# Goodman & Gilman's

# The Pharmacological Basis of Therapeutics

14TH EDITION

LAURENCE L. BRUNTON
BJÖRN C. KNOLLMANN

Mc Graw Hill

*Goodman & Gilman's*

# THE PHARMACOLOGICAL BASIS OF THERAPEUTICS

## FOURTEENTH EDITION

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*Another World is Possible*
Painting by Camilo, a member of the artists' collective of the EZLN, the Ejército Zapatista de Liberación Nacional, in Chiapas, Mexico.
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# Contents

Contents

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# Preface

This is the 14th edition of book that began as a collaboration between two friends and professors at Yale, Louis Goodman and Alfred Gilman. Over the years, "G&G" has been acclaimed as the "blue bible" of pharmacology. Surely much of that acclaim reflects the book's purpose, delineated by the original authors and steadily adhered to over 81 years: to correlate pharmacology with related medical sciences, to reinterpret the actions and uses of drugs in light of advances in medicine and the basic biomedical sciences, to emphasize the application of pharmacodynamics to therapeutics, and to create a book that would be useful to students of pharmacology and to healthcare practitioners.

Following these principles is demanding: the sheer volume and unremitting growth of knowledge in the basic biomedical sciences and their clinical applications continue to amaze, challenging editors and contributors who are trying to produce a one-volume work, and surely challenging students. To create a book that reflects our times, we have updated all chapters and have added five new chapters: drug response and the gastrointestinal biome, pharmacovigilance, the blood-brain barrier (it is not simply a lipid sheath), cannabinoids, and immunotherapies for cancer, plus a novel appendix on drug-drug interactions. Advances in immunomodulation are presented in most sections. In addition, we have continued to reach out to younger contributors who are on the forefront of pharmacological investigation and clinical practice. As a result, we have, in this edition, 56 new contributors, drawn from diverse backgrounds, who will ensure the book's vigor into the future.

A multi-authored work such as *Goodman & Gilman* grows by accretion, deletion, addition, replacement, and repair. The current text reflects over eight decades of such activity, with wisdom, memorable pearls, new material, and flashes of wit, hopefully edited to meet the present and to be forward looking. End-of-chapter notes acknowledge retired contributors to the 13th edition, but I am happy to acknowledge that several generations of editors and contributors have helped to bring this 14th edition to its present form. As in the 13th edition, we have used a larger page size, no extract type, and more mechanistic figures as we attempt to explain the pharmacodynamics of new agents. Some readers have complained that the book is getting too complex. We believe that a thorough understanding of a drug's actions and interactions at multiple physiological sites and with other drugs is essential to modern therapeutics. However, we also prominently summarize the mechanisms of action, ADME, and clinical use of individual agents and drug classes. Not wanting to favor one manufacturer's product over that of another, we continue generally to avoid using trade names except as needed to distinguish multiple formulations of the same agent that have distinct pharmacokinetic or pharmacodynamic properties or that are known only by a trade name. The full text is available online at many medical, pharmacy, and nursing schools by institutional subscription to *AccessMedicine.com* and *AccessPharmacy.com*, where we publish regular updates. Feel free to contact the editors by email if you have comments on the book or the websites.

Editing this book brings to mind a number of larger issues, both positive and negative, relating to health care; among them: the remarkable explosion of molecular genetic techniques, the proliferation of therapeutic agents affecting the immune system, and the power of computer-aided drug design; antibiotic resistance promoted by the continuing misuse and overuse of antibiotics in healthcare and animal husbandry; the adverse environmental effects of human activity to life on Earth; the effects of global warming and the sheer size of the human population on global health and nutrition; the ease with which infectious diseases can spread around the world; the fragility of truth and fact, and the difficulty of promoting health based on science and data in the face of determined conspiracy theories and political ideology. A better world is possible.

A number of people have contributed to the preparation of this edition of *Goodman & Gilman*. Many thanks to: my co-editor, Bjorn Knollmann, and to the clinical pharmacology fellows at Vanderbilt whom he recruited to read the first drafts of chapters as they honed their editorial skills; our attentive publisher at McGraw Hill, Michael Weitz, and his colleagues Christina Thomas and Melinda Avelar; consulting pharmacist Nelda Murri; Nitesh Sharma at KnowledgeWorks Global Ltd, who tirelessly oversaw the transformation of Word documents into a printed book; Jason McAlexander of MPS North America, whose rapid-response artwork brightens the pages; and the eagle-eyed Becky Hainz-Baxter, who saw what the editors had missed.

My special thanks to Lynne Larson, a novelist, artist, and grants management specialist who managed this enterprise and kept the editors organized. Lynne managed the production of the 11th edition of *Goodman & Gilman* when I first became the editor, when everything was done with hard copy and Word files submitted by mail, when galley proofs were actual long sheets of paper on which corrections were handwritten and then transcribed to new Word files. I was delighted when Lynne agreed to manage this all-electronic project. We would not have this 14th edition without her.

*Laurence L. Brunton*
*San Diego, CA*
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# Section I

## General Principles

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Chapter 1

# Drug Discovery: From Medicinal Plants to Computer-Aided Drug Design

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The first edition of *Goodman & Gilman*, published in 1941, helped to organize the field of pharmacology, giving it intellectual validity and an academic identity. That edition began: "The subject of pharmacology is a broad one and embraces the knowledge of the source, physical and chemical properties, compounding, physiological actions, absorption, fate, and excretion, and therapeutic uses of drugs. A *drug* may be broadly defined as any chemical agent that affects living protoplasm, and few substances would escape inclusion by this definition." In practice, of course, a chemical or biological agent is considered a legal drug only if it has been approved as such by a national regulatory agency, such as the U.S. Food and Drug Administration (FDA) or the European Medicines Agency; these approved compounds are the focus of this book.

This first nine chapters of this book, ***General Principles***, provide the underpinnings for these definitions of pharmacology and drugs by exploring the physiological, biochemical, and molecular mechanisms of drug action. This section covers drug invention, development, and regulation, as well as how drugs act in biological systems, i.e., *pharmacodynamics*, *pharmacokinetics* (including *drug transport* and *metabolism*), *the influence of the gastrointestinal microbiome*, and *pharmacogenetics*, with brief forays into *pharmacovigilance* and *drug toxicity and poisoning*. Subsequent sections deal with the use of specific classes of drugs as therapeutic agents in human subjects. The present chapter is an introduction to pharmaceuticals, their development, and the activities of the pharmaceutical industry and government surrounding the discovery, production, and use of therapeutic agents. The processes of discovery and invention of drugs have changed substantially with the general progress of biomedical sciences, the advent and improvement of computer-aided drug design, and technical advances in biochemistry and molecular biology. Some of these new capabilities are reviewed below.

## From Medicinal Plants to Computer-Aided Drug Design

### Early Experiences With Plants

The human fascination—and sometimes infatuation—with chemicals that alter biological function is ancient and begins with our long experience with and dependence on plants. Because most plants are rootbound, many produce defensive compounds that animals learn to avoid and humans to exploit or abuse. Thus, the prior of an Arabian convent came to appreciate coffee (caffeine) after noting the behavior of goats that gamboled and frisked through the night after eating the berries of the coffee plant; women sought to enhance their beauty by using an extract of the deadly nightshade plant, *Atropa belladonna* ("beautiful lady"), enriched in *atropine*, to produce pupillary dilation; the Chinese herb *ma huang* (*ephedrine*) was used as a stimulant; indigenous people of South American used curare to paralyze and kill animals hunted for food; and poppy juice (opium), containing *morphine* (from the Greek *Morpheus*, the god of dreams), has long been used for pain relief and control of diarrhea. *Morphine*, of course, has well-known addicting properties, as do other psychoactive natural products, such as nicotine, cocaine, and ethanol. Note that these drugs did not derive from a search for a druggable target or any knowledge of a target. Rather, drug discovery in the past often resulted from serendipitous observations of the effects of plant extracts or individual chemicals on animals or humans. Drugs were selected based on effect, with no understanding of mechanism as we use the term today. In the 20th century, the hunt for natural products broadened, driven in part by the discovery of antibiotics, such as *penicillin* and the cephalosporins, which fungi and microbes make to compete with each other.

# Abbreviations

**ADME:** absorption, distribution, metabolism, and excretion
**BLA:** Biologics License Application
**CADD:** computer-aided drug discovery
**DEL:** DNA-encoded compound library
**DHHS:** U.S. Department of Health and Human Services
**DMPK:** drug metabolism and pharmacokinetics
**FBDD:** fragment-based drug discovery
**FDA:** U.S. Food and Drug Administration
**GPU:** graphics processing unit
**HCV:** hepatitis C virus
**HDL:** high-density lipoprotein
**HMG-CoA:** 3-hydroxy-3-methylglutaryl coenzyme A
**HTS:** high-throughput screening
**IND:** Investigational New Drug
**LDL:** low-density lipoprotein
**mRNA:** messenger RNA
**NDA:** New Drug Application
**NIH:** National Institutes of Health
**NMEs:** new molecular entities
**PDUFA:** Prescription Drug User Fee Act
**SBDD:** structure-based drug design
**siRNA:** small interfering RNA

## Drug Discovery or Drug Invention?

The conventional phrase *drug discovery* makes sense for therapeutic compounds obtained from plants and other organisms. Today, however, only a fraction of the new drugs introduced each year are discovered in nature. Instead, most drugs are not discovered, but are totally new compounds, painstakingly optimized against many criteria through an interplay of design and experimentation. In that sense, today's new drugs are more invented than discovered.

The current paradigm for drug development grew out of synthetic organic chemistry, which arose as the dye industry in the late 19th century and has continued to flourish. Dyes are colored compounds with selective affinity across various biological tissues. Study of these interactions stimulated Paul Ehrlich to postulate the existence of chemical receptors in tissues that interacted with and "fixed" the dyes. Similarly, Ehrlich thought that unique receptors on microorganisms or parasites might react specifically with certain dyes and that such selectivity could spare normal tissue. Ehrlich's work culminated in the invention of *arsphenamine* in 1907, which was patented as "salvarsan," suggestive of the hope that the chemical would be the salvation of humankind. This and other organic arsenicals were used to treat syphilis until the discovery of *penicillin*. Gerhard Domagk demonstrated that another dye, *prontosil* (the first clinically useful sulfonamide), was dramatically effective in treating streptococcal infections, thereby launching the era of antimicrobial chemotherapy. The collaboration of pharmacology with chemistry on the one hand and clinical medicine on the other has been a major contributor to the effective treatment of disease, especially since the middle of the 20th century.

Early on, new compounds could be tested for their activities only in whole organisms. This is how the nonsteroidal anti-inflammatory drug *indomethacin* was discovered, for example (Brune and Hinz, 2004). In the past 70 years, researchers have begun to understand in considerable detail the cellular and molecular mechanisms of disease. As a result of this basic biomedical research, it is possible to do initial testing of compounds *in vitro* ("in glass"), using cellular and molecular assays. For example, one could look for the cellular responses due to inhibition of a protein involved in a disease process. In this scenario, by testing enough appropriately chosen compounds, one could develop at least a partial understanding of which types of compounds are most likely to be active and then use this information to steer the program of chemical synthesis and testing toward increasingly potent compounds.

In the 1980s, it became practical to determine high-resolution three-dimensional structures of complex organic molecules and even larger molecules such as proteins, using and refining the techniques of X-ray crystallography pioneered by Hodgkin, Kendrew, and Perutz in the mid-20th century. It was already known that many drugs worked by binding tightly to a disease-related protein and thereby modulating (e.g., inhibiting or activating) its biological function, but the atomic details of these interactions had remained mysterious. As a consequence, the only way to advance a drug discovery project had been by synthesizing and testing one compound after another. Now, with the protein's three-dimensional structure in hand, one could finally hope to design a compound that would bind with high affinity by fitting snugly into a pocket in the protein, such as an enzyme's active site. Thus, protein crystallography enabled *structure-based drug design* (SBDD), where the three-dimensional structure of the drug target is used to guide creation of tight-binding compounds, often called *ligands*.

Around the same time, computer technology began to advance rapidly. This accelerated the data processing needed to go from X-ray diffraction patterns to protein structures (i.e., three-dimensional atomic coordinates) and enabled interactive visualization of complex protein structures comprising thousands of atoms. It also opened new vistas in *computer-aided drug discovery* (CADD), including the use of molecular simulations to model the physical interactions of compounds and proteins, and the development of tools to encode, archive, share, and analyze chemical and pharmacological data. In parallel, automation and miniaturization have dramatically increased experimental throughput, notably through robotic *high-throughput screening* (HTS), in which hundreds of thousands of compounds can be tested rapidly and at relatively low cost in cellular or molecular activity assays. Today, excitement about the power of artificial intelligence motivates wide-ranging efforts to apply these technologies to drug discovery.

The following section goes into more detail regarding the process of drug discovery, focusing on so-called *small-molecule* drugs, organic compounds with molecular weights typically less than 500 Da, which have traditionally been the most common type of drug. Subsequent sections introduce biological drugs, such as antibodies and other engineered biomolecules.

## Target Identification

Today, most small-molecule drug discovery projects grow out of basic research that implicates a specific macromolecule, usually a protein, as a key player in a disease and, further, suggests that a small molecule which binds this macromolecule could be used to treat the disease. The macromolecule thus becomes a candidate *drug target*. Many small-molecule drugs are inhibitors (antagonists), which work by reducing the activity of their macromolecular target. Examples include the statins, which reduce cholesterol synthesis by binding and inhibiting the enzyme 3-hydroxy-3-methylglutaryl (HMG) coenzyme A (CoA) reductase, and β-lactam antibiotics, which kill bacteria by inhibiting enzymes involved in the synthesis of bacterial cell walls. However, some small molecules are activators (agonists) rather than inhibitors. Activators frequently target proteins whose normal role involves cell signaling, such as hormone receptors. For example, the asthma medication *albuterol* dilates bronchi by binding and activating β adrenergic receptors on bronchial smooth muscle, thereby mimicking the effect of adrenaline (epinephrine; see Chapter 10).

Candidate drug targets have been identified in many ways (Hughes et al., 2011). For example, the enzymes targeted by the β-lactam antibiotics were unknown in advance and were discovered precisely because they are bound by these naturally occurring antibiotics. In contrast, the target of the statins, HMG-CoA reductase, was identified by elucidation of the pathways of cholesterol synthesis (Tobert, 2003), and this information was used to help discover the first statins. Similarly, as researchers have determined the regulatory functions of human protein kinases—enzymes that change the activities of other proteins by covalently attaching phosphate groups to their hydroxyl-containing side

chains—specific kinases have been targeted for small-molecule drug discovery (Cohen et al., 2021). Many kinase inhibitors are anticancer agents that work by inhibiting protein kinases that accelerate cell proliferation. Some of these targeted kinases carry abnormal, cancer-associated mutations that make them hyperactive, so inhibiting them returns their regulatory activities toward normal. The pioneering example of this scenario is the drug *imatinib*, which inhibits a cancer-associated mutant protein kinase, the Bcr-Abl tyrosine kinase, and is used to treat chronic myelogenous leukemia (Buchdunger et al., 2002).

In recent years, technological advances enabling genome-wide experimentation (*omics*) have opened new approaches to identifying candidate targets (Lindsay, 2003; Paananen and Fortino, 2020). Fast, inexpensive genome sequencing facilitates genome-wide association studies, in which variations in the susceptibility to a disease across many people are correlated with variations in specific genes, leading to suggestions for gene products (i.e., proteins), that may be suitable drug targets. The growing availability of patient genomic data in the context of patients' electronic medical records will likely open new opportunities for data mining in support of target discovery in the coming years. It has also become routine to measure the quantities of messenger RNA (mRNA) transcribed from thousands of genes simultaneously (the *transcriptome*) and to quantify thousands of translated proteins (*proteomics*). By comparing such data between, for example, cancer cells and normal cells, one can identify proteins transcribed or present at elevated or depressed levels in the disease state. Mining data about these proteins from sources such as biomedical databases, scientific articles, and patents, and integrating it with the omics data, may suggest certain proteins as candidate drug targets.

A totally different approach starts with the use of high-throughput instrumentation and robotics to test a large collection of small molecules (a *chemical library*) for biological activity in a *phenotypic screen* (Swinney and Lee, 2020), which might use automated microscopy and image analysis to determine which compounds produce desired biological effects, such as the activation of a desired gene in cultured human cells or the death of a parasitic microorganism in culture. Various methods may then be used for *target deconvolution* (i.e., to determine how the active small molecules work). For example, candidate targets of compounds found to kill the malarial parasite *Plasmodium falciparum* were identified by cultivating these organisms in gradually increasing concentrations of the compound to select for resistant protozoa and then using omics methods to determine which genes had changed. The proteins encoded by these genes may then become candidate drug targets (Flannery et al., 2013).

## Target Validation

After a candidate drug target has been identified, additional research is usually warranted to *validate* it by seeking stronger evidence that a small molecule that binds and modulates it will actually treat the disease (Jones, 2016; Lansdowne, 2018; see Box 1–1). For example, the fact that a protein is more abundant in cancer cells than normal cells by no means proves that it is a suitable drug target. Instead, this might be a correlate rather than a cause, so further research is needed to assess its role. Accordingly, *target validation* aims to "de-risk" a project by lowering the probability that a compound carefully developed to hit the targeted protein will fail in clinical trials, whether because hitting the target does not influence the disease as expected or because the compound generates unanticipated toxicity, termed *on-target* or *mechanism-based* toxicity.

There are no absolute criteria for target validation, nor is there a single method. One approach is to use a *chemical probe*, a small molecule that binds the target, and study its biological effects (Quinlan and Brennan, 2021). This approach requires that such a probe be available, and the fields of *chemical genetics* (Stockwell, 2000) and *chemogenomics* (Bredel and Jacoby, 2004) aim to create selective chemical probes for as many proteins in the human genome as possible. Alternatively, one may use gene silencing via small interfering RNA (siRNA) to block production of the target protein, thereby mimicking the effect of an inhibitor of the protein's activity. Additional insight into the biological role of a candidate drug target may sometimes be obtained by studying genetically modified mice, including *knockout mice,* in which the gene coding for the target has been disabled entirely, and *transgenic mice*, in which expression of the target's gene is placed under the control of a promoter that can be turned on by feeding the animals a specific compound, such as *tetracycline* (Lindsay, 2003).

**BOX 1–1 ■ Target Validation: The Lesson of Leptin**

Biological systems frequently contain redundant elements or can alter expression of drug-regulated elements to compensate for the effect of the drug. *In general, the more important the function, the greater the complexity of the system.* For example, many mechanisms control feeding and appetite, and drugs to control obesity have been notoriously difficult to find. The discovery of the hormone leptin, which suppresses appetite, was based on mutations in mice that cause loss of either leptin or its receptor; either kind of mutation results in enormous obesity in both mice and people. Leptin thus appeared to be a marvelous opportunity to treat obesity. However, on investigation, it was discovered that obese individuals have high circulating concentrations of leptin and appear insensitive to its action.

## Target Druggability

It is important to know whether the candidate target is *druggable*, that is, whether it can, in principle, bind a small molecule with sufficient affinity. If the protein has been the target of a prior drug discovery effort, there may be informative small-molecule binding data in a public database, such as BindingDB (Gilson et al., 2016), PubChem (Kim et al., 2021), or ChEMBL (Gaulton et al., 2012), or in an article or patent not yet curated by one of these databases. One may also check the Protein Data Bank (Berman et al., 2000; Berman and Gierasch, 2021) for a crystal structure of the target, which may assist in locating a suitable binding pocket for the small molecule to be developed as a drug. This is frequently true for metabolic enzymes and receptors that have evolved to bind small substrate and transmitter molecules. Many proteins belong to families, such as the protein kinases, whose members have similar properties (e.g., an ATP binding pocket), so that if one member of a family is druggable, then the others probably are also. In contrast, receptors for proteins often have large, relatively flat binding surfaces, rather than small binding pockets suitable for a small-molecule drug, and are thus less likely to be druggable and influenced by small molecules. Efforts are under way to systematically search for all druggable targets encoded by the human genome (Nguyen et al., 2017; Finan et al., 2017; Hopkins and Groom, 2002) and to gain traction against targets hitherto considered *undruggable* (Dang et al., 2017).

The ultimate validation of a candidate target is the successful development of a novel drug that works by binding to it. Such a novel drug is termed *first-in-class*. A first-in-class drug is a true innovation and may represent a medical breakthrough, so one might expect first-in-class to be the goal of every drug discovery project. In fact, however, pharmaceutical companies often engage in less innovative, more predictable projects by developing *me-too drugs* against old targets that are already fully validated by a first-in-class drug. Such projects aim to improve on the first-in-class drug through, for example, greater potency, reduced side effects, or more convenient dosing (e.g., oral instead of intravenous), and ideally to produce a new drug considered *best-in-class*. For example, Merck's *lovastatin* broke ground as the first statin, the first in a class of drugs that lower cholesterol by inhibiting the enzyme HMG-CoA reductase (see Chapter 37); but other statins, such as *atorvastatin*, have also achieved enormous commercial success.

## Beyond Single-Protein Drug Targets

A number of drugs, whether by accident or by design, hit multiple protein targets, a phenomenon termed *polypharmacology* (Peters, 2013). This phenomenon is particularly common when the target is a member of a family of proteins with similar binding sites. For example, the

full physiological effect of an adrenergic antagonist is determined by its actions across the family of adrenergic receptor types and subtypes. Similarly, many protein kinase inhibitors inhibit multiple kinases, each to a different degree. There are instances where hitting multiple targets is fruitful, such as inhibiting sequential reactions in a series. Modulating multiple proteins in a single biochemical pathway or signaling network overcomes the evolved redundancy of a robust biological system and hence leads to greater efficacy than modulating only one protein. A single compound may, alternatively, hit two entirely different targets in different pathways, although this is more challenging to achieve without going to larger compounds. The analysis of complex molecular systems in relation to drug action is termed *systems pharmacology*.

Polypharmacology is not always beneficial, and indeed, it can lead to toxicity. Some of the unintended effects of a drug will be termed side effects or even major adverse drug responses. For example, a number of initially promising compounds have proven to bind and inhibit hERG, the $K^+$ channel in the heart that mediates repolarization (the $I_{Kr}$ current; see Chapter 34); inhibition of hERG can lead to potentially fatal arrhythmias. The hERG channel has, therefore, become a notorious *anti-target* that must be scrupulously avoided by drug discovery projects (Garrido et al., 2020).

Some small-molecule drugs do not bind to proteins at all. For example, platinum anticancer drugs, such as *carboplatin*, kill cancer cells by binding covalently to DNA; the aminoglycoside antibiotics block bacterial protein synthesis by binding to RNA within the bacterial ribosome; and antiviral nucleoside analogues are incorporated into viral DNA in place of normal nucleosides and then block DNA replication. The drug *sugammadex* has both an unusual purpose and an unusual mechanism. Surgical patients often receive not only general anesthesia but also the nondepolarizing neuromuscular blocking agent *rocuronium*, which prevents involuntary movements of skeletal muscle during surgical procedures (see Chapter 13). *Sugammadex*, a larger, cup-shaped molecule, binds and sequesters *rocuronium*. Thus, injection of *sugammadex* rapidly reduces the concentration of unbound *rocuronium* in the blood and promptly reverses paralysis when a procedure is complete.

## Protein-Drug Binding: Affinity and Allostery

A successful drug with a protein target must bind to its target with high affinity so that even a small dose of the drug will yield a blood concentration high enough to bind a large fraction of the targeted protein. If the affinity were low, then a high concentration of drug would be needed for a substantial fraction of the target sites to be occupied, and a large dose of drug would need to be administered, leading to inconvenience and an increased risk of side effects. The affinity of a small molecule for a protein is generally given as the dissociation constant, the concentration of free drug molecules in solution at which 50% of the targeted protein has bound drug; the lower this concentration, the higher the affinity (see Figure 3–3). Drug design projects typically aim for a dissociation constant on the order of $10^{-9}$ mol/L (1 nM); such a "nanomolar drug" is typically dosed in milligrams to grams per day. A successful drug should also exhibit a high degree of specificity for its target protein, meaning that the drug does not interact with other proteins that could lead to undesired side effects and toxicity. In some cases, the effectiveness of a drug may be influenced by not just the affinity but also the kinetic rate constants for drug-protein binding and dissociation, which determine the drug's residence time at its receptor (Copeland, 2016).

Most drugs bind their targeted proteins via attractive, intermolecular interactions that do not involve a covalent chemical bond. These *noncovalent interactions* typically include:

- Hydrogen bonding, in which an electronegative atom with a bound hydrogen atom, such as a hydroxyl group, partly shares its hydrogen with an electronegative atom on the other molecule
- Attractive electrostatic interactions between atoms of opposite charge, such as between a negatively charged carboxylic acid belonging to the drug and a positively charged arginine side chain of the protein
- The hydrophobic effect, in which nonpolar or "greasy" parts of the drug and protein associate with each other to reduce their energetically unfavorable exposure to water, much as oil droplets coalesce in salad dressing
- Dispersion forces—the attractive part of van der Waals interactions—short-ranged attractive interactions between the instantaneous electrical dipoles that result from the constant fluctuations of negatively charged atomic electron clouds around positively charged atomic nuclei

These attractive forces need to overcome the entropic tendency of the drug and protein to wander apart, due to thermal energy. There are also, inevitably, forces that oppose binding and that must be overcome by the attractive ones. For example, there is an energy penalty for stripping water from polar chemical groups of the ligand and protein as they come together to bind. Thus, the overall affinity of a drug-protein interaction reflects a delicate and hard-to-predict balance of attractive and repulsive interactions.

Small-molecule drugs do not bind to the relatively smooth, exterior surfaces of their protein targets, but instead are enfolded by binding pockets in the protein (see Figure 1–4). This structural arrangement makes it possible to form the extensive, short-ranged, physical interactions that are needed to hold the two molecules together tightly. Druggable binding pockets (i.e., ones that enable small-molecule binding) usually are available in enzymes whose substrates are small molecules and in receptors that bind small-molecule hormones and transmitters. However, many proteins lack a concave pocket and therefore are difficult or impossible to drug with a small molecule. In such cases, one may instead consider developing a protein therapeutic, such as an engineered antibody that targets the protein of interest. Because proteins are large, they can form extensive, short-ranged, physical interactions even with the relatively flat exterior surface of a targeted protein, and thus can achieve adequate binding affinity where a small-molecule drug cannot. These considerations also help explain why it is difficult to develop a small-molecule drug that will block a protein-protein interaction: protein-protein binding usually involves a large number of interactions on a relatively flat binding interface between the two proteins, and a small molecule cannot get sufficient purchase on such a flat surface.

Note that a drug must not only bind to its target but also have the desired effect upon it. If the goal is to inhibit an enzyme, then a drug that binds in the active site should easily accomplish this by simply blocking association of the enzyme with molecules of substrate. In contrast, when a cell-surface receptor is the target, a small molecule might interact at the agonist binding site but without inducing an activating conformational change and thus might function as an antagonist or inverse agonist (see Chapter 3). A drug may also inhibit the function of a protein by binding in a pocket outside the active site, and thereby modifying the three-dimensional conformation of the targeted protein; this is an allosteric effect. Such a drug must not only bind in a suitable pocket but also induce the desired conformational change. *Efavirenz* and *nevirapine*, used in treating HIV-AIDS, are nonnucleoside reverse transcriptase inhibitors that act allosterically to inhibit viral transcription of viral RNA to DNA (see Figure 65–5). Similarly, a number of ligands interact with allosteric sites on $GABA_A$ receptors (see Figure 16–11) and other Cys-loop receptors to modulate receptor/channel function. Allostery can also offer a sophisticated strategy to target a single enzyme from among a family of similar enzymes. Thus, in designing a drug, one might take advantage of the fact that, even within a family of related proteins with similar active sites, the members will likely have other regions of their structure that are more variable and possibly unique. Designing a small ligand that binds to such a site might produce an agent that is a quite selective allosteric modifier of enzyme function. This approach is being used to target selected protein phosphatases (Mullard, 2018).

A few small-molecule drugs react chemically with their protein targets to form *irreversible*, *covalent* bonds, rather than relying entirely on the noncovalent attractions discussed above. Such covalent drugs bond to a

specific chemical group of the protein target, often a relatively reactive amino acid side chain within an enzyme's catalytic site. In principle, covalent drugs should require smaller, less frequent dosing, because a covalently bound drug will not dissociate from the protein as the concentration of free drug dwindles over time following a dose (but note that some boron-containing compounds form *reversible* covalent bonds [Diaz and Yudin, 2017]). Drug developers have tended to avoid covalent drugs because they necessarily possess chemically reactive groups that risk reacting not only with the desired target but also with other proteins and biomolecules, with the potential for causing undesired biological effects. However, selectivity can be achieved by specific noncovalent interactions between the drug and the protein that pull the compound into a location and conformation where it is poised to form the desired covalent bond.

Covalent binding has been used to successfully target and inhibit a member of the RAS GTPase family, KRAS G12C, which had been viewed as virtually undruggable. As a result of such targeted positioning, the cancer drug *sotorasib* gains both potency and specificity by forming a covalent bond with a cysteine side chain present in an oncogenic mutant form of KRAS but not in normal KRAS (Lanman et al., 2020).

## Experimental Approaches to Drug Discovery

Given a validated target, the next major milestone in a drug discovery project is arrival at a *clinical candidate*, a small molecule that binds the target with high affinity and specificity, has the desired effect on it, and meets a range of other criteria for a safe, efficacious drug (Hefti, 2008). Some of these criteria relate to *pharmacokinetics*: How well will the compound be absorbed if given orally? How well does it distribute to the targeted organs and tissues? How rapidly and by what mechanisms is it eliminated? Is it metabolized to an active metabolite? These properties are often lumped together as absorption, distribution, metabolism, and excretion (*ADME*) or drug metabolism and pharmacokinetics (*DMPK*).

It is also essential to confirm that the compound does not show evidence of toxicity. Both pharmacokinetics and toxicity can be initially studied *in vitro*. For example, there are *in vitro* methods that examine the ease with which the compound enters cells (see Chapter 4) and the likelihood that liver enzymes (see Chapter 5) will chemically modify the compound. Compounds also can be evaluated *in vitro* for evidence of toxicity and mutagenicity. However, *in vitro* studies cannot fully model the complexities of a living organism; animal studies are still required to minimize the chances that a compound will be problematic when first given to human subjects. For example, toxicity is usually assessed by long-term monitoring of the health of two species of animals, generally one rodent (usually mouse) and one nonrodent (often rabbit), when dosed with the compound. A good clinical candidate should also meet some nonbiological criteria. In particular, it must be amenable to large-scale synthesis and high-grade purification at acceptable cost, and it should be possible to create a formulation (e.g., a tablet or injection) that is sufficiently water soluble and stable.

Sophisticated technologies have been developed to speed the process of generating a clinical candidate. These mainly focus on the discovery or design of compounds that will bind the protein target with high affinity (*potent ligands*). Less progress has been made toward designing in safety and favorable pharmacokinetics. These properties pose more complex challenges, because they go far beyond how a small molecule and a protein interact with each other and instead involve the interactions of the small molecule with thousands of different biomolecules in a living system. The technologies for ligand discovery are both experimental and computational, and different methods are applicable in different settings. The following subsections touch on broad approaches but are not comprehensive. Note, too, that various approaches can be used in combination, so the distinctions made here are ultimately somewhat artificial.

### Medicinal Chemistry

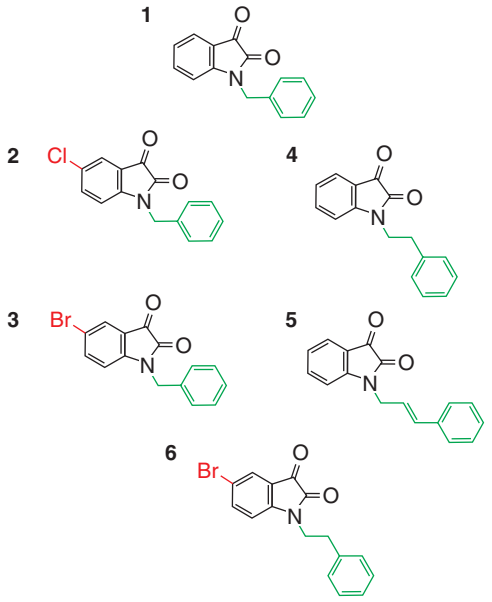
Synthetic organic chemistry remains at the heart of small molecule drug discovery, where it is specialized and known as medicinal chemistry. Medicinal chemists typically are part of a project team that includes, among others, biologists, assay specialists, and computational chemists; their role is to reduce chemical concepts to practice by synthesizing and purifying compounds that may ultimately lead to a new drug. In addition to providing the expertise needed to synthesize compounds of interest, they also help guide the design and selection of the compounds to be made. A key consideration is the complexity of a compound's synthesis, or "synthetic accessibility", which must be balanced against the level of interest in the compound. For example, it can be difficult to generate pure stereoisomers of compounds with multiple chiral carbon atoms, and certain chemical structures can by synthesized only via demanding, multi-step syntheses. A compound that is too difficult to make or purify will not only slow down the research effort but may also lead to a drug that is too costly to manufacture.

Medicinal chemists also inform the drug design process by providing insights into the properties of various chemical groups that might be incorporated into a drug, such as the attractive or repulsive interactions they may form with the targeted protein, their susceptibility to metabolic changes following administration, their potential to spontaneously form undesired covalent bonds with biomolecules, and their influence on the compound's ability to cross the blood-brain barrier (which may be desirable or undesirable, depending on the goal of the project). This expertise comes into play, for example, when a compound binds the target well but is rapidly metabolized by the liver into an inactive product. In this setting, the medicinal chemist may try substituting the part of the compound that is metabolized with a "bioisostere", a different chemical group with a similar shape and ability to interact with the protein but with reduced susceptibility to metabolic modification. More broadly, decades of experience have led to a number of rules of thumb for what makes a compound "drug-like", such as the "rule of five" (Lipinski, et al., 2001). These may be useful guides during drug discovery projects, but there are also many exceptions to the rules (Zhang et al., 2007).

### High-Throughput Screening

If nothing is known about the structure of the target protein and what small molecules can bind it, it is common to turn to HTS, in which thousands or millions of compounds are tested using automation and robotics (Wildley et al., 2017). Tiny samples of each compound are drawn from a stored *chemical library* and deposited into multiwell plates for testing. Substantial effort often must be invested to devise an assay that works reliably in miniature and without user intervention. Most provide an optical readout, such as a change in luminescence, fluorescence, or color, as these can be efficiently measured with an optical plate reader. The compounds screened can range from part of the vast, in-house compound collection that a major pharmaceutical company has assembled over the years to a smaller set purchased from a commercial vendor. A screening library is often designed for the particular application. For example, one can purchase libraries tuned for activity against protein kinases, libraries with reactive groups that can form covalent bonds to the protein, and libraries designed to sample a wide range of compounds through high *chemical diversity*. A compound chosen at random from a screening library has a very low probability, typically 0.1% or less, of being active against a given target (Shun et al., 2011), and HTS measurements are subject to experimental error. Therefore, many of the compounds that appear active on an initial screen (*hit compounds*) are false positives, so careful data analysis and confirmatory testing are essential.

Even the confirmed hits from a high-throughput screen are far from being drugs. Their affinity for the target usually is orders of magnitude too weak, they may lack the desired specificity, and they do not meet DMPK or safety criteria. However, they offer an initial toehold on the challenge of finding a potent drug candidate. The next step is to purchase (*analogue by catalog*) and/or synthesize (*medicinal chemistry*) similar compounds that ultimately give a picture of how various changes in




| Compound | ALDH1A1 | ALDH2 | ALDH3A1 |
|---|---|---|---|
| 1 | 0.02 | 82 | 7.7 |
| 2 | 0.06 | 2.1 | 16 |
| 3 | 0.58 | 2.1 | 69 |
| 4 | 0.07 | 3.5 | 0.45 |
| 5 | 0.07 | >100 | 0.31 |
| 6 | 2.0 | 0.05 | 18 |

**Figure 1–1** *Structure-activity relationship: scaffolds and substituents.* Five inhibitors of the aldhyde dehydrogenase family of enzymes have a common chemical scaffold (black) while having different chemical substituents at two positions (red, green). The table lists the $IC_{50}$ (μM) of each compound for three members of the aldehyde dehydrogenase family of enzymes: ALDH1A1, ALDH2, and ALDH3A1; i.e., the concentration of compound needed to provide 50% inhibition of each enzyme. The lower the $IC_{50}$, the more potently the compound inhibits the enzyme. Focusing first on compounds **1**, **2**, and **3**, one can see that adding an increasingly bulky halogen atom (Cl, Br) on the six-membered ring tends to reduce the compound's potency against ALDH1A1 and ALDH3A1 but to increase it against ALDH2. Focusing next on compounds **1**, **4**, and **5**, one can see that adding increasingly bulky, nonpolar, aromatic substituents at the nitrogen modestly reduces the potency against ALDH1A1, initially improves but then destroys potency against ALDH2, and consistently improves potency against ALD3A1. Such patterns can guide the design of new compounds with desired potency and selectivity. For example, the substituents in compounds **3** and **4** each reduce potency against ALDH1A1 while increasing potency against ALDH2, so it is not surprising that compound **6**, which combines both substituents, has particularly low potency against ALDH1A1 and high potency against ALDH2. Note, however, that this kind of reasoning can only offer guidelines; its predictions are not always borne out by experiment. Data drawn from Kimble-Hill et al., 2014.

the chemical structure influence activity against the target (*structure-activity relationships, or SAR*) and other properties (Figure 1–1). This information is used to guide the synthesis of often hundreds of compounds with gradually improving properties. The most promising early molecules (*lead compounds*) serve as starting points for further improvement (*lead optimization*), ultimately generating, hopefully, a clinical candidate, potentially accompanied by several *backup compounds* in case the leading candidate fails.

## Fragment-Based Drug Discovery

Even a large-scale screen can fail to provide useful hits (Keserü and Makara, 2009). This result becomes understandable when one recognizes that the number of stable, drug-sized, organic compounds is on the order of $10^{60}$ (Reymond et al., 2010), so a screen of even $10^6$ compounds scarcely touches the vastness of *chemical space*. This vastness results from the combinatorial explosion of ways of connecting various chemical substructures, such as benzene rings, hydroxyl groups, and cycloalkanes. To be a good binder, a compound has to get multiple substructures positioned so they all form favorable interactions with complementary groups in the targeted binding pocket. If it has two chemical components suitable for binding the target but a third that is inappropriate or in the wrong place on the compound, it may fail to bind the target. This perspective motivates another method of discovering binders, *fragment-based drug discovery* (FBDD) (Erlanson, 2012; Lamoree and Hubbard, 2017). In FBDD, one conceptually breaks down drug-sized compounds into their substructures (*fragments*) and tests simple substructures against the target. Although such fragment-like molecules can bind only very weakly, such studies can, nonetheless, identify a small set of chemical substructures that are suitable for the target, and one can then buy or synthesize larger compounds assembled from these components. When either X-ray crystallography (Patel et al., 2014) or nuclear magnetic resonance spectroscopy (Shuker et al., 1996) is used to detect or analyze fragment binding, specific information is usually available about where each fragment binds to the protein. This information can be used to stitch together designed compounds that place the appropriate fragments at the right places in the protein's binding pocket (*fragment linking*) or to optimize and expand one selected fragment (*fragment growing*). In this way, FBDD avoids the combinatorial explosion of possible compounds made from various chemical components and allows researchers to focus quickly on compounds made from only a productive subset of chemical components. The drug *vemurafenib*, which targets an oncogenic mutation of B-Raf kinase and was developed with a fragment-growing strategy, is usually referenced as the first FBDD success story (Bollag et al., 2012).

## Emerging Experimental Technologies

The difficulty and cost of drug discovery, coupled with the market and human need for new medications, have driven ongoing innovation in drug discovery technologies. For example, DNA-encoded compound libraries (DELs) dramatically expand the number of compounds that can be tested, relative to conventional HTS (Halford, 2017). Unlike a traditional HTS compound library, where each compound is kept in its own separate container or well, a DEL is a mixture of compounds in a single container and can include far more compounds—into the billions and even trillions. Each unique compound in the mixture is covalently bound to a corresponding unique short DNA molecule, which serves as an identification tag. Such libraries can be synthesized and tagged with the methods of *combinatorial chemistry*, where a mixture of compounds is split into multiple portions, each portion is modified with a different chemical step and its DNA tags modified accordingly, and the portions are mixed again. This process is iterated until the synthesis is complete. To screen the DEL for active compounds, one may immobilize the target of interest on a solid surface, expose the surface to the DEL mixture, and then wash the surface to remove all the DEL compounds that have not bound tightly to the target. The binders are then removed from the target by more aggressive washing, and the active compounds in the wash are identified by sequencing the DNA tags they carry.

Another emerging technology, sometimes termed *clinical trials in a dish* (Alpeeva et al., 2017; Fermini et al., 2018; Strauss and Blinova, 2017), aims to predict the effects of a compound in humans more accurately than is possible with standard cell culture or animal models. This approach involves creating specific cell types of interest from human pluripotent stem cells and using them to create three-dimensional organoids in culture (Fligor et al., 2018; Liu et al., 2021; Sato and Clevers, 2013) or artificial tissue architectures via three-dimensional bioprinting (Ferrer and Simeonov, 2017). These relatively intricate *in vitro* constructs promise to better recapitulate the properties of the corresponding *in vivo* tissues and may be used to test compounds for activity, DMPK properties, compound metabolism, and toxicity.

## Computer-Aided Drug Discovery

The rise of information technology has enabled the research community to store and move large quantities of information, to write and maintain complex software, and to do calculations at unprecedented speed and scale. These continually improving capabilities are used in a variety of ways to support and accelerate drug discovery. Thus, chemical informatics enables compact databasing of information on hundreds of millions of compounds and rapid recovery of chemical data for a specific compound and/or chemically similar compounds (Willett et al., 1998), while the Internet makes chemical (Gaulton et al., 2012; Gilson et al., 2016; Kim et al., 2021), macromolecular (Benson et al., 1994; Berman et al., 2000; Berman and Gierasch, 2021; UniProt Consortium, 2015), biomolecular pathway (Croft et al., 2014; Ogata et al., 2000; Oughtred et al., 2021; Wishart et al., 2020), and other databases readily accessible to researchers worldwide. These data are useful in their own right and also support the development and evaluation of computer models used in drug discovery.

In parallel, exponential increases in computer speed, measured as the number of mathematical operations executed per second, have made more and more detailed molecular simulations feasible. Ideally, a computational chemist could design a compound, hand the design to a medicinal chemist to synthesize, and the compound would prove to bind the target with nanomolar affinity. When this level of accuracy becomes feasible, one might go further and compute the affinity of a candidate drug to all known human proteins in order to check for unwanted interactions. This level of accuracy is not possible today, but existing methods have predictive value, and growing computer power may make this vision achievable in the coming years.

Approaches to predicting the interactions of a small molecule with a protein may be broadly divided into *ligand-based* and *structure-based approaches*, as explained below.

### Using Chemical Similarity to Discover Targeted Ligands

If the targeted protein is an enzyme with a small-molecule substrate or a receptor for a small-molecule transmitter (e.g., histamine), then compounds chemically similar to the substrate or transmitter may be active against the target and thus useful starting points for drug design (Figure 1–2). For some targets, more extensive information about ligands for the target may be available from prior drug discovery efforts and may be used to guide a new project. As noted above, even if a drug has already been developed against the target, there may still be room for a me-too drug with better properties, such as less frequent oral dosing or reduced side effects. Large quantities of data to support this ligand-based drug discovery approach are available in the scientific literature, patents, and public databases (Gaulton et al., 2012; Gilson et al., 2016; Kim et al., 2019).

Metrics of chemical similarity abstract the detailed chemical structures of compounds into characteristics that can be computed and compared across molecules. One approach computes a compound's molecular fingerprint, which indicates whether various molecular substructures are present (Muegge and Mukherjee, 2016). Other similarity metrics jettison such details and, instead, compute and compare the overall shapes of the two molecules and the electrical fields they generate (Bajorath, 2017). In a third approach, even molecular shape is set aside and one instead computes tens or hundreds of quantitative *descriptors* for each compound. Examples include simple descriptors, such as molecular weight or number of aromatic rings, and more complex descriptors such as electrical dipole and quadrupole moments. If one imagines descriptors as Cartesian coordinates in a multidimensional space, one can then quantify the similarity of two molecules in terms of how close they are in this *descriptor space* (Wale et al., 2008).

Similarity metrics such as these enable *virtual screening*, a fast, inexpensive, computational alternative to experimental HTS (Figure 1–3). In this approach, every compound in a chemical library—a large set of compounds that are available or synthesizable—is assessed for its similarity to one or more known ligands of the protein target. The most similar compounds are tested in an experimental assay, and confirmed hits become candidates for further chemical optimization. This approach is most relevant when the three-dimensional structure of the targeted protein has not been determined. When the structure is known, powerful structure-based methods become applicable.

### Structure-Based Drug Design

The detailed three-dimensional structure of a targeted protein opens up a range of additional computational methods for designing a small molecule that binds the target with high affinity (Figure 1–4). The applicability of such SBDD methods has grown continually, due to rapid increases in computer power and the development of technologies that make determining protein structures easier and faster. One example is the use of synchrotrons (e.g., the Advanced Photon Source at Argonne National Laboratory) to generate high-quality X-ray beams for use in protein X-ray crystallography. Another is the development of methods to solve the structures of membrane-bound proteins, such as ion channels and cell-surface receptors. These can be high-quality drug targets because a drug does not need to enter the cell to access them and because they regulate many cellular processes. However, their structures were virtually impossible to solve until methods were developed in recent years to grow three-dimensional crystals of them. Since at least the 1980s, the promise of advances in SBDD methods has inspired the founding of multiple companies.

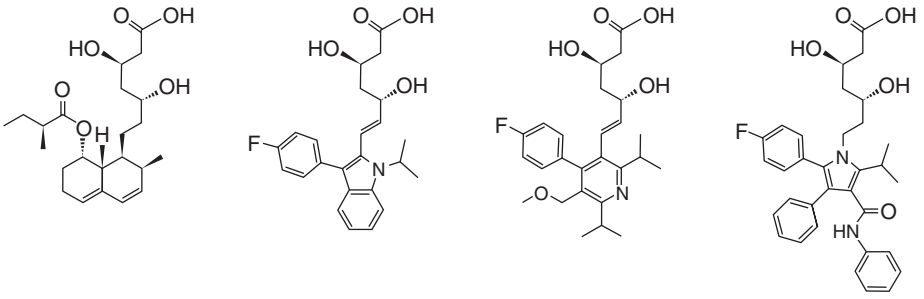
The field of physical chemistry tells us how to compute the binding affinity of two molecules in water (Gilson and Zhou, 2007). Ideally, one could use numerical solutions of Schrödinger's equation to obtain the electronic wave function for the compound, the target protein, and the aqueous solvent, for any given conformation of the system (i.e., given the Cartesian coordinates of all atoms). From the wave function, one could then compute the instantaneous force on every atom. Given this method of computing atomic forces, one could simulate the system at atomistic detail, computing the reversible work of gradually pulling the compound out of the protein binding site as all the atoms wiggled, jiggled, and shifted due to thermal motion (Feynman et al., 1963). This reversible work would equal the free energy of binding, $\Delta G^o$, which is directly related to the dissociation constant, $K_D$:

$$\Delta G^o = RT \ln K_D \qquad \text{(Equation 1–1)}$$

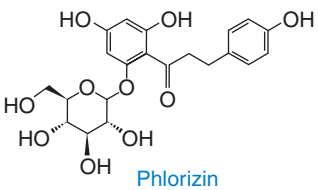
This would be a prohibitively massive calculation with existing computer technology. However, researchers have created fast approximations to such an ideal calculation, each with its own strengths and weaknesses in terms of accuracy, range of applicability, and the computer power required (Figure 1–5).

An important approximation used in molecular modeling is the force field or potential function, a mathematical model for the atomic forces that can be evaluated orders of magnitude faster than solving Schrödinger's equation (Dauber-Osguthorpe and Hagler, 2019). Force fields often contain adjustable parameters fitted to give agreement with reference solutions of Schrödinger's equation. With a force field in hand, it becomes practical to use molecular simulations to estimate protein-ligand binding free energies (Tembe and McCammon, 1984; Kollmann, 1993; Gilson et al., 1997; Simonson et al., 2002). Such *free energy methods* are among the most accurate approaches available to predict protein-ligand binding affinities (Schindler et al., 2020), and their use by the drug

**A. Statins**




**B. SGLT Inhibitors**




| SGLT inhibitor | $IC_{50}$ (nM) at SGLT1 | $IC_{50}$ (nM) at SGLT2 | Relative selectivity for SGLT2 (col2/col3) |
|---|---|---|---|
| Phlorizin | 290 | 21 | ~14 |
| Canagliflozin | 710 | 2.7 | ~260 |
| Dapagliflozin | 1400 | 1.2 | ~1200 |
| Empagliflozin | 8300 | 3.1 | ~2700 |
| Ertugliflozin | 2000 | 0.9 | ~2200 |

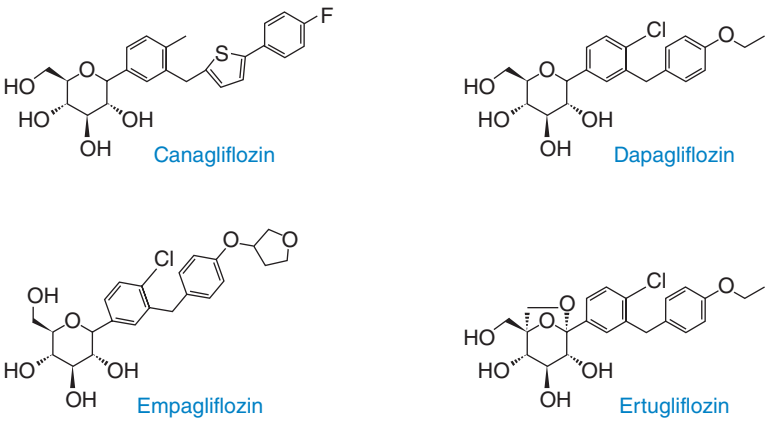



**Figure 1–2** *Using chemical similarity to develop ligands.* **A.** *Statins.* Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), the rate-limiting enzyme in cholesterol synthesis. These inhibitors are widely used to lower blood levels of cholesterol (see Chapter 37). Mevastatin is a natural product that inspired development of the three FDA-approved statins shown here. Each compound has a polycyclic lower part linked to a common hydroxyacid moiety, which can also exist as a cyclic lactone. **B.** *SGLT inhibitors.* Sodium-glucose cotransporters (SGLTs) facilitate glucose ingress in the gastrointestinal tract (SGLT1) and the kidney (SGLT2). The natural product phlorizin inhibits both SGLTs to varying extents. Modifications of the phlorizin structure led to the four FDA-approved relatively specific SGLT2 inhibitors, the gliflozins, shown here. Gliflozins reduce renal reabsorption of glucose, thereby lowering blood sugar concentrations, and thus are used to treat type 2 diabetes (see Chapter 51). Each compound has a glucose moiety (except *ertugliflozin*, which has a glucose-similar moiety), sensible for compounds that interact with transporters that bind glucose. Phenyl-containing moieties endow each inhibitor with varying activities against each of the two protein forms, as shown in the table. Activities are given as $IC_{50}$, the concentration of drug (nM) that reduces the transporter's activity by 50%. Data adapted from Fediuk et al. (2020) and Wright (2021).

discovery community has been enabled by the acceleration of molecular simulations on graphics processing units (GPUs) (Salomon-Ferrer R, et al., 2013). Even with GPUs, though, the simulations are too slow to replace an experimental high-throughput screen of millions of compounds. Instead, simulations are most commonly used to help medicinal chemists decide which chemical variations on a promising starting compound are worth synthesizing and testing. Fast molecular simulations also are used to explore the various conformations that a protein can adopt. For example, if a simulation shows that a new binding pocket could form as a result of thermal protein motions, it may be possible to design a drug that will bind this hitherto unrecognized site.

Another computational approach, *molecular docking* (Guedes et al., 2014; Huang, 2010; Meng, 2011), is fast enough to substitute for (or supplement) a large-scale experimental high-throughput screen. In docking, most or all of the protein is held rigid, and the software tries a vast number of different locations and conformations—*poses*—of a small molecule in the target's binding site, searching for the one that is lowest in energy and hence most stable. Because docking leaves out so many known contributions to the free energy of binding (e.g., protein flexibility and entropy), the energy model usually must be tuned against experimental binding data to make it more predictive. The resulting model is often called a docking *score*, to differentiate

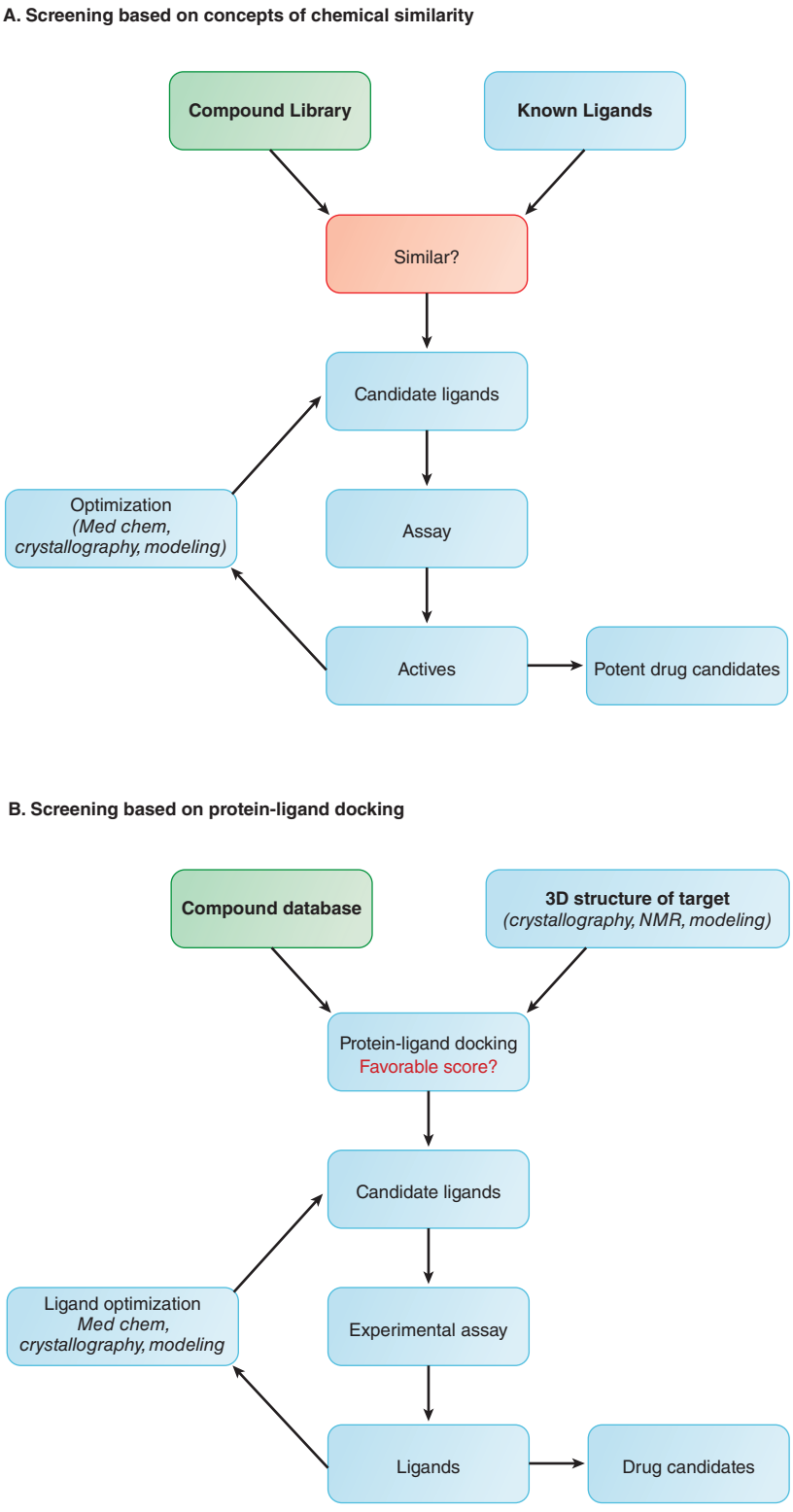



**Figure 1–3** *Virtual screening.* **A.** *Virtual compound screening based on concepts of chemical similarity.* Using available similarity metrics, the compounds in a database (green) are computationally tested for chemical similarity to the known ligands (binders) of the targeted protein. Compounds that are above some threshold similarity are considered candidate ligands and so are experimentally assayed for binding to the protein target. Those found to be inactive are set aside, while "actives" are subjected to iterative rounds of ligand optimization where structure-activity relationships are defined and used to guide the design of new compounds by medicinal chemists. When sufficiently active compounds are found, these become early-stage drug candidates. **B.** *Virtual compound screening based on protein-ligand docking.* The compounds in a database (green) are computationally docked; i.e., optimally fitted into the binding site of a target protein of known three-dimensional structure. Compounds whose computed stabilizing interactions with the binding site are above a threshold similarity are considered candidate ligands and so are experimentally assayed for binding to the protein target. Those found to be inactive are set aside, while "actives" are subjected to iterative rounds of ligand optimization. This typically involves using the protein structure to design new compounds that can form better interactions with the binding site and solving crystal structures of the protein with selected compounds to determine whether the designed compounds bind as hoped and to guide further rounds of chemical design and synthesis. Advanced computational methods, such as simulation-based free energy calculations, may also be used at this stage. When sufficiently active compounds are found, these become early-stage drug candidates.

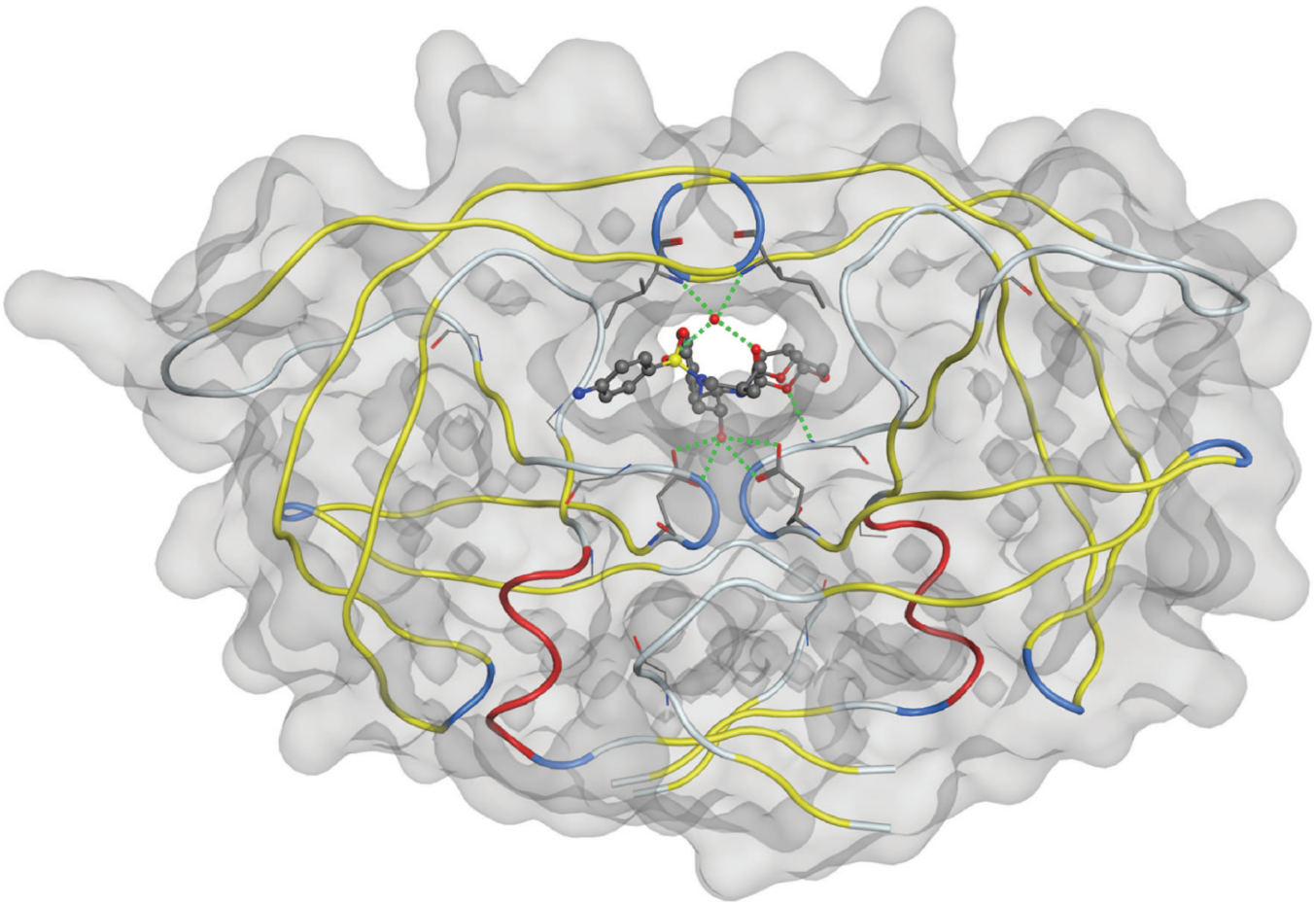


**Figure 1–4** *Crystal structure of the human immunodeficiency virus 1 protease (HIV-1 protease) with the protease inhibitor darunavir bound in the active site.* Colored tubes: protein backbone of the enzyme, a symmetric dimer made up of two identical subunits, where color indicates secondary structure (yellow, β-sheet; red, α-helix; blue, turn; white, none). Translucent gray: overall surface of the protein, including both side-chain and backbone atoms. Ball and stick: *darunavir* in the tunnel-shaped active site, with atoms colored by element (gray, carbon; red, oxygen; blue, nitrogen), with hydrogen atoms omitted for simplicity. Key hydrogen bonds are shown as dashed green lines, and the oxygen of a water molecule that bridges between the drug and the protein is shown as a red ball. Atomic coordinates from Protein Data Bank (Wang et al., 2011).

it from a true force field. Docking calculations are typically used for *virtual HTS* (see Figure 1–3), in which thousands or millions of compounds in a chemical library are rapidly fitted into the binding site of the targeted protein. Tens or hundreds of the top-scoring compounds may then be subjected to more detailed calculations or tested experimentally. Although not all of the top-scoring compounds will be good binders, the fraction of binders will normally be enriched relative to the chemical library as a whole. In addition, the predicted binding poses may provide mechanistic insight and serve as starting points for molecular simulations (Guest et al., 2022; Heinzelmann and Gilson, 2021). The empirically tuned scoring functions used in docking can also be used to guide manual chemical editing of a known binder with graphical molecular modeling software. For example, one may manually edit an existing compound in the context of a three-dimensional rendering of the binding pocket to design a new compound that reaches into a neighboring subpocket and forms stabilizing hydrophobic and hydrogen-bonding interactions with the protein. This interactive work may be aided by immersive visualization and manipulation technologies, such as virtual reality.

## Artificial Intelligence in Drug Discovery

Deep neural networks have proven their power in wide-ranging artificial intelligence tasks such as image recognition and language translation, and researchers are now exploring their use in drug discovery. These methods may be *trained* on existing data, such as on existing collections of protein–small-molecule binding data, the results of DELs, and protein structures, to enable direct prediction of protein–small-molecule binding and automated design of ligands for a targeted protein. They may also support drug discovery in other ways, such as by predicting the three-dimensional structures of proteins (AlQuraishi, 2021; Baek et al., 2021; Jumper et al., 2021), the energies of molecules as a function of conformation (Smith et al., 2017), and molecular properties such as whether a compound is water soluble (Francoeur and Koes, 2021). Artificial intelligence and machine learning will undoubtedly play an expanding role in drug discovery in the coming years.

## Designing Large Molecules as Drugs: The Rise of Biopharmaceuticals

Large molecules are increasingly important as therapeutic agents. For example, antisense oligonucleotides are used to block gene transcription or translation, as are siRNAs and modified mRNAs (as in several vaccines for SARS-CoV-2 [severe acute respiratory syndrome coronavirus 2]). Important proteins used therapeutically include monoclonal antibodies, enzymes, and peptide hormones. Protein therapeutics were uncommon before the advent of recombinant DNA technology except for the few peptide hormones that could be isolated and purified in bulk. Insulin was

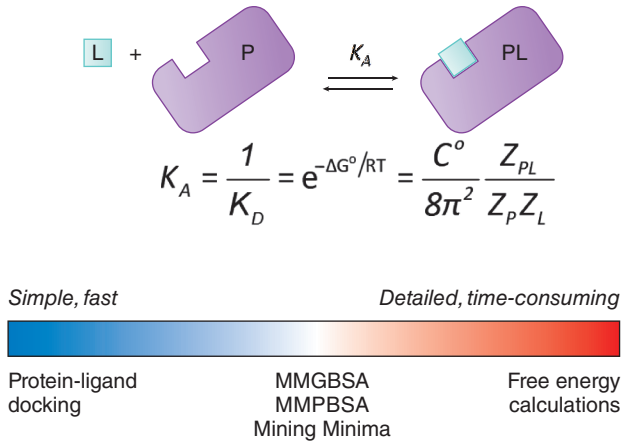



**Figure 1–5** *Physics-based computational methods for estimating protein-ligand binding affinities.* These methods provide an estimate of the association constant, $K_A$, for binding of a ligand, L, to a protein of known three-dimensional structure, P, to form a protein-ligand complex, PL, held together typically by noncovalent interactions such as hydrogen bonding and the hydrophobic effect. The equation relates $K_A$ to the standard free energy of binding $\Delta G^o$, the gas constant R, and the absolute temperature T, and further relates the binding free energy to the standard concentration $C^o$, and the configuration integrals of the protein-ligand complex ($Z_{PL}$), the unbound protein ($Z_P$), and the unbound ligand ($Z_L$). As more low-energy conformations are accessible to each molecular species (PL, P, L), the corresponding value of Z increases. Therefore, if the protein-ligand complex can access more low-energy conformations than the separate protein and ligand, the equilibrium constant will be large, favoring binding. Direct calculation of these configuration integrals is a computational challenge; however, researchers have created a spectrum of computational methods, ranging from fast, approximate methods that are expected to be less accurate, to more detailed, more computationally demanding methods that are typically more accurate (Gilson and Zhou, 2007). Docking, discussed in the text, is at the fast end of the spectrum; it treats the protein as mainly rigid, along with other approximations. Free energy calculations, also discussed in the text, are at the slow end of the spectrum; they treat the protein and ligand as fully flexible. In the middle of the spectrum are the molecular mechanics generalized Born/surface area (MMGBSA) (Srinivasan et al., 1998), molecular mechanics Poisson-Boltzmann/surface area (MMPBSA) (Gouda et al., 2003), and Mining Minima methods (Chen et al., 2010). These use various approaches to directly estimate the configuration integrals, $Z_{PL}$, $Z_P$, and $Z_L$. For example, Mining Minima searches for low-energy conformations of the protein, the ligand, and the complex; estimates their individual contributions to Z; and sums these contributions to provide an overall estimate of the configuration integral.

introduced into clinical medicine for the treatment of diabetes following the experiments of Banting and Best in 1921. Insulins purified from porcine or bovine pancreas are active in humans, although antibodies to the foreign proteins are occasionally problematic. Growth hormone, used to treat pituitary dwarfism, exhibits more stringent species specificity. Only the human hormone could be used after purification from pituitary glands harvested during autopsy, and such use had its dangers—some patients who received the human hormone developed Creutzfeldt-Jakob disease (the human equivalent of mad cow disease), a fatal degenerative neurological disease caused by prion proteins that contaminated the drug preparation.

Thanks to gene cloning, expression of the cloned gene in bacteria or eukaryotic cells, and large-scale production techniques, protein therapeutics now use highly purified preparations of human (or humanized) proteins. Rare proteins can be produced in quantity, and immunological reactions are minimized. Proteins can be designed, customized, and optimized using genetic engineering techniques.

Proteins used therapeutically include hormones, growth factors (e.g., erythropoietin, granulocyte colony-stimulating factor), cytokines, and a number of monoclonal antibodies used in the treatment of cancer and autoimmune diseases (see Chapters 38–40, 45, and 72). Murine monoclonal antibodies can be "humanized" (by substituting human for mouse amino acid sequences). Alternatively, mice have been engineered by replacement of critical mouse genes with their human equivalents, such that they make completely human antibodies. Protein therapeutics are administered parenterally, and their receptors or targets must be accessible extracellularly.

Using some of the strategies outlined above, nonantibody therapeutic proteins and peptides can now be optimized for stability, activity, and targeting to particular cell types. Peptides are being developed as therapeutics, especially in the area of interrupting protein-protein interactions where the large contact surfaces may defy small-molecule action. Computational methods are proving very useful in the design of peptide therapeutics (Belvisi et al., 2021). Therapeutic proteins are usually close copies of naturally occurring proteins that are optimized for high stability (both during manufacture and after administration) and optimized to avoid rapid degradation, to have low immunogenicity, and to have high potency when administered to a patient. Strategies include optimizing expression of a protein's gene sequence in multiple hosts, exploring close relatives of the protein of interest and mutations (random and rational), introduction of posttranslational modifications, and exploring biological modifications such as fusion with macromolecules (Dellas et al., 2021). Conjugation strategies (e.g., PEGylation) can be used to improve pharmacokinetic properties of therapeutic proteins (Moncalvo et al., 2020). The roster of recently engineered proteins that are not antibodies includes agents for cancers, gout, clotting disorders and hemophilia, inherited metabolic diseases, lysosomal storage disorders, pancreatic exocrine deficiency, insufficiencies of hormones and growth factors, and macular degeneration, among others. The number of nonantibody FDA-approved therapeutic proteins and peptides is growing rapidly (see a database of FDA-approved proteins and peptides at https://webs.iiitd.edu.in/raghava/thpdb/index.html; Usmani et al., 2017). A few protein therapeutics are administered topically or orally, but most are administered by injection. However, this is changing with the development of liposomal drug delivery systems, which are administered parenterally but are proving amenable to inhalation, ocular, and topical routes.

## The Investigational New Drug Application

Before the drug candidate can be administered to human subjects in a clinical trial, the sponsor must file an Investigational New Drug (IND) application, a request to the FDA for permission to use the drug for human research (see Clinical Trials, below). The IND describes the rationale and preliminary evidence for efficacy in experimental systems, as well as pharmacology, toxicology, chemistry, manufacturing, and so forth. It also describes the plan (protocol) for investigating the drug in human subjects. The FDA has 30 days to review the IND application, by which time the agency may disapprove it, ask for more data, or allow initial clinical testing to proceed.

## Clinical Trials

### Role of the FDA

The FDA, a federal regulatory agency within the U.S. Department of Health and Human Services (DHHS), is responsible for protecting the public health by ensuring the safety, efficacy, and security of human and veterinary drugs, biological products, medical devices, our nation's food supply, cosmetics, and products that emit radiation (FDA, 2018). The FDA also is responsible for advancing public health by helping to speed innovations that make medicines and foods more effective, safer, and more affordable and by helping people obtain the accurate, science-based information they need to use medicines and foods to improve their health.

The first drug-related legislation in the U.S., the Federal Pure Food and Drugs Act of 1906, was concerned only with the interstate transport of adulterated or misbranded foods and drugs. Motivations for federal regulation included the prominence of "patent medicines" and

their adulteration, the journalism of S. H. Adams (via articles in *Colliers Weekly*), and Upton Sinclair's novel *The Jungle* (Law, 2004). In the 1906 act, there were no obligations to establish drug efficacy or safety. This act was amended in 1938 after the deaths of over 100 children from "elixir sulfanilamide," a solution of *sulfanilamide* in *diethylene glycol*, an excellent but highly toxic solvent and an ingredient in antifreeze. The enforcement of the amended act was entrusted to the FDA, which began requiring toxicity studies as well as approval of a New Drug Application (NDA) (see The Conduct of Clinical Trials, below) before a drug could be promoted and distributed. Although a new drug's safety had to be demonstrated, no proof of efficacy was required.

In the 1960s, *thalidomide*, a hypnotic drug with no obvious advantages over others, was introduced in Europe. Epidemiological research eventually established that this drug, taken early in pregnancy, was responsible for an epidemic of what otherwise is a relatively rare and severe birth defect, phocomelia, in which limbs are malformed. In reaction to this catastrophe, the U.S. Congress passed the Harris-Kefauver amendments to the Food, Drug, and Cosmetic Act in 1962. These amendments established the requirement for proof of efficacy as well as documentation of relative safety in terms of the risk-to-benefit ratio for the disease entity to be treated (the more serious the disease, the greater the acceptable risk). Today, the FDA faces an enormous challenge, especially in view of the widely held belief that its mission cannot possibly be accomplished with the resources allocated by Congress. Moreover, harm from drugs that cause unanticipated adverse effects is not the only risk of an imperfect system; harm also occurs when the approval process delays the approval of a new drug with important beneficial effects.

## The Conduct of Clinical Trials

Clinical trials of drugs are designed to acquire information about the pharmacokinetic and pharmacodynamic properties of a candidate drug in humans and to establish the efficacy and safety of the drug prior to its sale in the U.S. The U.S. National Institutes of Health (NIH) identifies seven ethical principles that must be satisfied before a clinical trial can begin (NIH, 2021):

1. Social and clinical value
2. Scientific validity
3. Fair selection of subjects
4. Informed consent
5. Favorable risk-benefit ratio
6. Independent review
7. Respect for potential and enrolled subjects

The FDA-regulated clinical trials typically are conducted in four phases. Phases I to III are designed to establish safety and efficacy. Phase IV postmarketing trials and surveys gather additional data from larger populations and increasing numbers of administered doses. This phase provides information regarding new indications, risks, and optimal doses and schedules, as presented in Chapter 8. Table 1–1 and Figure 1–6 summarize the important features of each phase of clinical trials; note the attrition at each successive stage over a relatively long and costly process. When initial phase III trials are complete, the sponsor (usually a pharmaceutical company) applies to the FDA for approval to market the drug; this application is called either an NDA or a BLA (Biologics License Application). These applications contain comprehensive information, including individual case report forms from the hundreds or thousands of individuals who have received the drug during its phase III testing. Applications are reviewed by teams of specialists, and the FDA may call on the help of panels of external experts in complex cases.

Under the provisions of the Prescription Drug User Fee Act (PDUFA; enacted in 1992 and renewed every 5 years, most recently in 2017), pharmaceutical companies now provide a significant portion of the FDA budget via user fees, a legislative effort to expedite the drug approval review process by providing increased resources. The PDUFA also broadened the FDA's drug safety program and increased resources for review of television drug advertising. Under PDUFA, review typically takes 6 to 10 months after an NDA is submitted to the FDA. During this time, numerous review functions are usually performed, including advisory committee meetings, amendments, manufacturing facility inspections, and proprietary name reviews (FDA, 2013). Before a drug is approved for marketing, the company and the FDA must agree on the content of the "label" (package insert)—the official prescribing information. This label describes the approved indications for use of the drug and clinical pharmacological information, including dosage, adverse reactions, and special warnings and precautions (sometimes posted in a "black box"). Promotional materials used by pharmaceutical companies cannot deviate from information contained in the package insert. Importantly, the physician is not bound by the package insert; a physician in the U.S. may legally prescribe a drug for any purpose that he or she deems reasonable. However, third-party payers (insurance companies, Medicare, and so on) generally will not reimburse a patient for the cost of a drug used for an "off-label" indication unless the new use is supported by a statutorily named compendium (e.g., the American Hospital Formulary Service–Drug Information [AHFS-DI]). Furthermore, a physician may be vulnerable to litigation if untoward effects result from an unapproved use of a drug.

**TABLE 1–1 ■ TYPICAL CHARACTERISTICS OF THE PHASES OF CLINICAL TRIALS REQUIRED BY THE FDA BEFORE THE MARKETING OF NEW DRUGS***

| PHASE I<br>FIRST IN HUMAN | PHASE II<br>FIRST IN PATIENT | PHASE III<br>MULTISITE TRIAL | PHASE IV<br>POSTMARKETING |
|---|---|---|---|
| 10–100 participants | 50–500 participants | A few hundred to a few thousand participants | Many thousands of participants |
| Usually healthy volunteers; occasionally patients with advanced or rare disease | Patient-subjects receiving experimental drug | Patient-subjects receiving experimental drug | Patients in treatment with approved drug |
| Open label | Randomized and controlled (can be placebo controlled); may be blinded | Randomized and controlled (can be placebo controlled) or uncontrolled; may be blinded | Open label |
| Safety and tolerability | Efficacy and dose ranging | Confirm efficacy in larger population | Adverse events, compliance, drug-drug interactions |
| 1–2 years | 2–3 years | 3–5 years | No fixed duration |
| U.S. $10 to 15 million | U.S. $20 to 40 million | U.S. $50–150 million | Variable |
| Success rate: 50% | Success rate: 30% | Success rate: 25%–50% | — |

*Costs of clinical trial phases vary widely with a drug's therapeutic area, size and complexcity of trial, whether trial must prove non-inferiority to existing agents, etc. Overall cost to develop a new molecular entity (NME) from laboratory to FDA approval is estimated at $1 billion to $4 billion.

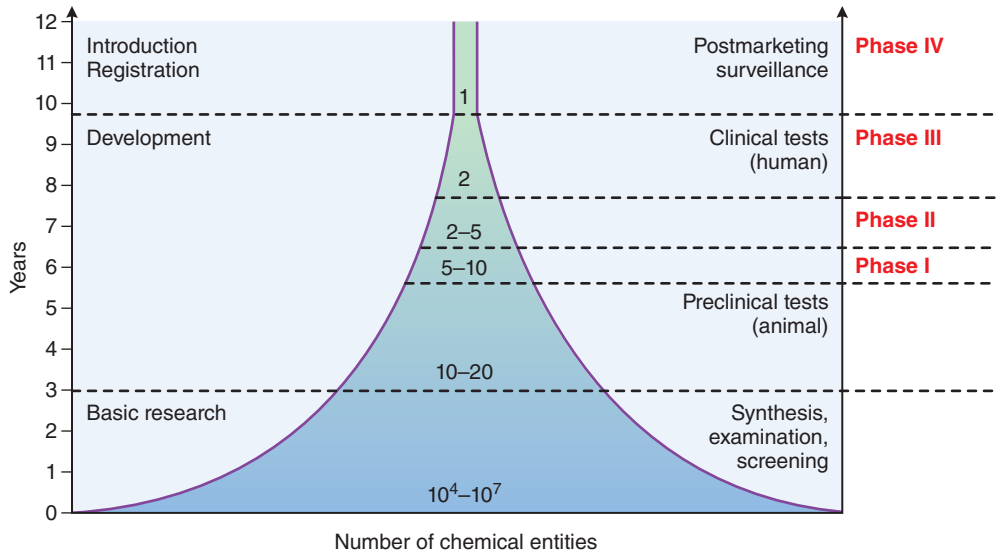



**Figure 1–6** *The phases, timelines, and attrition that characterize the development of new drugs.* See also Table 1–1.

## Determining "Safe" and "Effective"

Demonstrating efficacy to the FDA requires performing "adequate and well-controlled investigations," generally interpreted to mean two replicate clinical trials that are usually, but not always, randomized, double-blind, and placebo (or otherwise) controlled. Is a placebo the proper control? The World Medical Association's Declaration of Helsinki (World Medical Association, 2013) discourages use of placebo controls when an alternative treatment is available for comparison because of the concern that study participants randomized to placebo in such a circumstance would, in effect, be denied treatment during the conduct of the trial. What must be measured in the trials? In a straightforward trial, a readily quantifiable parameter (a secondary or surrogate end point), thought to be predictive of relevant clinical outcomes, is measured in matched drug- and placebo-treated groups. Examples of surrogate end points include low-density lipoprotein (LDL) cholesterol as a predictor of myocardial infarction, elevated high-density lipoprotein (HDL) cholesterol as a predictor of reduced risk of myocardial infarction (see Box 1–2), bone mineral density as a predictor of fractures, or hemoglobin $A_{1c}$ as a predictor of the complications of diabetes mellitus. More stringent trials would require demonstration of reduction of the incidence of myocardial infarction in patients taking a candidate drug in comparison with those taking an HMG-CoA reductase inhibitor (statin) or other LDL cholesterol–lowering agent or reduction in the incidence of fractures in comparison with those taking a bisphosphonate. Use of surrogate end points significantly reduces cost and time required to complete trials, but there are many mitigating factors, including the significance of the surrogate end point to the disease that the candidate drug is intended to treat.

Some of the difficulties are well illustrated by experiences with *ezetimibe*, a drug that inhibits absorption of cholesterol from the gastrointestinal tract and lowers LDL cholesterol concentrations in blood, especially when used in combination with a statin. Lowering of LDL cholesterol was assumed to be an appropriate surrogate end point for the effectiveness of *ezetimibe* to reduce myocardial infarction and stroke, and the drug was approved based on such data. Surprisingly, a subsequent clinical trial (ENHANCE) demonstrated that the combination of *ezetimibe* and a statin did not reduce intima media thickness of carotid arteries (a more direct measure of subendothelial cholesterol accumulation) compared with the statin alone, despite the fact that the drug combination lowered LDL cholesterol concentrations substantially more than did either drug alone (Kastelein et al., 2008). Critics of ENHANCE argued that the patients in the study had familial hypercholesterolemia, had been treated with statins for years, and did not have carotid artery thickening at the initiation of the study. Should *ezetimibe* have been approved? Must we return to measurement of true clinical end points (e.g., myocardial infarction) before approval of drugs that lower cholesterol by novel mechanisms? The costs involved in such extensive and expensive trials must be borne somehow (see below). A follow-up 7-year study involving over 18,000 patients (IMPROVE-IT) vindicated the decision to approve *ezetimibe* (Jarcho and Keaney, 2015). Taken in conjunction with a statin, the drug significantly reduced the incidence of myocardial infarction and stroke in high-risk patients.

No drug is totally safe; all drugs produce unwanted effects in at least some people at some dose. Many unwanted and serious effects of drugs occur so infrequently, perhaps only once in several thousand patients, that they go undetected in the relatively small populations (a few thousand) in the standard phase III clinical trial (see Table 1–1). To detect and verify that such comparatively rare effects are, in fact, drug-related would require administration of the drug to tens or hundreds of thousands of people during clinical trials, adding enormous expense and time to drug development and delaying access to potentially beneficial therapies. In general, the true spectrum and incidence of untoward effects become known only after a drug is released to the broader market and used by a large number of people (phase IV, postmarketing surveillance). Drug development costs and drug prices could be reduced substantially if the public were willing to accept more risk. This would require changing the way we think about a pharmaceutical company's liability for damages from an unwanted effect of a drug that was not detected in clinical trials deemed adequate by the FDA. Would the public accept a drug with extremely severe unwanted effects, including death, if its therapeutic effect were sufficiently unique and valuable? Such dilemmas are not simple and can become issues for great debate.

Several strategies exist to detect adverse reactions after marketing of a drug. Formal approaches for estimation of the magnitude of an adverse drug response include the follow-up or cohort study of patients who

### BOX 1–2 ■ A Late Surprise in the Search for a Blockbuster

*Torcetrapib* elevates HDL cholesterol (the "good cholesterol"). Higher levels of HDL cholesterol are statistically associated with (are a surrogate end point for) a lower incidence of myocardial infarction. Surprisingly, clinical administration of *torcetrapib* caused a significant *increase* in mortality from cardiovascular events, ending a development path of 15 years and $800 million. In this case, approval of the drug based on this secondary end point would have been a mistake (Cutler, 2007). A computational systems analysis suggested a mechanistic explanation of this failure (Xie et al., 2009).