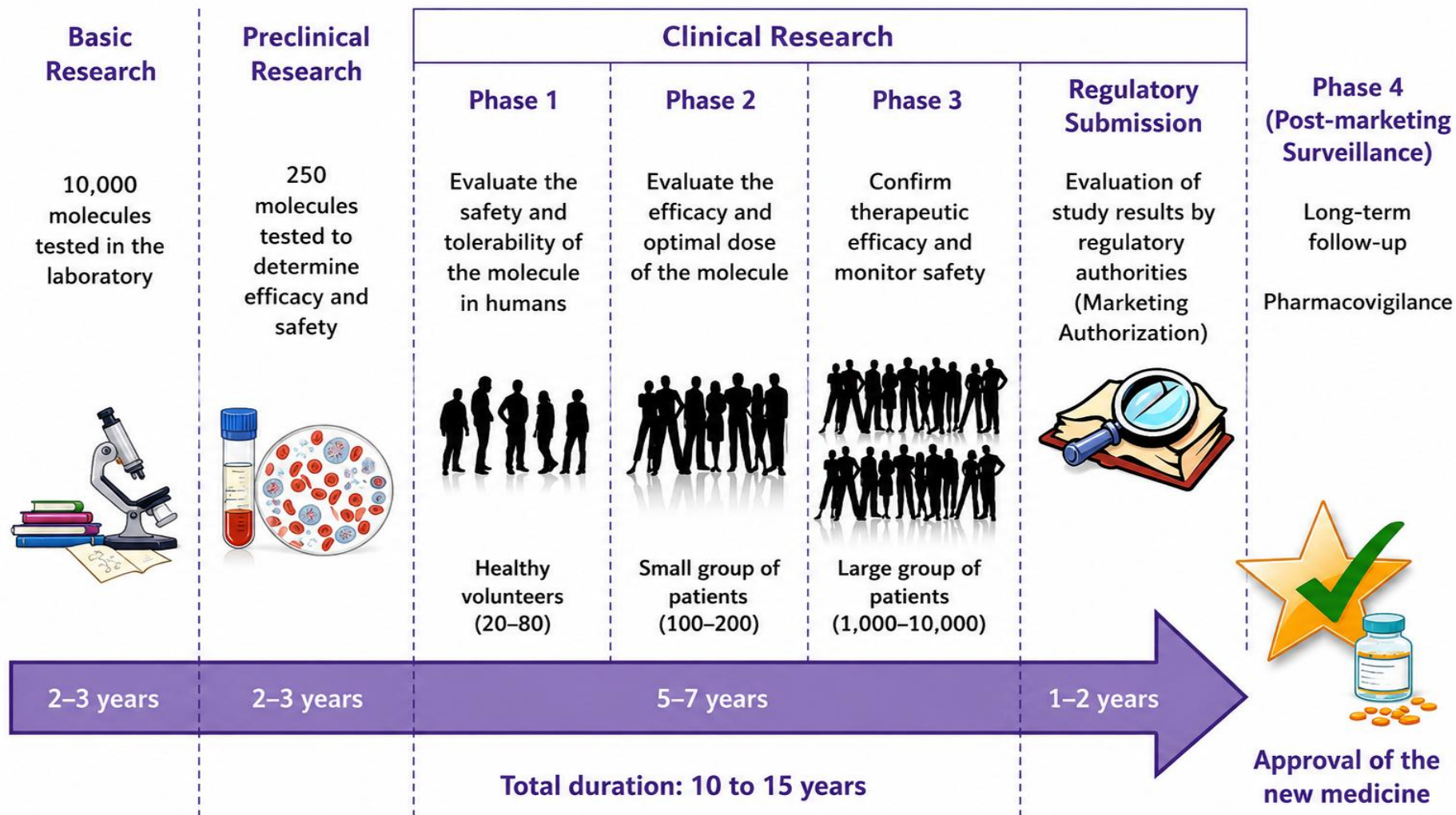


Figure 2. Major stages of drug development and regulatory approval. Adapted from the original figure published by COLCOT (MHCC website), with modifications by the author.

Chapter 2: Medicinal chemistry

I. Classification of drugs

Drugs can be classified according to different criteria depending on the scientific, therapeutic, or regulatory objectives pursued. No single classification system is universally satisfactory, as each approach presents both advantages and limitations.

1. Classification according to origin

This classification is based on the source from which the drug is obtained (figure 3).

1.1. Mineral-derived drugs

Mineral-derived drugs are medicinal products composed of substances obtained directly from minerals or derived from mineral sources. Although they represent only a small proportion of modern pharmaceuticals, they continue to play an important role in clinical practice, particularly in gastroenterology and dermatology. Common examples include **kaolin**, used as an adsorbent in gastrointestinal disorders; **talc**, widely employed in dermatological and pharmaceutical formulations; **calcium carbonate**, used as an antacid and calcium supplement; and **magnesium sulfate**, which is utilized as a laxative, electrolyte replacement therapy, and anticonvulsant in specific clinical situations.

1.2. Plant-derived drugs

Plant-derived drugs have been the primary source of therapeutic agents for centuries and have played a fundamental role in the development of modern pharmacology. Many important medicines were originally isolated from medicinal plants and continue to be used in clinical practice today. Notable examples include **morphine**, obtained from the opium poppy (*Papaver somniferum*), which is widely used for the management of severe pain; **quinine**, extracted from the bark of *Cinchona* species and historically employed in the treatment of malaria; **digoxin**, derived from the foxglove plant (*Digitalis purpurea*) and used in certain cardiac disorders; and **paclitaxel**, isolated from the Pacific yew (*Taxus brevifolia*), an important anticancer agent.

1.3. Chemically derived drugs

Chemically derived drugs currently constitute the majority of medicines used in modern therapeutics. They include compounds obtained either by total chemical synthesis, in which the entire molecule is produced through chemical reactions, or by semi-synthesis, where naturally occurring substances are chemically modified to improve their pharmacological properties. Advances in organic chemistry and medicinal chemistry have enabled the development of numerous safe and effective synthetic drugs. Representative examples include **paracetamol** (acetaminophen), widely used as an analgesic and antipyretic; **omeprazole**, a proton pump inhibitor employed in the treatment of acid-related gastrointestinal disorders; **atorvastatin**, a lipid-lowering agent used to prevent cardiovascular diseases; and **ibuprofen**, a nonsteroidal anti-inflammatory drug (NSAID) commonly prescribed for pain, inflammation, and fever.

1.4. Animal-derived drugs

Animal-derived drugs represent a relatively small category of therapeutic agents obtained from animal tissues or biological products. Despite their limited number, they remain clinically important in several medical fields. Examples include **heparin**, an anticoagulant traditionally extracted from porcine intestinal mucosa and widely used for the prevention and treatment of thromboembolic disorders; **immunoglobulins**, which are purified antibodies used in the

management of immune deficiencies and certain autoimmune diseases; and various **blood-derived products**, such as clotting factors and albumin, which are essential for the treatment of coagulation disorders, hemorrhagic conditions, and other serious medical situations.

1.5. Biotechnology-derived drugs

Biotechnology-derived drugs, also known as biopharmaceuticals, are produced using advanced techniques such as genetic engineering, recombinant DNA technology, and cell culture systems. Unlike conventional small-molecule drugs synthesized chemically, these medicines are typically large and complex biological molecules designed to mimic or modulate natural physiological processes. Important examples include recombinant **insulin**, used in the treatment of diabetes mellitus; **erythropoietin**, which stimulates red blood cell production and is prescribed for certain forms of anemia; **monoclonal antibodies**, which specifically target disease-associated molecules and have revolutionized the treatment of cancer, autoimmune diseases, and inflammatory disorders; and **recombinant coagulation factors**, used in the management of hemophilia and other bleeding disorders. Owing to their high specificity and therapeutic efficacy, biopharmaceuticals now occupy a central position in modern medicine and play a major role in the treatment of cancers, autoimmune diseases, rare genetic disorders, and many chronic conditions.

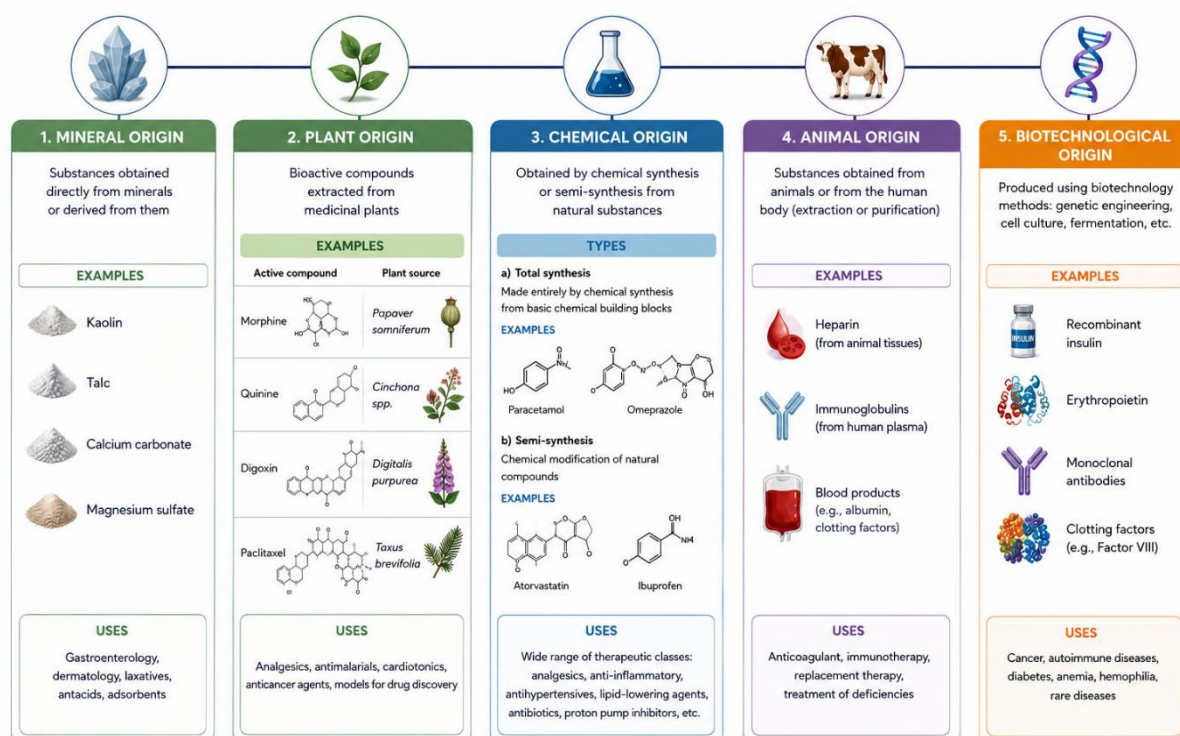


Figure 3. Origins of modern medicines. Figure created by the author based on information adapted from Atanasov et al. (2021).

2. Chemical classification

Chemical classification organizes drugs according to similarities in their molecular structures. This approach is particularly valuable in medicinal chemistry because it facilitates drug discovery, structure–activity relationship (SAR) studies, and the prediction of pharmacological properties. However, drugs belonging to the same chemical family may exhibit markedly different biological activities and therapeutic applications, highlighting the importance of three-dimensional structure and functional groups in determining biological activity.

Example: Sulfonamides

Sulfonamides belong to the same chemical family but have diverse therapeutic applications:

- Sulfamethoxazole: antibacterial agent
- Furosemide: diuretic
- Acetazolamide: carbonic anhydrase inhibitor
- Sulfasalazine: anti-inflammatory agent

3. Pharmacological classification

Pharmacological classification categorizes drugs according to their mechanism of action or their specific molecular targets, making it particularly relevant in molecular pharmacology. This approach groups together drugs that interact with the same biological target, such as **β -blockers** acting on β -adrenergic receptors, **ACE inhibitors** targeting angiotensin-converting enzyme, **statins** inhibiting HMG-CoA reductase, and **calcium channel blockers** acting on Ca^{2+} channels. By focusing on drug–target interactions, this classification provides valuable insight into the cellular and molecular mechanisms underlying therapeutic effects.

4. Therapeutic classification

This classification groups drugs according to the diseases they treat or the physiological systems they affect. It encompasses major therapeutic areas such as cardiology and angiology, infectious diseases, neurology and psychiatry, endocrinology, oncology, pulmonology, gastroenterology, dermatology, rheumatology, hematology, and immunology. Because it is directly related to clinical indications and patient management, this classification is the most widely used in everyday medical practice and serves as a practical tool for healthcare professionals when selecting appropriate treatments.

II. Chemical structure and therapeutic properties**1. Structure–activity relationships (SAR)**

Structure–activity relationships describe the relationship between the chemical structure of a molecule and its biological activity. The pharmacological properties of a drug are directly influenced by its molecular architecture, and even small structural modifications can lead to significant changes in potency, selectivity, efficacy, or toxicity. SAR studies aim to identify the specific structural features responsible for a drug's interaction with its biological target and therapeutic effects. A classic example is the modification of **morphine**, which has led to the development of related compounds such as **codeine**, **hydromorphone**, and **oxycodone**. Although these drugs share a common chemical scaffold, differences in their structures result in distinct pharmacological profiles, demonstrating the critical role of molecular structure in determining drug activity.

2. Pharmacophore

A pharmacophore represents the minimal three-dimensional arrangement of structural features required for a molecule to be recognized by and interact effectively with a specific biological target. These essential features may include positively or negatively **charged groups**, **hydrophobic regions**, **hydrogen-bond donors**, and **hydrogen-bond acceptors**, which collectively determine the strength and specificity of ligand–target interactions (figure 4). Identification of the pharmacophore is a fundamental step in modern drug discovery, as it

facilitates the design, optimization, and virtual screening of compounds with desired biological activities while improving their selectivity toward the target of interest.

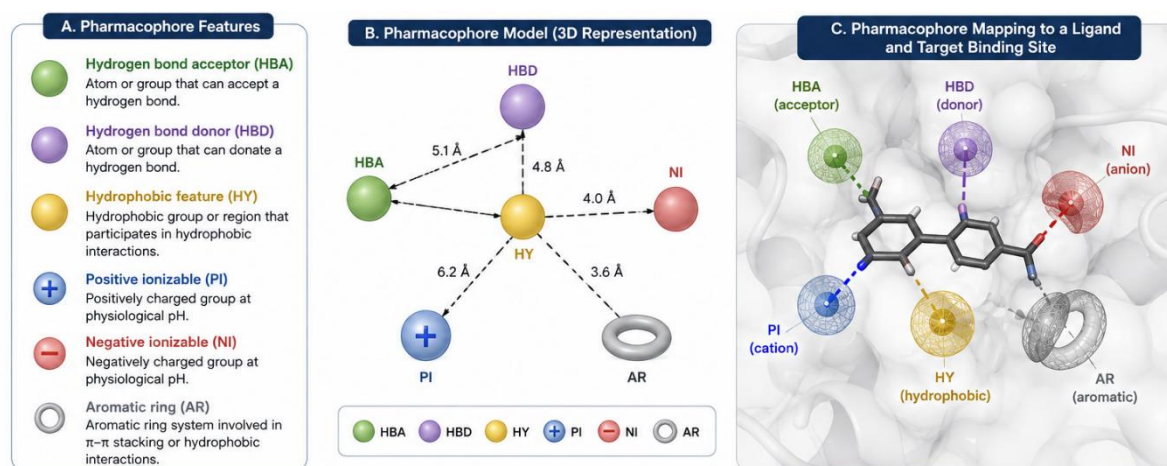


Figure 4. Example of a pharmacophore and its interactions with a biological target. A. Pharmacophore features. B. Pharmacophore model (3D representation). C. Pharmacophore mapping to a ligand and target binding site. Figure created by the author based on information adapted from Tyagi et al. (2022).

3. Physicochemical properties influencing therapeutic activity

Physicochemical properties play a fundamental role in determining the therapeutic activity of a drug by influencing its absorption, distribution, interaction with biological targets, and elimination from the body. One of the most important parameters is **lipophilicity**, which reflects a drug's ability to cross biological membranes and is commonly expressed by the partition coefficient (LogP), defined as the logarithm of the concentration ratio of the drug in octanol and water. Another key property is the **pK_a**, which determines the ionization state of a drug under different physiological conditions. Because only the ionized or non-ionized forms of a molecule may readily cross membranes, pK_a significantly affects drug absorption, distribution, and elimination. **Solubility** is also crucial, as adequate solubility generally improves drug dissolution and bioavailability, thereby enhancing therapeutic efficacy. Finally, **molecular weight** influences membrane permeability, with smaller molecules generally diffusing more easily across biological barriers than larger compounds. Together, these physicochemical characteristics are critical determinants of a drug's pharmacokinetic behavior and overall therapeutic performance.

4. Rational drug design

Rational drug design is a cornerstone of modern drug discovery and relies extensively on detailed structural information about therapeutic targets. According to Fischer's classical **lock-and-key model**, a drug interacts with its biological target through a precise complementarity of shape and chemical properties. Consequently, knowledge of the three-dimensional structure of a target protein enables researchers to design molecules that bind with high affinity and selectivity to specific binding sites. This strategy, known as **Structure-Based Drug Design (SBDD)**, has become a powerful approach for the discovery and optimization of new therapeutic agents (figure 5). Advances in structural biology techniques, including X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, cryo-electron microscopy

(cryo-EM), and molecular modeling, have greatly enhanced the ability to characterize drug targets at the atomic level. These technologies facilitate the identification of binding sites, the virtual screening of large compound libraries, the optimization of ligand–target interactions, and the prediction of ADMET properties (Absorption, Distribution, Metabolism, Excretion, and Toxicity), thereby accelerating the development of safer and more effective drugs.

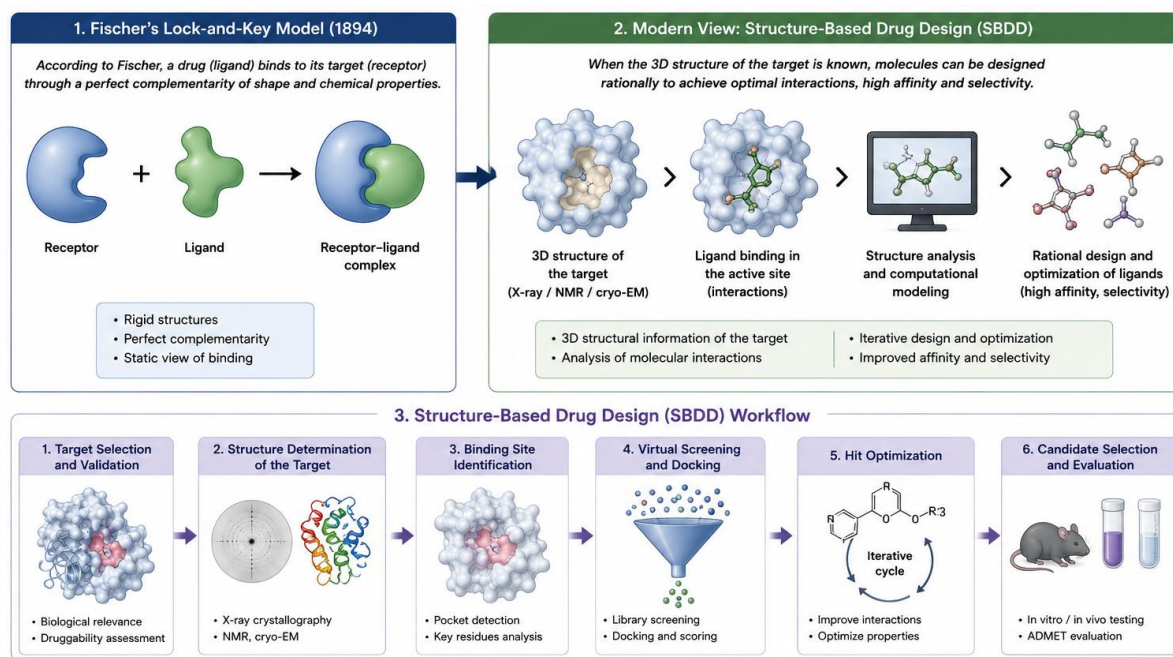


Figure 5. Principle of rational drug design from the classical lock-and-key model to modern structure-based drug design (SBDD). 1. Fischer's lock-and-key model (1894). 2. Modern view (SBDD). 3. SBDD workflow. The goal of SBDD is to use the structural information of the target to design molecules that bind with high affinity and selectivity, leading to safer and more effective drug candidates. Figure created by the author based on information adapted from Anderson (2003).

Chapter 3: Mechanisms of drug action

Introduction

The therapeutic effect of a drug results from its interaction with a specific biological target. In most cases, this target is a receptor, but it may also be an enzyme, ion channel, transporter protein, nucleic acid, structural protein, or another cellular component. Drug action depends on the ability of the molecule to recognize and bind its target with sufficient affinity and selectivity. Once bound, the drug may activate, inhibit, block, replace, or modulate the function of its target, thereby altering cellular physiology and producing a therapeutic response.

At the molecular level, drug action can be broadly classified into six major mechanisms: replacement of deficient endogenous substances, modulation of endogenous metabolic pathways, interaction with physiological receptors, modulation of ion channels and transport systems, inhibition of pathogens, and interaction with intracellular structures such as DNA and cytoskeletal proteins (figure 6).

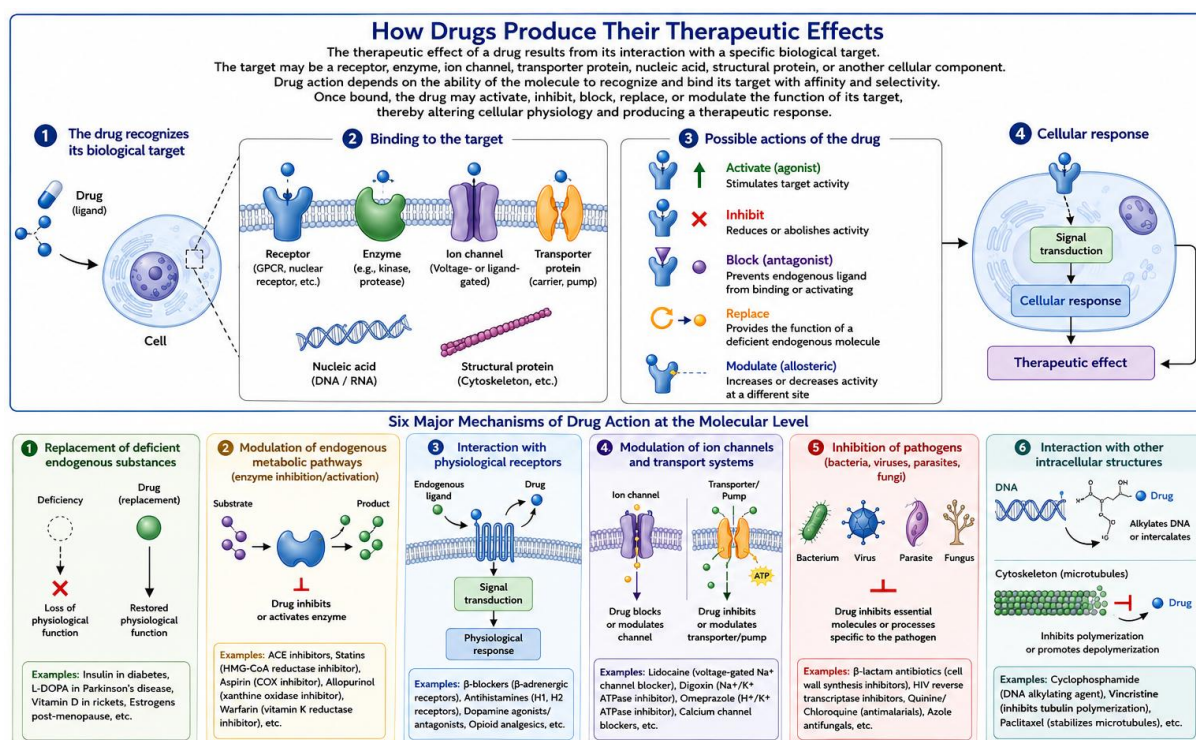


Figure 6. Major molecular targets and mechanisms of drug action. Author-created figure based on information adapted from Santos et al. (2016) and Kamepalli et al. (2025).

I. Major molecular targets and mechanisms of drug action

1. Replacement of essential endogenous substances (replacement therapy)

Replacement therapy consists of administering a substance that is absent, deficient, or produced in insufficient quantities by the body. Rather than modifying a physiological pathway, these drugs restore normal biological function by compensating for the deficiency.

1.1. Replacement of deficient hormones or mediators

Hormone replacement therapy provides some of the most important examples. Recombinant human **insulin** is used in diabetes mellitus to compensate for deficient insulin secretion, while **levodopa** serves as a precursor of dopamine in Parkinson's disease, where dopaminergic

neurons progressively degenerate. **Coagulation factors** VIII and IX are administered in hemophilia to restore normal blood clotting. Additional examples include levothyroxine in hypothyroidism, growth hormone in growth hormone deficiency and cortisol replacement in adrenal insufficiency

1.2. Replacement of nutritional deficiencies

Replacement therapy is also commonly used to correct nutritional deficiencies. **Vitamin D** supplementation is employed in rickets and osteomalacia, whereas **vitamin B12** is administered to patients with pernicious anemia. **Iron** supplementation is required in iron-deficiency anemia.

1.3. Replacement of physiological hormonal decline

Replacement therapy may also be used to compensate for the natural decline in hormone production that occurs with aging or as a consequence of physiological changes. In such cases, the objective is not to correct a pathological deficiency caused by impaired synthesis, but rather to restore hormone levels that have decreased below those required to maintain normal physiological functions and quality of life. Common examples are **estrogen** replacement therapy in postmenopausal women and **testosterone** replacement therapy in men with hypogonadism, a condition characterized by insufficient testosterone production by the testes.

2. Modulation of endogenous metabolic pathways

Many drugs exert their effects by modifying metabolic pathways controlled by enzymes. Because **enzymes** regulate the synthesis and degradation of endogenous molecules, they constitute major pharmacological targets.

Angiotensin-converting enzyme (ACE) inhibitors such as captopril, enalapril, and lisinopril reduce the production of angiotensin II, a potent vasoconstrictor involved in blood pressure regulation. Similarly, **statins such as** simvastatin, atorvastatin and rosuvastatin inhibit **HMG-CoA reductase**, the rate-limiting enzyme of cholesterol biosynthesis, thereby lowering plasma cholesterol concentrations and reducing cardiovascular risk.

Another important example involves **vitamin K antagonists**, such as **warfarin**, which inhibit **vitamin K epoxide reductase** and prevent the activation of clotting factors.

Aspirin exerts its pharmacological effects through the irreversible inhibition of **cyclooxygenase enzymes** (COX-1 and COX-2). Inhibition of **COX-2** contributes primarily to its anti-inflammatory, analgesic, and antipyretic effects by reducing the synthesis of pro-inflammatory **prostaglandins**. In contrast, inhibition of platelet **COX-1** decreases the production of **thromboxane A₂** (TXA₂) by preventing the formation of its precursor, prostaglandin H₂ (PGH₂). The resulting reduction in TXA₂ synthesis inhibits platelet activation and aggregation, thereby producing an antithrombotic effect.

Allopurinol inhibits **xanthine oxidase** (XO), reducing uric acid production and thereby treating gout and hyperuricemia.

Additional examples include **acetylcholinesterase inhibitors** used in Alzheimer's disease and **DPP-4 inhibitors** used in type 2 diabetes mellitus.

3. Interaction with physiological receptors

A large proportion of therapeutic agents act by interacting with **receptors** that normally bind endogenous mediators. These receptors convert extracellular signals into intracellular responses through complex signaling pathways.

Adrenergic receptors constitute a major receptor family targeted by numerous drugs. These G protein-coupled receptors (GPCRs) are activated by catecholamines such as epinephrine and norepinephrine. **β -blockers**, including **propranolol**, **metoprolol**, and **atenolol**, act as antagonists of β -adrenergic receptors and are widely used in hypertension, heart failure, ischemic heart disease, and arrhythmias. **Salbutamol** and **dobutamine** are examples of adrenergic receptor **agonists**; salbutamol selectively activates β_2 -adrenergic receptors to induce bronchodilation, whereas dobutamine primarily stimulates β_1 -adrenergic receptors to enhance cardiac contractility and output.

Histamine receptors represent another important pharmacological target (figure 7). **H1 receptor antagonists** such as **cetirizine** and **loratadine** are used in allergic disorders, whereas **H2 receptor antagonists** such as **famotidine** reduce gastric acid secretion. Histamine itself is a key inflammatory mediator that promotes vasodilation, increases vascular permeability, stimulates gastric secretion, and contributes to bronchoconstriction. Histamine receptor **agonists** have limited therapeutic applications. The only commercially available histaminergic agonist, **betahistine** (Serc®), acts as a partial agonist at H1 receptors and an antagonist at H3 receptors and is primarily used for the symptomatic treatment of Ménière's disease and recurrent vertigo.

Other clinically important receptor systems include **cholinergic** receptors, **dopamine** receptors, **opioid** receptors, and **serotonin** receptors.

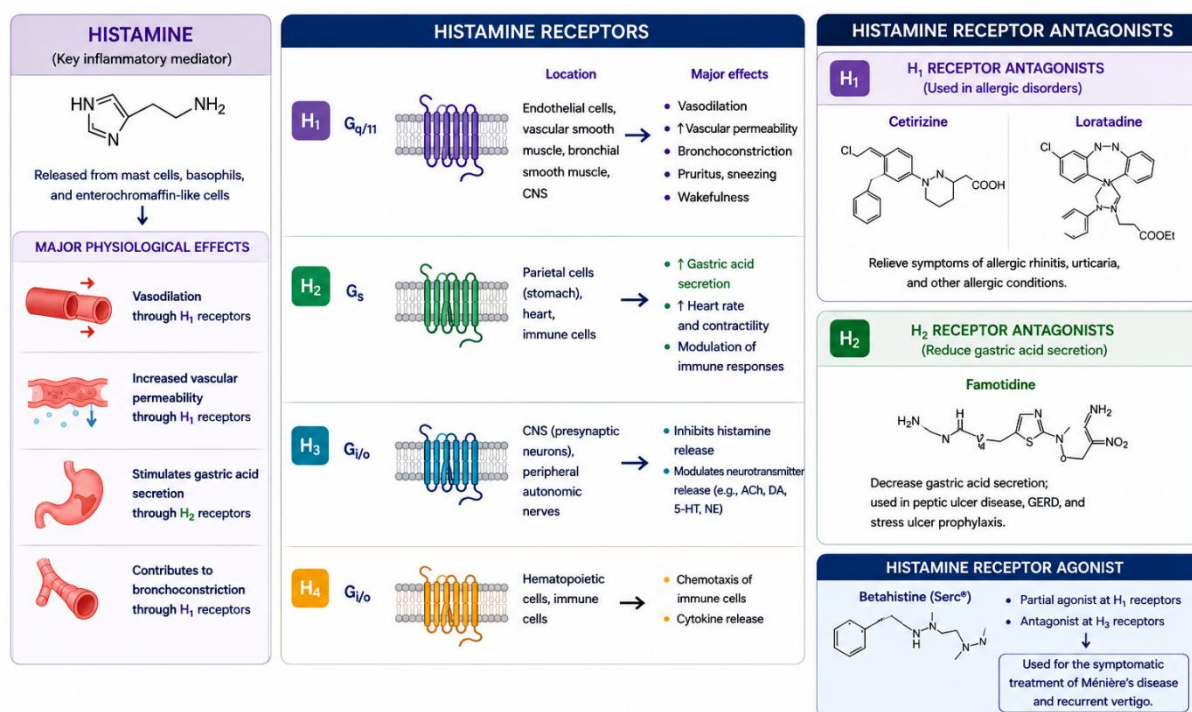


Figure 7. Histamine receptors as pharmacological targets. Author-created figure based on information adapted from Sriram et al. (2018).

4. Interaction with ion channels and membrane transporters

Ion channels and membrane transporters are essential for maintaining cellular excitability and ionic homeostasis. Many drugs act by modulating these proteins (figure 8).

Cardiac glycosides such as **digoxin** inhibit the **Na⁺/K⁺-ATPase pump**. This inhibition increases intracellular sodium concentrations, indirectly promoting calcium accumulation and enhancing myocardial contractility.

Proton pump inhibitors, including **omeprazole** and **pantoprazole**, block the **gastric H⁺/K⁺-ATPase** and are widely used in acid-related gastrointestinal disorders.

Local anesthetics such as **lidocaine** and **articaïne** reversibly block **voltage-gated sodium channels**, preventing the propagation of action potentials and thereby producing local anesthesia.

Calcium channel blockers, including **verapamil** and **amlodipine**, reduce calcium influx into excitable cells and are commonly used in cardiovascular diseases.

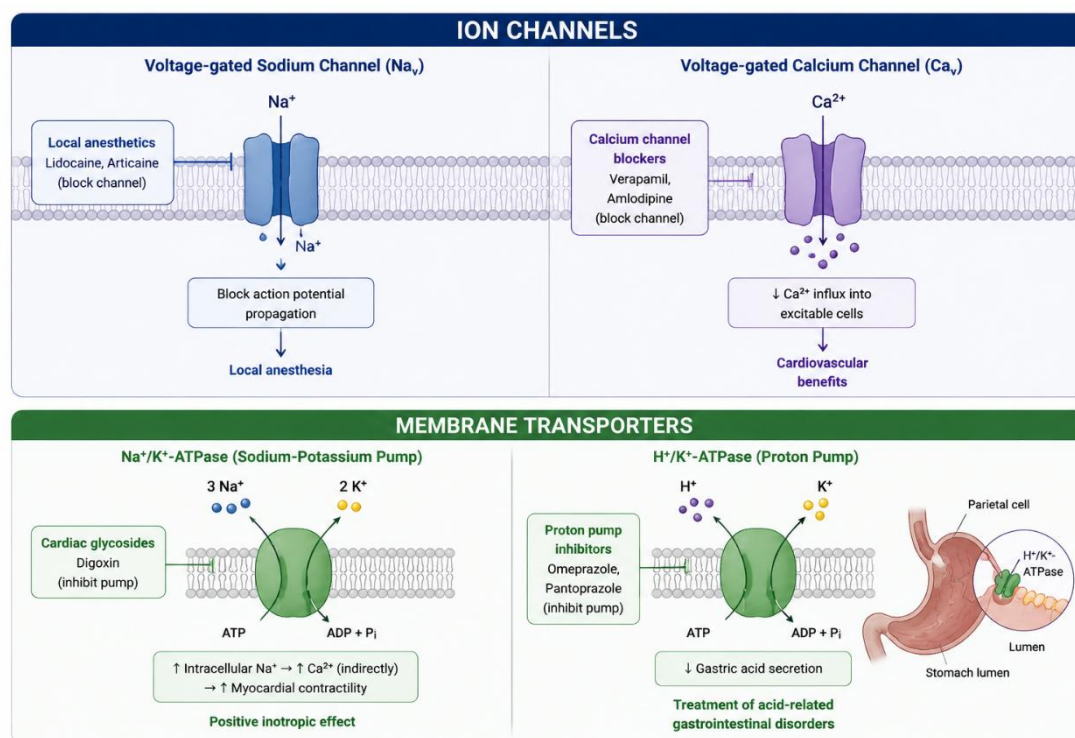


Figure 8. Examples of ion channels and membrane transporters targeted by therapeutic drugs. Created by the author based on information adapted from Picci et al. (2022).

5. Drugs targeting pathogens

Many antimicrobial agents selectively target molecules essential for the survival of pathogens while minimizing toxicity to host cells. **Antiretroviral** drugs such as **zidovudine** and **tenofovir** inhibit **HIV reverse transcriptase**, preventing viral replication (figure 9). **β-lactam** antibiotics inhibit bacterial cell wall synthesis by targeting **penicillin-binding proteins**, leading to bacterial death (figure 10). **Antimalarial** agents such as **quinine**, **chloroquine**, and **artemisinin** interfere with critical stages of the *Plasmodium* life cycle. Additional examples include azole **antifungals**, which inhibit ergosterol synthesis, and **antiparasitic** agents such as ivermectin.

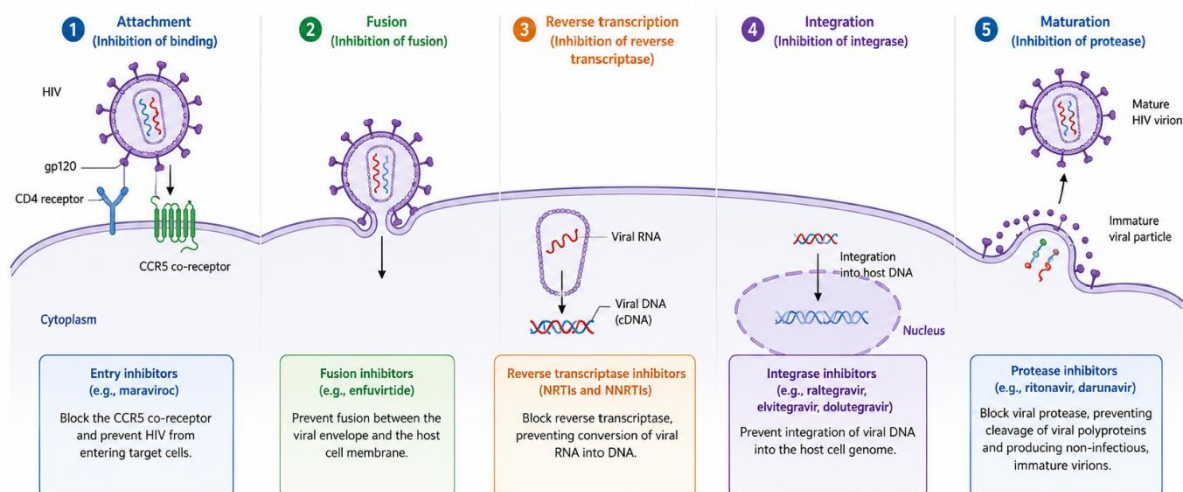


Figure 9. Mechanisms of action of antiretroviral drugs targeting the HIV life cycle. Author-created figure based on information adapted from Pharmacomédicale (n.d.).

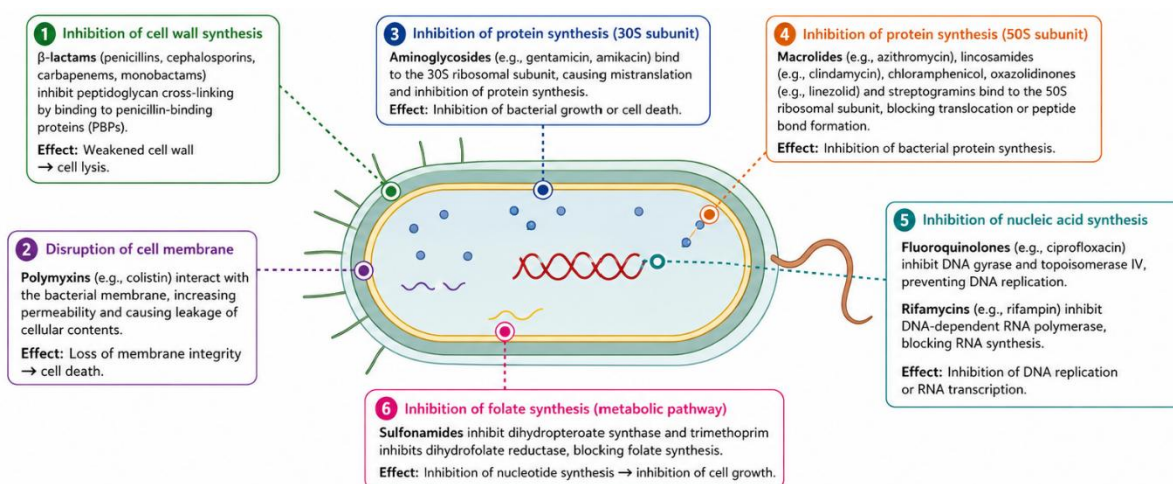


Figure 10: Major targets of antibiotic drugs. Created by the author based on information adapted from Kapoor et al. (2017).

6. Other cellular targets

Certain drugs act directly on intracellular structures essential for cell proliferation and survival. These mechanisms are particularly important in cancer chemotherapy.

6.1. DNA-targeting drugs

Cyclophosphamide belongs to the class of **alkylating agents** and exerts its antitumor activity by forming covalent bonds with DNA. These DNA cross-links prevent replication and transcription, ultimately triggering apoptosis.

6.2. Microtubule-targeting drugs

Vincristine targets the cytoskeleton by inhibiting **tubulin polymerization** and preventing mitotic spindle formation. As a result, cells become arrested during mitosis and undergo programmed cell death.

Chapter 4: Receptors involved in pharmacological action

1. Pharmacological receptors: general concepts

A pharmacological receptor is a biological macromolecule that specifically recognizes a ligand and converts this interaction into a cellular response. Receptors act as molecular sensors that receive, process, and transmit information through intracellular signaling pathways. This specific recognition takes place at a distinct region of the macromolecule known as the "active site". The interaction between a ligand and its receptor is driven by low-intensity, non-covalent forces, rendering the binding event characteristically labile and reversible. High specificity of fixation is achieved because the structural configurations, chemical functional groups, and electrical charges of both the ligand and the active site strictly correspond to one another (figure 11). Drug binding induces a conformational change in the receptor, generating a stimulus that is translated into a biological effect through signal transduction mechanisms (figure 12). Most therapeutic drugs act through a limited number of molecular targets, including receptors, enzymes, ion channels, transporters, and nucleic acids. Among these targets, receptors represent the largest and most important class.

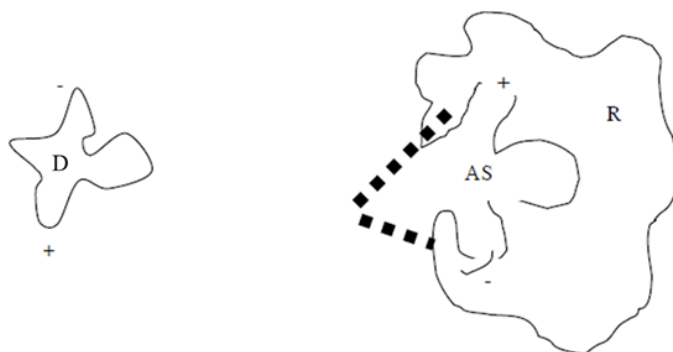


Figure 11: Receptor and binding site. A receptor is a macromolecule whose active site constitutes the region to which the drug (active ingredient) binds, resembling a "lock and key" mechanism. R: receptor, AS: active site, D: drug. Adapted from Dangoumau et al. (2006).

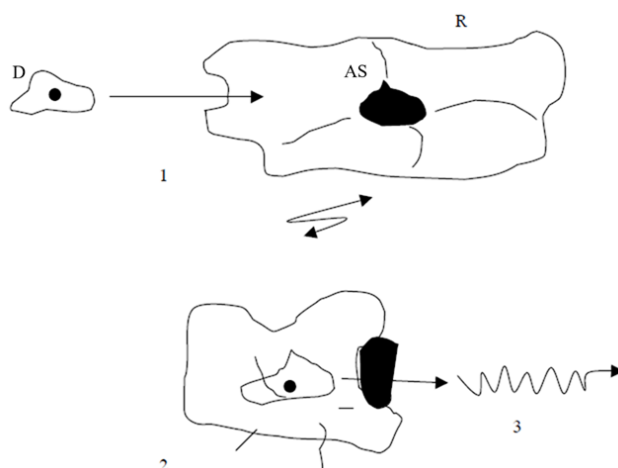


Figure 12: The stimulus. Binding of the drug (1) to the active site induces a conformational change in the receptor (2) that enables it to initiate a downstream biochemical process known as coupling (3). Adapted from Dangoumau et al. (2006).

2. Functional consequences of drug–receptor interactions

Drug binding does not necessarily produce the same biological outcome. Depending on its intrinsic efficacy, a ligand may activate, inhibit, or modulate receptor signaling.

2.1. Full agonists

Agonists are molecules that bind to and activate receptors, thereby producing a biological response. They possess both affinity for the receptor and intrinsic efficacy. Agonist binding stabilizes the active receptor conformation and initiates intracellular signaling. Examples include dobutamine at β_1 -adrenergic receptors and salbutamol at β_2 -adrenergic receptors.

2.2. Competitive antagonists

Competitive antagonists bind reversibly to the same receptor site as the agonist but do not activate the receptor. By occupying the binding site, they prevent agonist binding and reduce receptor activation. Their inhibitory effect can be overcome by increasing the concentration of the agonist. Propranolol is a classical example of a competitive β -adrenergic receptor antagonist.

2.3. Partial agonists

Partial agonists bind to and activate receptors but produce a weaker response than full agonists, even when all receptors are occupied. In the presence of a full agonist, they may reduce the overall response by competing for receptor binding. Buprenorphine, a partial μ -opioid receptor agonist, is a well-known example.

2.4. Inverse agonists

Some receptors display constitutive activity in the absence of a ligand. Inverse agonists bind preferentially to the inactive receptor state and reduce basal receptor activity below normal levels. Unlike antagonists, they actively suppress receptor signaling. Many H1 antihistamines, such as cetirizine and loratadine, act as inverse agonists.

2.5. Allosteric modulators

Allosteric modulators bind to receptor sites distinct from the orthosteric binding site of the endogenous ligand. Positive allosteric modulators enhance receptor activity, whereas negative allosteric modulators reduce it. Benzodiazepines are positive allosteric modulators of GABAA receptors.

2.6. Receptor desensitization

Prolonged or repeated receptor stimulation may lead to a progressive reduction in receptor responsiveness, a phenomenon known as desensitization. This process may involve receptor phosphorylation, internalization, or downregulation. Receptor desensitization contributes to the development of drug tolerance during chronic treatment.

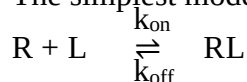
2.7. Receptor occupancy and pharmacological response

The intensity of a drug response generally increases with receptor occupancy. However, maximal effects are often achieved before all receptors are occupied because of the presence of **spare receptors**. Receptor reserve increases tissue sensitivity and provides functional compensation in pathological conditions.

3. Receptor–ligand kinetics

The interaction between a ligand and its receptor is generally reversible and can be described by the law of mass action.

The simplest model is:



where R represents the receptor, L the ligand, and RL the receptor–ligand complex.

Binding is characterized by:

- Association rate constant (k_{on})
- Dissociation rate constant (k_{off})

This dynamic equilibrium relies on two distinct velocity rates:

- **Association rate (v_{on}):** The velocity at which new receptor-ligand complexes form per unit of time. It depends directly on the concentration of both free species and the association rate constant (k_{on} , expressed in $\text{M}^{-1} \cdot \text{min}^{-1}$).

$$v_{\text{on}} = [R] \cdot [L] \cdot k_{\text{on}}$$

- **Dissociation rate (v_{off}):** The velocity at which existing complexes spontaneously break apart per unit of time. It depends strictly on the concentration of the complex and the dissociation rate constant k_{off} , expressed in min^{-1}).

$$v_{\text{off}} = [RL] \cdot k_{\text{off}}$$

3.1. Equilibrium dissociation constant (K_d)

At dynamic equilibrium, the rate of association equals the rate of dissociation ($v_{\text{on}} = v_{\text{off}}$). Rearranging this equality highlights a fundamental constant in pharmacology:

$$[R] \cdot [L] \cdot k_{\text{on}} = [RL] \cdot k_{\text{off}}$$

$$[R] \cdot [L] / [RL] = k_{\text{off}} / k_{\text{on}}$$

The affinity of a ligand is quantified by:

$$K_d = k_{\text{off}} / k_{\text{on}}$$

A lower K_d indicates stronger receptor affinity.

K_d is expressed in molar units (M), typically falling within the nanomolar (nM) or picomolar (pM) range for high-affinity biological systems.

3.2. Receptor occupancy

According to the law of mass action:

$$[RL] / R_T = [L] / K_d + [L]$$

This equation generates the classical saturation binding curve.

To see exactly how R_T fits into the equation, we can look at the conservation of mass for receptors:

$$R_T = [R] + [RL]$$

Where:

- R_T : Total concentration of receptors.
- $[R]$: Concentration of free (unbound) receptors.
- $[RL]$: Concentration of the receptor-ligand complex (bound receptors).

The ratio $[RL] / R_T$ represents the **fractional occupancy** (the proportion of total receptors that are successfully bound by the ligand).

- If $[RL] / R_T = 0$, no receptors are bound.
- If $[RL] / R_T = 1$ (or 100%), the system has reached **saturation**, meaning every single available receptor R_T is occupied by a ligand $[L]$.

This is why, as the ligand concentration $[L]$ becomes exceptionally high, the curve asymptotically approaches a plateau at 1, yielding the classic hyperbolic saturation binding curve.

3.3. Maximum binding capacity (B_{max})

In experimental radioligand binding assays, R_T value is explicitly designated as B_{max} (maximum binding capacity). It represents the total receptor population available for ligand binding. It provides information about receptor density and expression. It is expressed contextually as the number of binding sites per cell, or normalized to tissue mass (e.g., fmol/mg of membrane proteins).

3.4. Mathematical derivation of fractional occupancy

By isolating the free receptor concentration from our mass conservation equation ($[R] = R_T - [RL]$) and substituting it into the equilibrium affinity equation, we can derive the relationship governing fractional occupancy:

$$K_d = \frac{(R_T - [RL])[L]}{[RL]}$$

$$K_d [RL] = R_T [L] - [RL] [L]$$

$$[RL] (K_d + [L]) = R_T \cdot [L]$$

This yields the fundamental equation for **fractional occupancy**:

$$\frac{[RL]}{R_T} = \frac{[L]}{K_d + [L]}$$

Alternatively, when expressing specific binding directly as a function of B_{max} :

$$[RL] = \frac{B_{max} \cdot [L]}{K_d + [L]}$$

3.5. Biological analysis of the hyperbolic curve

Plotted graphically ($[RL]$ on the y-axis vs. $[L]$ on the x-axis), this relationship generates a classic **hyperbolic saturation curve** (figure 13):

- **Zero occupancy:** When no ligand is present ($[L] = 0$), the fraction $[RL] / R_T = 0$.
- **Half-saturation (K_d half-maximal value):** When the concentration of free ligand perfectly matches the dissociation constant ($[L] = K_d$), the equation simplifies:

$$\frac{[RL]}{B_{\max}} = \frac{K_d}{K_d + K_d} = \frac{1}{2}$$

Hence, K_d is operationally defined as the concentration of free ligand at which exactly 50% of the total receptor sites are occupied.

- **Asymptotic saturation:** As free ligand concentration reaches exceptionally high levels ($[L] \gg K_d$), the term $(K_d + [L])$ approaches $[L]$. The fraction $[RL] / R_T$ asymptotically approaches 1 (or 100% occupancy), where every available receptor is bound.

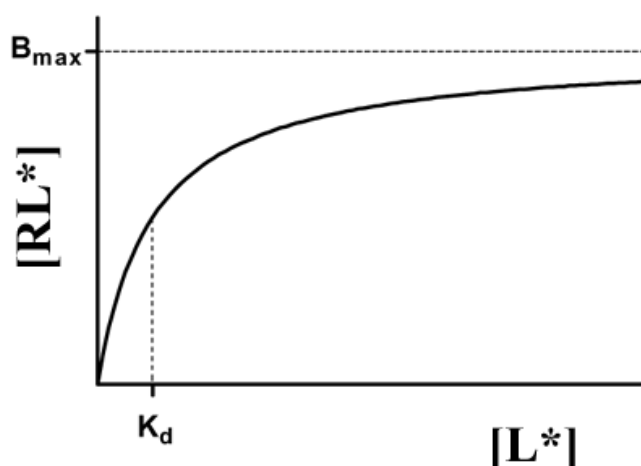


Figure 13: Representative saturation binding curve demonstrating the interaction between a radiolabeled or fluorescent ligand and its specific receptor. The concentration of bound ligand $[RL^*]$ is plotted against the concentration of free ligand $[L^*]$. $B_{\max}$ represents the maximum binding capacity of the receptor population, and K_d denotes the equilibrium dissociation constant, indicating the ligand concentration at half-maximal binding.

3.6. Graphical linearization: the Scatchard transformation

Because resolving exact asymptotes ($B_{\max}$) from hyperbolic curves can introduce statistical errors, pharmacologists linearize saturation data using the **Scatchard transformation** (figure 14).

By algebraically rearranging the saturation binding equation:

$$[RL] \cdot K_d + [RL] \cdot [L] = B_{\max} \cdot [L]$$

$$[RL] \cdot K_d = (B_{\max} \cdot [L]) - ([RL] \cdot [L])$$

Dividing both sides by $[L]$ and then by K_d yields:

$$\frac{[RL]}{[L]} = \frac{B_{\max} - [RL]}{K_d}$$

$$\frac{[RL]}{[L]} = \left(-\frac{1}{K_d}\right) \cdot [RL] + \frac{B_{\max}}{K_d}$$

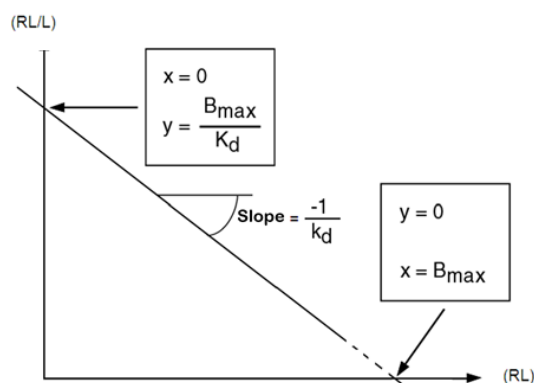


Figure 14: Scatchard transformation of equilibrium binding data. Plotting the ratio of bound-to-free ligand (RL/L) against bound ligand (RL) yields a linear relationship for a single class of independent, non-interacting binding sites. The negative reciprocal of the slope defines the equilibrium dissociation constant ($-1/K_d$), the x-intercept yields the maximum binding capacity (B_{max}), and the y-intercept represents B_{max}/K_d .

3.7. Competitive binding metrics (IC_{50} vs. K_i)

When evaluating inhibitors or antagonists that compete with an endogenous ligand for the same receptor binding site, potency and affinity are determined using specialized parameters:

- **IC_{50} (Half-maximal inhibitory concentration):** The concentration of an antagonist required to displace or inhibit 50% of specific radioligand binding under specific assay conditions.
- **K_i (Inhibitory K_i constant):** The true thermodynamic equilibrium dissociation constant for the competitor. It reflects the absolute affinity of the inhibitor and is independent of experimental parameters.

4. Structure and classification of pharmacological receptors

The physiological effect of any drug, hormone, or neurotransmitter is fundamentally initiated by its interaction with its receptor. To satisfy the biological criteria of a true receptor, the molecule must execute two primary, integrated functions. The first is the recognition function, which involves the high-affinity binding of the signaling molecule or ligand. The second is the effector function, which is responsible for triggering the downstream biochemical coupling process.

Researchers classify receptors based on their pharmacological profiles, tissue distribution, or structural and functional characteristics. While a pharmacological classification focuses on organizing receptors by their comparative sensitivity to selective agonists and antagonists, and an anatomical classification looks at tissue localization (such as differentiating between central and peripheral or pre-synaptic and post-synaptic receptors), the structural and functional method is the most robust for molecular biochemistry. This classification paradigm groups receptors into four major superfamilies based on their amino acid sequence homologies, transmembrane topologies, and the precise molecular nature of their downstream effector systems.

4.1. Superfamily I: Ionotropic receptors (ligand-gated ion channels)

Ionotropic receptors, or ligand-gated ion channels, are membrane-bound glycoprotein complexes that directly couple ligand binding to the opening or closing of an intrinsic ion channel (figure 15). These receptors are localized on the extracellular face of the plasma membrane, allowing them to interact directly with hydrophilic chemical messengers. The channel structures themselves are transmembrane proteins that facilitate the passive, selective passage of specific ions, such as sodium, potassium, calcium, or chloride, moving down their established electrochemical gradients.

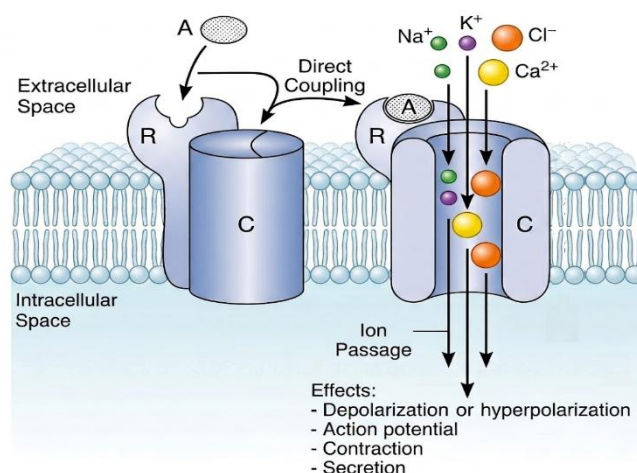


Figure 15: Ionotropic receptors. The binding of an extracellular agonist ligand (A) to its specific receptor (R) on the cell surface directly triggers the opening of an associated ion channel (C), allowing specific ions to pass through and leading to various cellular effects. Author created based on information adapted from Bylund (2014).

Depending on the ion selectivity of the activated channel, ligand binding results in either an influx of cations, causing membrane depolarization and cellular excitation, or an influx of anions, leading to hyperpolarization and cellular inhibition. Because the binding site and the ion channel pore are physically integrated into the same protein complex, the activation kinetics are remarkably rapid, operating on a millisecond timescale. This makes them perfectly suited for fast synaptic transmission throughout the nervous system.

Ionotropic receptors are key targets for numerous therapeutic agents due to their rapid signaling properties and distribution throughout the nervous system. A classic example is the nicotinic acetylcholine receptor (nAChR), a cation channel that mediates fast excitatory transmission. Varenicline, a first-line therapy for smoking cessation, acts as a partial agonist at the $\alpha 4\beta 2$ subtype of the nAChR. This mechanism is therapeutically beneficial because varenicline binds with higher affinity than nicotine but has lower efficacy. It thus antagonizes the rewarding effects of smoked nicotine by preventing its binding, while its partial agonist activity provides sufficient stimulation to minimize withdrawal symptoms and cravings.

Another major family is the GABA_A receptor, a chloride channel that mediates most fast inhibitory neurotransmission in the brain. This receptor is a common target for sedatives, anxiolytics, and anesthetics. For instance, benzodiazepines (such as diazepam) and barbiturates (such as phenobarbital) are positive allosteric modulators (PAMs) that bind to distinct sites on the GABA_A receptor to enhance the chloride current induced by GABA, leading to increased neuronal inhibition.

4.2. Superfamily II: G-protein coupled receptors (GPCRs)

GPCRs (also called 7-transmembrane receptors) are the largest and most diverse family of membrane-bound receptors in humans and are the target for most clinical drugs. They function on an intermediate timescale (seconds). Structurally, they consist of a single polypeptide with seven hydrophobic transmembrane alpha-helices.

Signal transmission depends entirely on interaction with **heterotrimeric G-proteins**, which act as molecular switches. These G-proteins are composed of three subunits: an **α subunit** (with intrinsic GTPase activity) and a **$\beta\gamma$ dimer** (figure 16).

- **Resting state:** The α subunit binds GDP and remains associated with $\beta\gamma$, forming an inactive heterotrimer.
- **Activation:** An agonist binds the GPCR, causing a conformational change. The activated receptor acts as a **guanine nucleotide exchange factor (GEF)**, promoting GDP release from the α subunit and GTP binding in its place.
- **Dissociation:** GTP binding destabilizes the heterotrimer. The GTP-bound α subunit dissociates from both the receptor and the $\beta\gamma$ dimer. Both the α subunit and the $\beta\gamma$ complex then diffuse along the membrane to independently activate downstream effectors (enzymes or ion channels) (figure 17, table 3).
- **Termination:** The α subunit hydrolyzes GTP to GDP via its intrinsic GTPase activity (accelerated by RGS proteins). This causes the α subunit to reassociate with the $\beta\gamma$ complex, reforming the inactive heterotrimer and resetting the system.

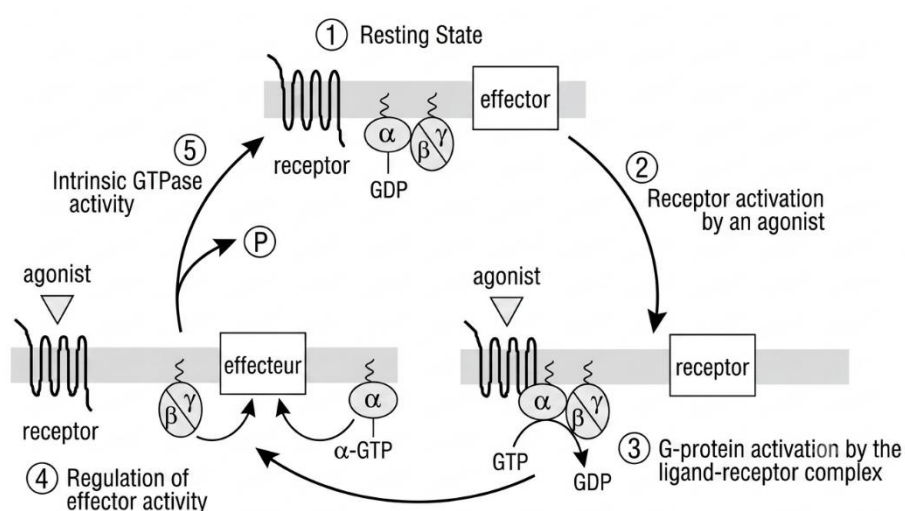


Figure 16: Activation of G proteins. G proteins are composed of three protein subunits (α , β , and γ). When the receptor is activated, the α subunit releases its GDP and binds to GTP. This binding to GTP causes the dissociation of the three subunits into two active components (the α subunit and a $\beta\gamma$ complex), which then stimulate either enzymes or ion channels in the plasma membrane. The α subunit possesses GTPase activity. When it hydrolyzes its GTP to GDP, it reassociates with a $\beta\gamma$ complex to reform an inactive trimer. Adapted from Récepteurs Couplés aux Protéines G (n.d.).

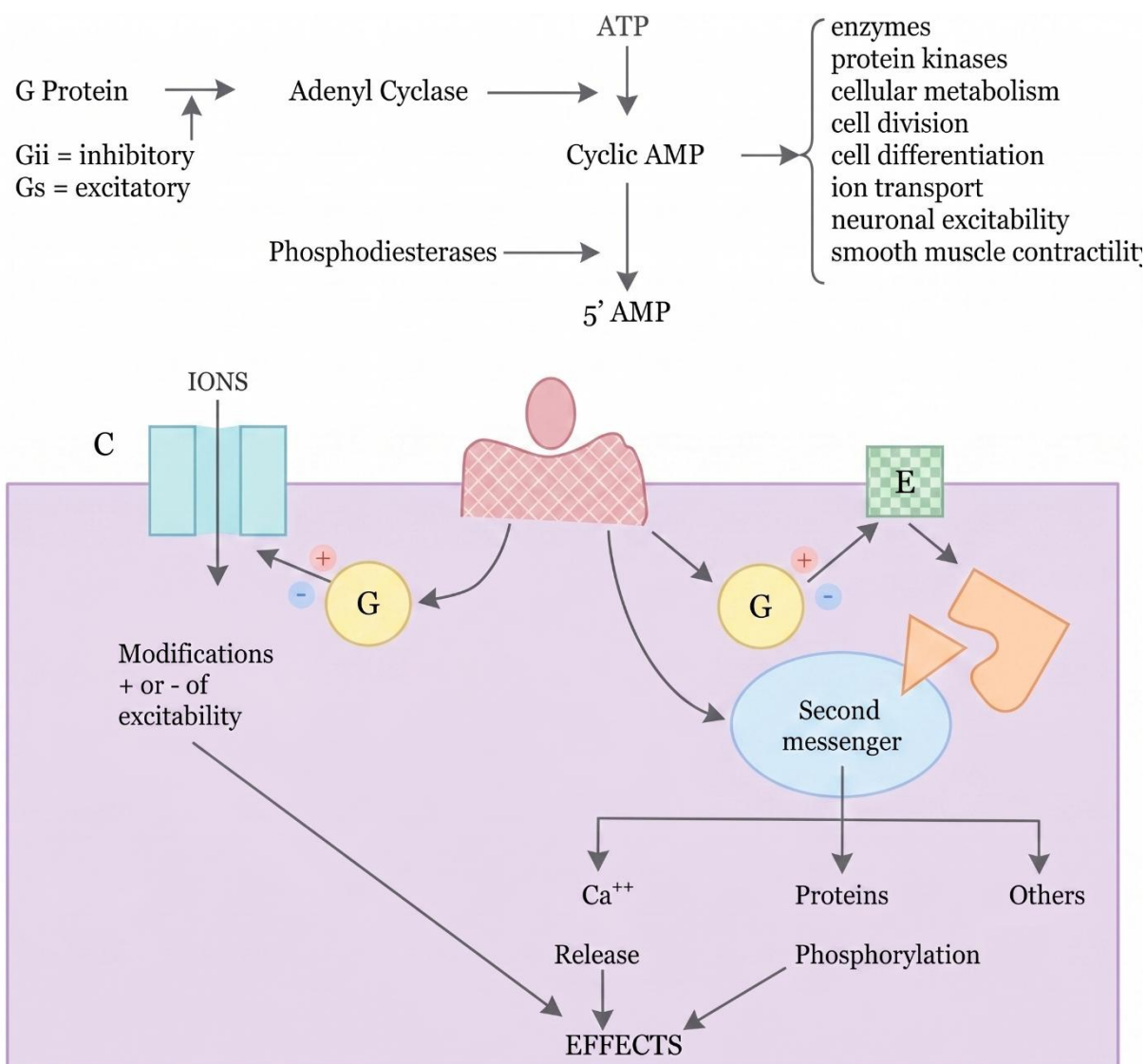


Figure 17: G-protein coupled receptors. R receptor, G G protein, E enzyme, C channel. This type is characterized by the involvement of proteins called G proteins. The receptor is located on the external face of the cytoplasmic membrane. Receptor activation leads to a modification (activation) of the G protein which acts on its target (enzyme, ion channel). Hydrolysis restores the original G protein. The time scale is in seconds. Author created based on information adapted from Alexander et al. (2025).

Table 3: Effectors modulated by G protein.

Effectors	Mechanisms
Adenylyl cyclase / cyclic AMP system	Certain G proteins act on an enzyme, adenylyl cyclase. Some stimulate it (Gs), others inhibit it (Gi). Adenylyl cyclase produces cyclic AMP (second messenger) from ATP. Depending on the case, receptor stimulation leads to either an increase or a decrease in second messenger formation. Cyclic AMP can activate protein kinases, which, depending on the cellular equipment, leads to cell division or differentiation, ion transport, modification of neuronal excitability or muscle contractility, metabolism changes, etc. The time scale is minutes. Cyclic AMP is inactivated by conversion to non-cyclic AMP via phosphodiesterases.
Phospholipase C / inositol phosphate system	Certain G proteins activate or inhibit enzymes that act on membrane phospholipids: * When the enzyme is phospholipase C, the second messenger is inositol triphosphate (IP3). IP3 triggers the release of intracellular calcium, which is responsible, depending on the case, for smooth muscle or myocardial contraction, exocrine or hormonal secretions. * In other cases, the second messenger is diacylglycerol (DAG), which activates protein kinases, responsible, depending on the case, for activation or inhibition of hormone or neuromediator release, smooth muscle contraction or relaxation, or substances involved in inflammation. * When the enzyme is phospholipase A2, the second messenger is GMC (or arachidonic acid); a cascade of reactions leads to the formation of local hormones called eicosanoids.
Ion channels	In this case, the G protein acts directly on an ion channel, without the involvement of a second messenger. The Go protein triggers the opening of calcium channels, while other G proteins trigger the opening of potassium channels, both resulting in cell hyperpolarization.

4.3. Superfamily III: Enzyme-linked receptors (catalytic receptors)

Enzyme-linked receptors are the third major superfamily of membrane-bound receptors. They control long-term cellular processes such as metabolism, growth, differentiation, and tissue proliferation over minutes to hours. Structurally, they are single-pass transmembrane proteins with three domains: an extracellular ligand-binding domain, a single transmembrane α -helix, and an intracellular domain that possesses enzymatic activity or binds directly to an enzyme.

The most studied class is the **Receptor Tyrosine Kinase (RTK)** family. In their resting state, RTKs exist as inactive monomers. Ligand binding drives two receptor monomers to form a dimer, bringing their intracellular kinase domains together. This triggers cross-phosphorylation (autophosphorylation), where each subunit phosphorylates the other, activating the receptor. The tyrosine kinase enzyme then phosphorylates target proteins on tyrosine residues. These target proteins can be structural proteins, regulatory proteins, or other enzymes, and they are involved in cell growth, differentiation, and gene transcription regulation (figure 18). Key examples include the **insulin receptor** and various **growth factor receptors**.

Other enzyme-linked receptors utilize different catalytic mechanisms. **Guanylate cyclase** is an enzyme that synthesizes the second messenger **cyclic GMP** from GTP. A key example is the **atrial natriuretic factor (ANF)** receptor, a polypeptide hormone primarily synthesized by the right atrium of the heart. ANF participates in sodium, potassium, and water homeostasis by acting on renal excretion and also has a vasodilatory action. This hormone is normally produced in response to mechanical stretching of the right atrial wall during hypertension, and through its action, it helps lower blood pressure.

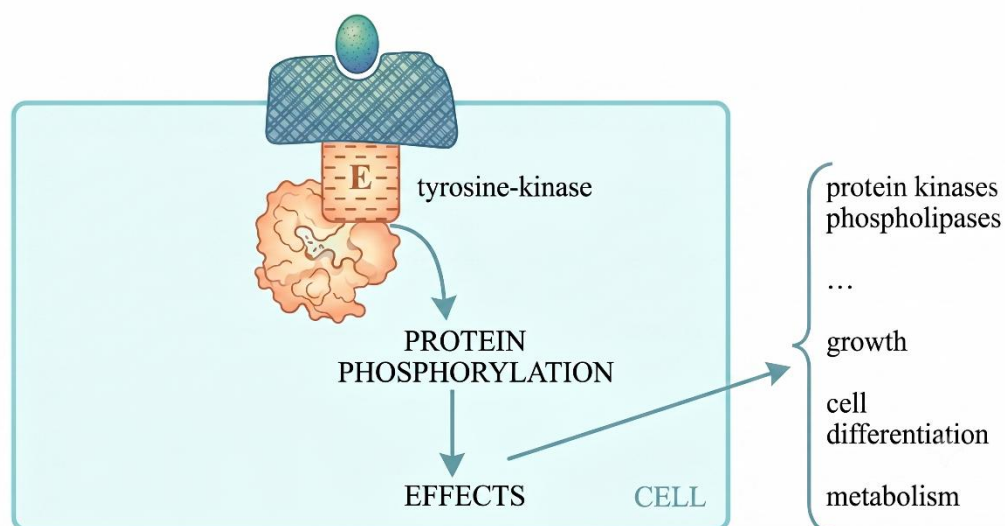


Figure 18: Enzyme-linked receptors. R receptor, E enzyme (tyrosine kinase). Located on the external face of the cell membrane, they are associated with an intracellular enzyme across the membrane. Agonist binding triggers an enzymatic reaction (catalytic receptors). Adapted from Schulz et al. (1989).

4.4. Superfamily IV: Nuclear receptors (regulators of DNA transcription)

Nuclear receptors differ completely from membrane-bound receptors by residing entirely inside the cell, either in the cytosol or the nucleus (figure 19). Because they are isolated from the extracellular environment, their ligands must be sufficiently lipophilic to passively diffuse

across the plasma membrane. Physiological ligands include steroid hormones (glucocorticoids, estrogens, progesterone), thyroid hormones, vitamin D, and retinoic acid derivatives. Their mechanism involves directly altering gene transcription, so their effects have a slow onset (hours to days).

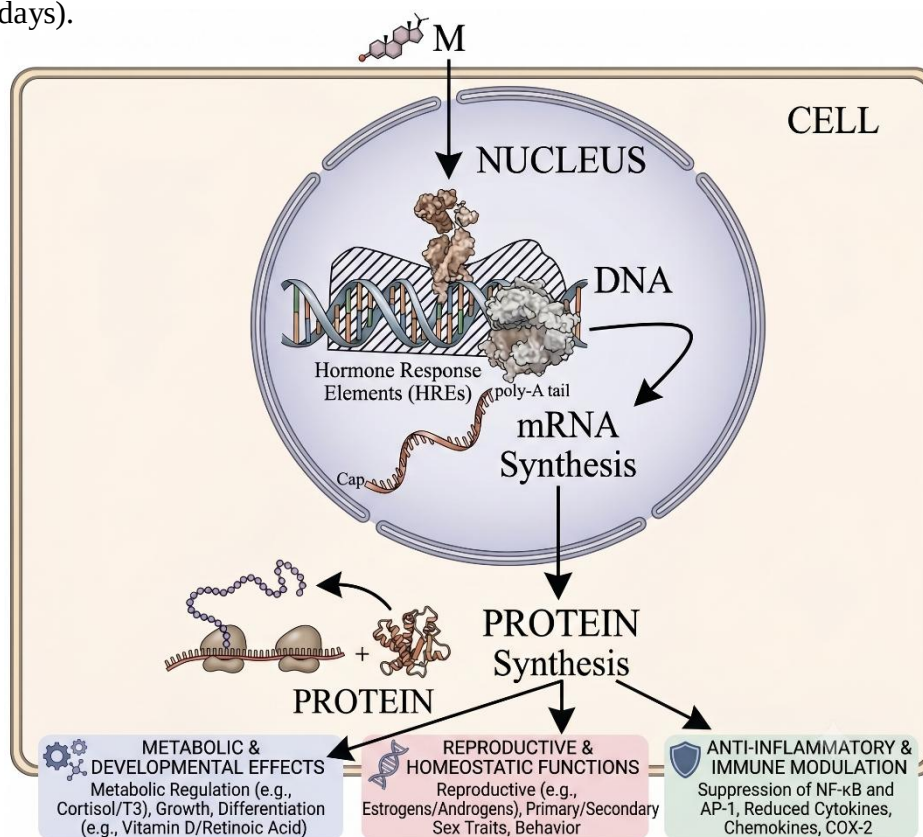


Figure 19: Nuclear receptors. Author created based on information adapted from Jin and Li (2010).

Nuclear receptors share a conserved modular organization with distinct functional domains:

- **A/B domain (N-terminal):** Contains Activation Function-1 (AF-1), which facilitates ligand-independent transcription by interacting with tissue-specific coactivators.
- **C domain (DNA-binding domain, DBD):** Contains two zinc finger motifs that recognize and bind specifically to Hormone Response Elements (HREs) in target gene promoters.
- **D domain (Hinge region):** Flexible region containing nuclear localization signals.
- **E domain (Ligand-Binding Domain, LBD):** Contains a hydrophobic pocket for ligand binding and Activation Function-2 (AF-2), which recruits coactivators or corepressors upon ligand binding.

In the basal state, soluble intracellular receptors (especially Class I steroid receptors) are maintained inactive in the cytosol, bound to heat shock proteins (HSPs). When a lipophilic hormone diffuses into the cell and binds the LBD, it triggers a structural rearrangement that causes HSP dissociation. The activated receptor monomers then form dimers, exposing their nuclear localization signals.

The dimer translocates into the nucleus, where its DBD binds to specific HREs on target gene promoters. The receptor dimer acts as a ligand-activated transcription factor, recruiting RNA polymerase II and either accelerating or suppressing transcription into mRNA. The newly synthesized mRNA is exported to the cytoplasm and translated into new functional proteins, ultimately altering the cell's physiological state.

Chapter 5: Pharmacological signaling pathways

Introduction

In a multicellular organism, coordinated communication is vital for survival. Every cell constantly receives a multitude of extracellular signals from other cells and the surrounding microenvironment. The successful integration of this information by the target cell depends not only on its specific surface or intracellular receptors but also on a complex network of intracellular signaling proteins that distribute, process, and propagate the signal within the cell.

1. Intracellular signaling

Signal transduction encompasses the complete sequence of intracellular molecular events triggered by the binding of an extracellular signaling molecule (ligand/drug) to its specific receptor, culminating in a distinct cellular response. The intracellular machinery executing this process relies heavily on an array of signaling proteins, including kinases, phosphatases, and guanosine triphosphate (GTP)-binding proteins, along with numerous scaffolding and adaptor molecules.

The ultimate outcome of these signaling networks manifests as a precise modulation of physiological actions, including cell growth, proliferation, differentiation, cell migration, structural remodeling, programmed cell death (apoptosis), immune and inflammatory responses, and other functions through the regulation of genes encoding growth factors, cytokines, chemokines, and proteases.

Many diseases arise from altered intracellular signaling; thus, a better understanding of these pathways enables the development of novel therapeutic targets using small molecules that regulate signaling proteins.

2. Signaling proteins as molecular networks

Intracellular signaling proteins do not function in isolated, linear tracks; rather, they form an intricate, overlapping network. This network relays extracellular messages across the plasma membrane and through the cytoplasm into the nucleus, where signaling molecules interact with specific transcription factors to alter target gene expression.

These signaling proteins exhibit extraordinary functional diversity and can be classified into several distinct categories based on their operational roles (figure 20):

- **Relay proteins:** Simply pass the signal along to the next downstream component in the chain.
- **Messenger/carrier proteins:** Transport the signal from one intracellular compartment to another (e.g., from the plasma membrane to the nucleus).
- **Adaptor proteins:** Link two or more signaling proteins together without directly participating in the enzymatic transduction themselves.
- **Amplifier proteins:** Greatly increase the intensity of the signal, either by producing large amounts of second messengers or by activating numerous downstream enzymatic targets.
- **Transducer proteins:** Convert the signal into a different molecular form (e.g., transforming an extracellular ligand-binding event into an intracellular cyclic adenosine monophosphate production).
- **Scaffolding proteins:** Anchor multiple interacting signaling components into a localized physical complex, enhancing speed, efficiency, and specificity while preventing unwanted cross-talk.

- **Modulator proteins:** Regulate the activity of other signaling molecules, fine-tuning the strength and duration of the pathway's activation.

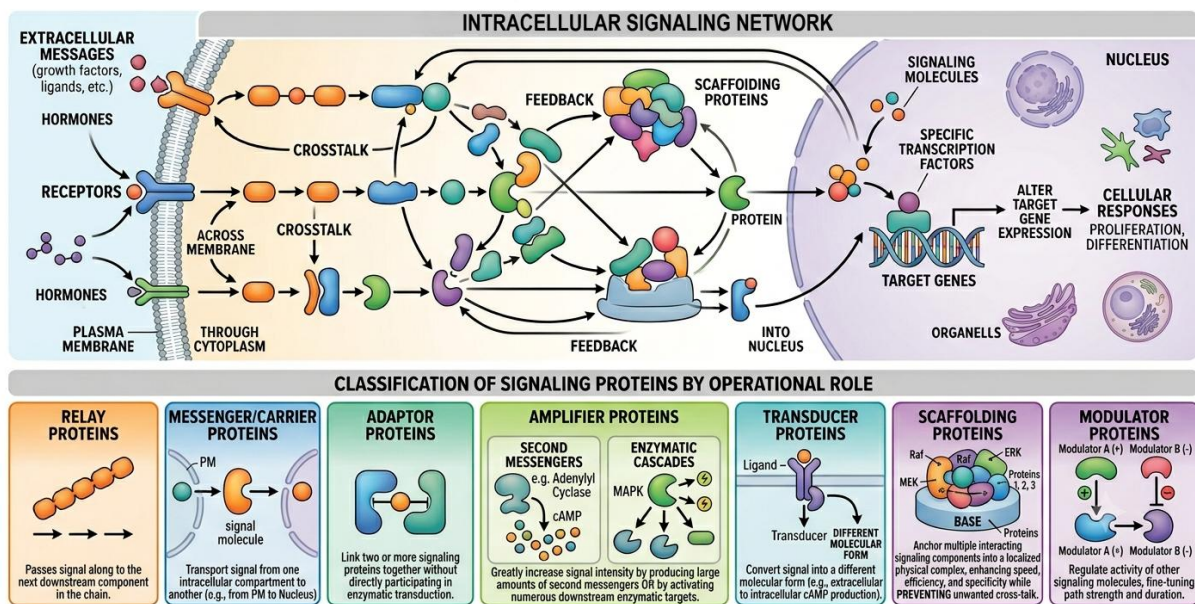


Figure 20: Intracellular signaling proteins as molecular network. Author created based on information adapted from Alberts et al. (2015).

3. Activation and deactivation mechanisms: molecular switches

The vast majority of intracellular signaling proteins operate as **molecular switches**. Upon receiving an upstream stimulus, they rapidly transition from an inactive conformation to an active conformation, and vice versa. These switches are strictly controlled, and in both major classes, the functional state of the protein is dictated by the precise addition or removal of a phosphate group.

3.1. Phosphorylation and dephosphorylation

Protein phosphorylation is one of the most common post-translational modifications used to regulate cell signaling.

- **Protein kinases:** Catalyze the transfer of a terminal phosphate group from ATP to a specific amino acid residue on the target signaling protein, typically triggering its activation.
- **Protein phosphatases:** Remove the phosphate group via hydrolysis, generally restoring the signaling protein to its inactive state.

Because many activated signaling proteins are themselves kinases, their stimulation often triggers **phosphorylation cascades**. An upstream kinase phosphorylates and activates a downstream kinase, which in turn phosphorylates a subsequent kinase. This sequential chain reaction serves to exponentially amplify, diversify, and distribute the signal throughout the cell.

Kinases are classified based on the specific amino acid residues they phosphorylate:

- **Serine/threonine kinases:** Phosphorylate hydroxyl groups on serine and threonine residues (e.g., PKA, PKC, MAPK).
- **Tyrosine kinases:** Phosphorylate phenolic hydroxyl groups on tyrosine residues (e.g., Src, JAK, and receptor tyrosine kinases like the insulin receptor).

3.2. GTP-binding proteins

The second major class of molecular switches consists of GTP-binding proteins (G-proteins). These proteins alternate between an active "on" state and an inactive "off" state depending on the nucleotide bound to them.

Active state: The protein is bound to **guanosine triphosphate (GTP)**.

Inactive state: the protein is bound to **guanosine diphosphate (GDP)**.

Once activated, these proteins possess an intrinsic **GTPase activity**, meaning they self-inactivate over time by hydrolyzing their bound GTP into GDP and an inorganic phosphate (P_i).

There are two primary subfamilies of GTP-binding proteins:

- **Heterotrimeric G-proteins**

Trimeric G-proteins (α , β , and γ subunits) relay signals from GPCRs. In the resting state, the α subunit binds GDP and the $\beta\gamma$ complex. Ligand-receptor binding triggers the α subunit to exchange GDP for GTP, causing the heterotrimer to dissociate into active α -GTP and $\beta\gamma$ components that modulate downstream effectors. Signaling terminates when the α subunit hydrolyzes GTP back to GDP, prompting trimer reassociation (for a detailed breakdown of these mechanisms, see Chapter 4, Section 4.2).

- **Small monomeric GTPases**

These are single-subunit proteins (e.g., Ras, Rho, Rab) that function similarly to the heterotrimeric α subunit. They rely on accessory proteins: **GEFs** (Guanine Nucleotide Exchange Factors) to drive activation by promoting GDP-to-GTP exchange, and **GAPs** (GTPase-Activating Proteins) to accelerate their intrinsic GTP hydrolysis, turning the pathway off.

4. Principles of cell signaling

Cellular signaling follows a common framework consisting of four key principles:

Reception: The initial event where an extracellular signaling molecule (hormone, neurotransmitter, or drug) binds with high affinity and specificity to its designated receptor (extracellular or intracellular).

Transduction: The structural transformation of the receptor upon ligand binding that converts the extracellular signal into an intracellular chemical message. This often involves generating second messengers or initiating protein recruitment.

Amplification: An enzymatic cascade where a small initial reception event is multiplied exponentially inside the cytoplasm, ensuring that a low concentration of extracellular ligand can evoke a robust cellular outcome.

Response: The ultimate phenotypic or physiological adaptation of the cell, such as altered metabolic activity, cytoskeletal rearrangements, or changes in gene transcription.

5. Major intracellular signaling pathways

5.1. The cAMP/PKA pathway

The cAMP (Cyclic Adenosine Monophosphate) / PKA (Protein Kinase A) pathway is a prototypical second messenger system, often activated by GPCRs (G Protein-Coupled Receptors).

Activation mechanism: An extracellular signal (e.g., epinephrine) binds to a GPCR, activating the heterotrimeric G protein Gs. The Gs α subunit exchanges GDP for GTP, dissociates from G $\beta\gamma$, and activates adenylyl cyclase. Adenylyl cyclase converts ATP to cAMP. cAMP then binds to the regulatory subunits of PKA, releasing the active catalytic subunits.

Downstream effects: PKA phosphorylates serine/threonine residues on various targets, including:

- Metabolic enzymes (e.g., glycogen phosphorylase kinase, leading to glucose release).
- Ion channels (e.g., CFTR [Cystic Fibrosis Transmembrane Conductance Regulator], L-type calcium channels).
- Transcription factors (e.g., CREB — cAMP Response Element-Binding Protein), regulating genes involved in memory, metabolism, and survival.

Termination: cAMP is degraded by PDEs (phosphodiesterases) to 5'-AMP (Adenosine 5'-Monophosphate). The signal is also terminated by GTP hydrolysis on Gs α (intrinsic GTPase activity).

Pharmacological modulation: Forskolin (direct adenylyl cyclase activator), PDE inhibitors (e.g., caffeine, sildenafil), and cholera toxin (irreversibly activates Gs α) are key tools and drugs.

5.2. The PLC / IP3 / DAG pathway

The PLC (Phospholipase C) / IP3 (Inositol Trisphosphate) / DAG (Diacylglycerol) pathway is another major GPCR and RTK (Receptor Tyrosine Kinase) effector system.

Activation mechanism: Activated GPCRs (via Gq/11) or RTKs recruit and activate PLC β (for GPCRs) or PLC γ (for RTKs). PLC hydrolyzes the membrane phospholipid PIP2 (Phosphatidylinositol 4,5-Bisphosphate) into two second messengers: IP3 (water-soluble) and DAG (lipid-soluble).

Downstream effects:

- **IP3:** diffuses to the ER (Endoplasmic Reticulum) and binds to IP3R (Inositol Trisphosphate Receptors), calcium channels that trigger the release of Ca²⁺ from ER stores into the cytoplasm.
- **DAG:** remains in the plasma membrane and activates PKC (Protein Kinase C). PKC phosphorylates numerous targets, including ion channels, cytoskeletal proteins, and transcription factors (e.g., NF- κ B [Nuclear Factor Kappa B]).

Termination: IP3 is dephosphorylated or phosphorylated; DAG is phosphorylated to phosphatidic acid or hydrolyzed; Ca²⁺ is pumped back into ER by SERCA (Sarco/Endoplasmic Reticulum Calcium ATPase) pumps.

Pharmacological modulation: Lithium (inhibits inositol monophosphatase, depleting PIP2), U-73122 (PLC inhibitor), and phorbol esters (DAG analogs that constitutively activate PKC).

5.3. Calcium (Ca²⁺) signaling

Calcium is a ubiquitous and versatile second messenger controlling processes from muscle contraction to synaptic plasticity.

Cytosolic Ca²⁺ regulation: Resting cytosolic [Ca²⁺] is very low (~100 nM) compared to extracellular (~2 mM) and ER/SR (Sarcoplasmic Reticulum) stores (~0.5 mM). This gradient

is maintained by pumps (PMCA [Plasma Membrane Ca^{2+} ATPase], SERCA) and exchangers (NCX [Sodium-Calcium Exchanger]).

Signal generation: Ca^{2+} signals arise from:

- **Release from intracellular stores:** Primarily via IP3R and RyR (Ryanodine Receptors) in ER/SR.
- **Influx from extracellular space:** Via store-operated Ca^{2+} channels (e.g., Orai1 with STIM1 [Stromal Interaction Molecule 1]), VGCCs (Voltage-Gated Calcium Channels), and ligand-gated channels (e.g., NMDA [N-methyl-D-aspartate] receptors).

Effectors: Ca^{2+} binds to sensor proteins, most notably CaM (Calmodulin). Ca^{2+} /CaM activates CaMKs (Ca²⁺/Calmodulin-dependent Protein Kinases), calcineurin (a phosphatase that activates NFAT [Nuclear Factor of Activated T-cells] transcription factor), and other proteins.

Pharmacological modulation: BAPTA / EGTA (Aminopolycarboxylic acids / Calcium chelators), thapsigargin (SERCA inhibitor, depletes stores), ryanodine (RyR modulator), and calcium channel blockers (e.g., verapamil, nifedipine) for hypertension.

5.4. The MAPK/ERK pathway

The MAPK (Mitogen-Activated Protein Kinase) / ERK (Extracellular signal-Regulated Kinase) cascade is a central pathway for cell proliferation, differentiation, and survival, typically downstream of RTKs and Ras (Rat Sarcoma).

Activation mechanism: Growth factors (e.g., EGF [Epidermal Growth Factor]) bind RTKs, leading to autophosphorylation. Adaptor proteins (Grb2 [Growth Factor Receptor-Bound Protein 2]) and GEFs (Guanine Nucleotide Exchange Factors) such as SOS (Son of Sevenless) recruit and activate the small GTPase Ras (GTP-bound). Ras-GTP activates the kinase Raf (Rapidly Accelerated Fibrosarcoma), which is a MAPKKK (MAPK Kinase Kinase). Raf phosphorylates and activates MEK (MAPK/ERK Kinase), which is a MAPKK (MAPK Kinase). MEK phosphorylates and activates ERK (the terminal MAPK).

Downstream effects: ERK phosphorylates numerous targets in the cytoplasm (e.g., cytoskeletal proteins, other kinases) and nucleus (e.g., transcription factors like Elk-1, c-Fos, c-Myc), driving gene expression for cell cycle progression and differentiation.

Termination: Ras hydrolyzes GTP to GDP (accelerated by GAPs [GTPase-Activating Proteins]); phosphatases (e.g., MKPs [MAPK Phosphatases]) dephosphorylate ERK.

Pharmacological modulation: This pathway is heavily targeted in cancer. Examples: Sorafenib (Raf inhibitor), Trametinib (MEK inhibitor). Inhibitors of RTKs (e.g., Erlotinib, Trastuzumab) also block this pathway.

5.5. The PI3K / Akt / mTOR pathway

The PI3K (Phosphoinositide 3-Kinase) / Akt (also historically known as PKB [Protein Kinase B]) / mTOR (Mammalian Target of Rapamycin) pathway is a master regulator of cell growth, metabolism, and survival.

Activation mechanism: RTKs (or activated Ras) recruit and activate PI3K. PI3K phosphorylates the membrane lipid PIP2 to produce PIP3 [Phosphatidylinositol 3,4,5-Trisphosphate]. PIP3 recruits Akt and its activator PDK1 (Phosphoinositide-Dependent Kinase 1) to the membrane. PDK1 phosphorylates and partially activates Akt; full activation requires additional phosphorylation by mTORC2 (mTOR Complex 2).

Downstream effects: Active Akt phosphorylates a broad range of targets:

- **Pro-survival:** Inactivates pro-apoptotic proteins (Bad [Bcl-2 Associated Agonist of Cell Death], FOXO [Forkhead Box O]).
- **Metabolism:** Promotes glucose uptake (via GLUT4 [Glucose Transporter Type 4] translocation) and glycolysis.
- **Cell growth:** Activates mTORC1 (mTOR Complex 1), which promotes protein synthesis (via S6K [Ribosomal Protein S6 Kinase] and 4E-BP1 [Eukaryotic Translation Initiation Factor 4E-Binding Protein 1]) and inhibits autophagy.

Termination: PTEN (Phosphatase and Tensin Homolog) dephosphorylates PIP3 back to PIP2, opposing PI3K. Akt is dephosphorylated by phosphatases (e.g., PHLPP [Pleckstrin Homology Domain Leucine-Rich Repeat Protein Phosphatase]). PTEN loss is a frequent oncogenic event.

Pharmacological modulation: This is a major cancer target. Inhibitors include: Wortmannin/LY294002 (PI3K inhibitors), Rapamycin and its analogs (rapalogs, e.g., everolimus) that inhibit mTORC1, and ATP-competitive mTOR inhibitors.

5.6. The JAK / STAT pathway

The JAK (Janus Kinase) / STAT (Signal Transducer and Activator of Transcription) pathway is the primary signaling mechanism for cytokines, interferons, and many growth hormones.

Activation mechanism: Cytokines bind to their cognate receptors (e.g., interferon- γ receptor, growth hormone receptor), which lack intrinsic kinase activity. The associated JAKs are brought into proximity and trans-phosphorylate each other and the receptor tail. The phosphorylated receptor tail serves as a docking site for STATs. JAKs phosphorylate the recruited STATs on a single tyrosine residue.

Downstream effects: Phosphorylated STATs dimerize (via SH2 domain [Src Homology 2 Domain]-phosphotyrosine interaction) and translocate to the nucleus. In the nucleus, STAT dimers bind specific DNA response elements and regulate transcription of target genes involved in immune responses, inflammation, cell survival, and proliferation (e.g., SOCS [Suppressors of Cytokine Signaling], Bcl-xL, c-Myc).

Termination: PTPs (Protein Tyrosine Phosphatases) dephosphorylate JAKs, receptors, and STATs. SOCS proteins feedback-inhibit the pathway. STATs also undergo nucleocytoplasmic shuttling.

Pharmacological modulation: JAK inhibitors (jakinibs) are successful therapeutics for inflammatory diseases and myeloproliferative neoplasms. Examples: Tofacitinib (JAK3-preferring, rheumatoid arthritis), Ruxolitinib (JAK1/2, polycythemia vera).

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