1 Introduction to Drug Design and Discovery

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CONTENTS

1.1 MEDICINAL CHEMISTRY: AN INTERDISCIPLINARY SCIENCE

Therapeutic agents are chemical entities that prevent disease, assist in restoring health to the diseased, or alleviate symptoms associated with disease conditions. Medicinal chemistry is the scientific discipline that makes such drugs available either through discovery or design processes. Throughout history, drugs were primarily discovered by empirical methods, investigating substances or preparations of materials, such as plant parts or plant extracts, found in the local environment. Over the previous centuries, chemists developed methods for the isolation and purification of the active principles in medicinal plants. The purification and structure determination of natural products like morphine, hyoscyamine, quinine, and digitalis glycosides represent milestones in the field of drug discovery and the beginning of medicinal chemistry as a fascinating independent field of research (Figure 1.1).

In the twentieth century, a very large number of biologically active natural products were structurally modified in order to optimize their pharmacology and drug properties in general, and novel drugs were prepared by an increasing use of advanced synthetic methods. Moreover, the rapidly growing understanding of the nature of disease mechanisms, how cells function, and how drugs interact with cellular processes has led to the rational design, synthesis, and pharmacological evaluation of new drug candidates. Most recently, new dimensions and opportunities have emerged from a deeper understanding of cell biology, genetics, and biostructures.

Modern medicinal chemistry draws upon many scientific disciplines, with organic chemistry, physical chemistry, and pharmacology being of fundamental importance. But other disciplines such as biochemistry, molecular biology, toxicology, genetics, cell biology, biophysics, physiology, pathology, and computer modeling approaches play important roles. The key research objective of medicinal chemistry is to investigate relationships between chemical structure and biological effects. When the chemical structure of a particular drug candidate has been optimized to interact

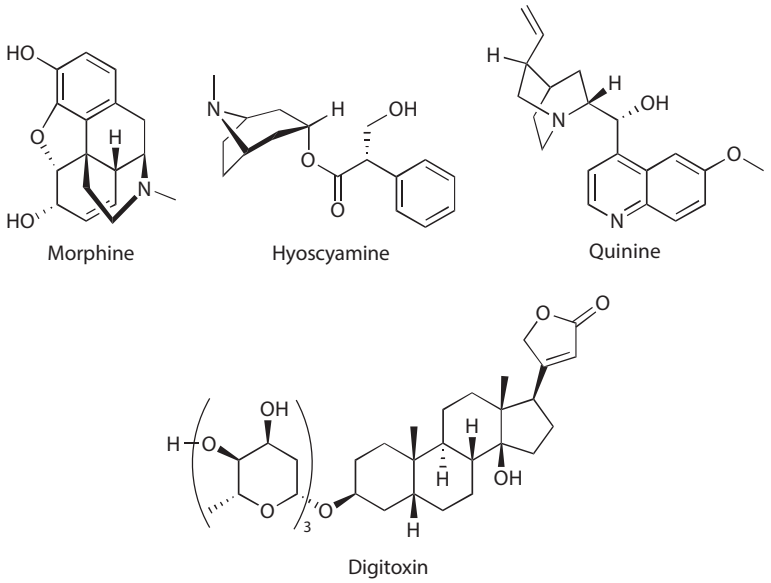


FIGURE 1.1 Chemical structures of four naturally occurring classical therapeutic agents.

with the biological target, the compound further has to fulfill a multifaceted set of criteria before it can be safely administered to patients. Absorption, distribution, metabolism, excretion (ADME), and toxicology studies in animals and humans are time-consuming research tasks which often call for redesign of the chemical structure of the potential therapeutic agent investigated. It is an iterative process which in reality ends up in an overall compromise with respect to multiple desired properties.

1.2 DRUG DISCOVERY: A HISTORICAL PERSPECTIVE

In early times, there was no possibility of understanding the biological origin of a disease. Of necessity, progress in combating disease was disjointed and empirical. The use of opium, ephedra, marijuana, alcohol, salicylic acid, digitalis, coca, quinine, and a host of other drugs still in use long predate the rise of modern medicine. These natural products are surely not biosynthesized by plants for our therapeutic convenience, but they normally have survival value to the plants in dealing with their own ecological challenges.

The presence of biologically active substances in nature, notably in certain plants, was in medieval times interpreted more teleologically. In the early sixteenth century, the Swiss-Austrian medical doctor and natural scientist Paracelsus formulated the "Doctrine of Signatures":

> Just as women can be recognized and appraised on the basis of their shape; drugs can easily be identified by appearance. God has created all diseases, and he also has created an agent or a drug for every disease. They can be found everywhere in nature, because nature is the universal pharmacy. God is the highest ranking pharmacist.

The formulation of this doctrine was in perfect agreement with the dominating philosophies at that time, and it had a major impact on the use of natural medicines. Even today, remanences of this doctrine can be observed in countries where herbal medical preparations are still widely used. Although the "Doctrine of Signatures" evidently is out of the conception of modern medicinal natural product research, the ideas of Paracelsus were the first approach to rational drug discovery.

More than 100 years ago, the mystery of why only certain molecules produced a specific therapeutic response was rationalized by the ideas of Emil Fischer and further elaborated by John Langley and Paul Ehrlich that only certain cells contained receptor molecules that served as hosts for the drugs. The resulting combination of drug and receptor created a new super molecule that had properties producing a response of therapeutic value. One extension of this conception was that the drug fits the target specifically and productively like "a key into its corresponding lock." When the fit was successful, a positive pharmacological action (agonistic) followed, analogous to opening the door. On the contrary, when the fit prevented the intrinsic key to be inserted an antagonist action resulted—i.e., the imaginative door could not be opened. Thus, if one had found adventitiously a ligand for a receptor, one could refine its fit by opportunistic or systematic modifications of the ligand's chemical structure until the desired function was obtained.

This productive idea hardly changed for the next half century and assisted in the development of many useful drugs. However, a less fortunate corollary was that it led to some limitations of creativity in drug design. The drug and its receptor (whose molecular nature was unknown when the theory was formulated) were each believed to be rigid molecules precrafted to fit one another precisely. Today, we know that receptors are highly flexible transmembrane glycoproteins accessible from the cell surface that often comprise more than one drug compatible region. Further complexities have been uncovered continually. For example, a number of receptors have been shown to consist of clusters of proteins either preassembled or assembled as a consequence of ligand binding. The component macromolecules may be either homo- or heterocomplexes. The challenge of developing specific ligands for systems of this complexity may readily be imagined (Chapters 4 and 12).

The opposite extreme to "the lock and key model" is "the zipper model." In this view, a docking interaction takes place (much as the end of a zipper joins the talon piece) and, if satisfactory complementarity is present, the two molecules progressively wrap around each other and adapt to the steric and electrostatic needs of each other. A consequence of accepting this mutual adaptation is that knowledge of the receptor ground state may not be particularly helpful as it adjusts its conformation to ligand binding. Thus, in many cases, one now tries to determine the 3D structure of the receptor–ligand complex. In those cases where X-ray analysis remains elusive, modeling of the interactions involved is appropriate. This is the subject of Chapters 2 through 4.

Earlier, it was also noted that enzymes could be modulated for therapeutic benefit. Enzymatic proteins share many characteristics with receptors, although enzymes catalyze biochemical reactions. Receptor ligands interact with the receptor glycoproteins or with the interfaces between the macromolecular subunits of di- or polycomponent receptor complexes and modify the conformation and dynamics of these complexes. Thus, neither receptor agonists nor antagonists directly interfere with chemical reactions and generally are dissociated from the receptor recognition sites structurally unchanged.

The reaction mechanisms underlying the function of the vast majority of enzymes have been elucidated in detail, and based on such mechanistic information, it has been possible to design a variety of mechanism-based enzyme inhibitors, notably k_{cat} inactivators and transition-state analogs, many of which are in therapeutic use (Chapter 11). Until very recently, it was usually only possible to inhibit enzyme action rather than facilitate it. Actually, diseases frequently result from excessive enzymatic action, making selective inhibition of these enzymes therapeutically useful.

Much later, further classes of receptors were disclosed, explored, and exploited as therapeutically relevant pharmacological targets. This heterogeneous group of receptors comprises nuclear receptors operated by steroid hormones and other lipophilic biochemical mediators, a broad range of membrane-ion channels (Chapter 13), DNA or RNA (Chapter 22), and a number of other biostructures of known or unknown functions. These aspects will be discussed in different chapters of this book.

1.3 DRUG DEVELOPMENT PROCESS: AN OUTLINE

The stages through which a drug discovery/development project proceeds from inception to marketing and beyond are illustrated in Figure 1.2 and described briefly in the following text. The discovery and development process can be described by a number of individual steps, but is also a continuous and iterative process not necessarily performed in a strict stepwise process. From this outline, the complexity of the task of finding new therapeutic agents is evident:

- Target discovery comprises identification and validation of disease-modifying targets. Two major strategies are used for target identification and validation: (1) the molecular approach, with focus on the cells or cell components implicated in the disease and the use of clinical samples and cell models, and (2) the systems approach based on target discovery through the study of diseases in whole organisms.
- Before or after identification of target disease, establishment of a multidisciplinary research team, selection of a promising approach, and decision on a sufficient budget. Initiation of chemistry normally involves synthesis based on available chemicals, in-house chemical libraries, or collection of natural product sources. Start of pharmacology includes suitable screening methods and choice of receptor or enzymatic assays.
- Confirmation of potential utility of initial class(es) of compounds in animals, focusing on potency, selectivity, and apparent toxicity.
- Analog syntheses of the most active compounds, planned after careful examination of literature and patents. More elaborated pharmacology in order to elucidate the mode of action, efficacy, acute and chronic toxicity, and genotoxicity. Studies of ADME characteristics. Planning of large-scale synthesis and initiation of formulation studies. Application for patent protection.

These first project phases which typically last 4–5 years, are followed by highly time- and resource-demanding clinical, regulatory, and marketing phases which normally last 6–10 years:

- Very-large-scale synthesis in parallel or before clinical studies
- Phase I clinical studies which include safety, dosage, and blood level studies on healthy volunteers
- Phase II clinical studies focusing on efficacy and side effects on delimited groups of patients
- Phase III clinical studies which involve studies of range of efficacy and long-term and rare side effects on large patient groups
- Regulatory review
- Marketing and phase IV clinical studies focusing on long-term safety
- Distribution, advertisement, and education of marketing and information personnel

After these project stages from initiation to successful therapeutic application after approval, the patent protection expires, normally after 17–25 years, and generic competition becomes a reality.

This outline of a drug discovery and development process illustrates that, it takes many years to introduce a new therapeutic agent, and it must be kept in mind that most projects are terminated before marketing, even at advanced stages of clinical studies. The later a project fails the more expensive, and many efforts are done in order to consider as many potential failure problems as early as possible in the process. Especially forward translation of preclinical data to possible clinical outcome and back translation of clinical data to "humanized" preclinical data of more predictive value are important issues in the desire to avoid late failures.

Some of the aspects of drug discovery phase are described in more detail in the following sections.

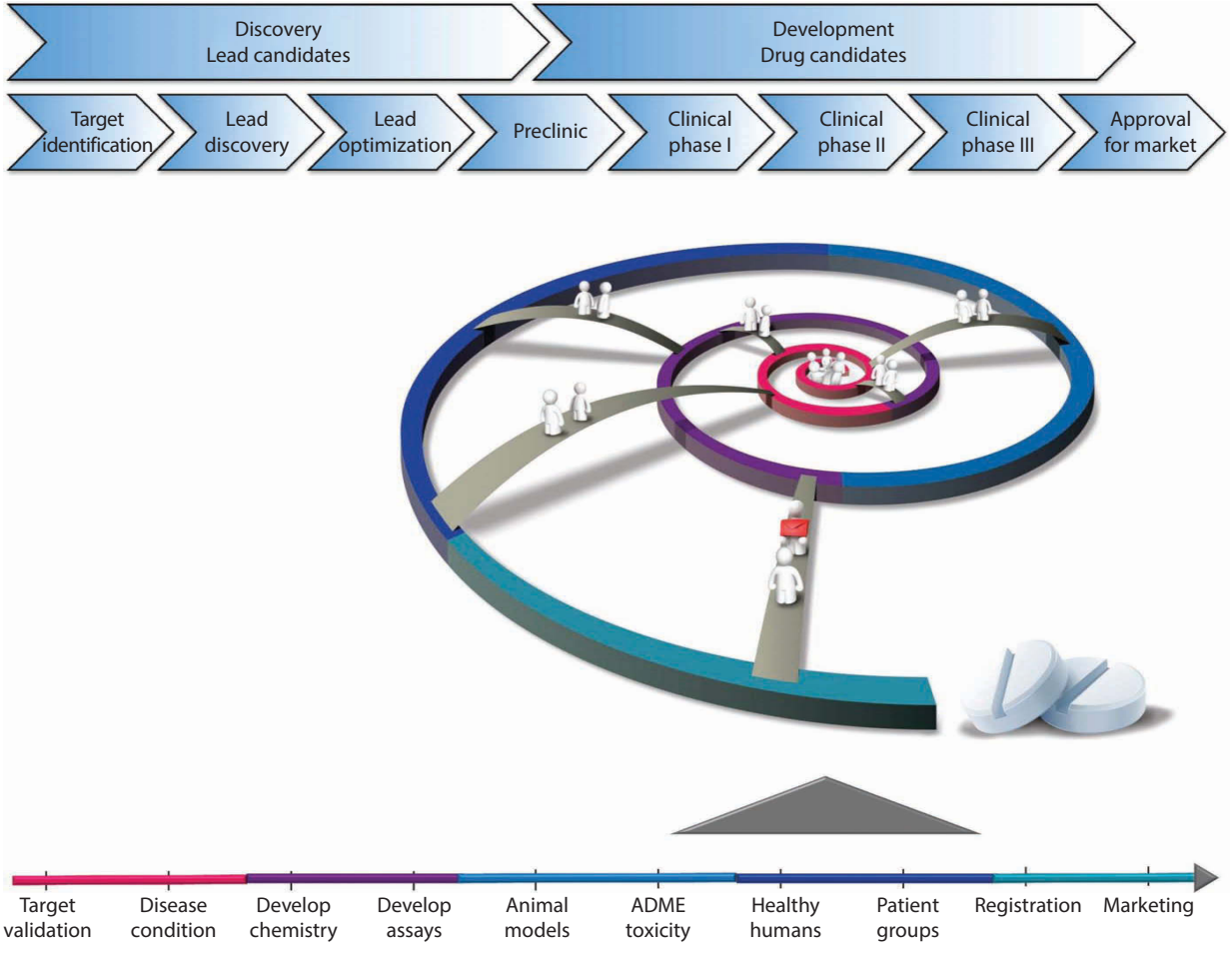


FIGURE 1.2 Outline of the drug discovery and development process with indication of individual steps (blue arrows). The lower multicolored timeline shows the process as a continuous flow which also involves iterative processes at all stages. The spiral representation illustrates such forward and backward translation of knowledge by bridges between the different stages of development.

1.4 DISCOVERY OF DRUG CANDIDATES

Prehistoric drug discovery started with higher plant and animal substances, and this continues today to be a fruitful source of biologically active molecules frequently belonging to unanticipated structural types. Adding to the long list of classical plant products that are still used in modern medicine, one can list many substances of more recent origin, including antibiotics such as penicillins, cephalosporins, tetracyclines, aminoglycosides, various glycopeptides, and many others (Chapter 23). Anticancer agents of natural origin comprise taxol, camptothecin, vinca alkaloids, doxorubicin, and bleomycin (Chapter 21). Among immunosuppressant agents, cyclosporine and tacrolimus deserve special mention.

Other sources of lead structures and drug candidates include endogenous compounds and other compounds with known activity at the target(s) in question, as well as screening programs. The role of natural products in target identification and as lead structures is further described in the following sections, whereas examples of use of other sources are described in different chapters throughout the textbook.

1.4.1 Natural Products: Role in Target Identification

Many naturally occurring compounds have potent and/or selective activity on different biological targets and are of potential therapeutic value. Most often these activities are toxic effects, since these compounds are either animal venoms (e.g., snake poison, spider, or wasp toxins) which can paralyze or kill prey or plant toxins preventing animals to eat the plants. However, toxicity is generally a matter of dose, and in some instances a toxin can be used as a drug in the appropriate dose.

Various biologically active natural products have played a key role in the identification and characterization of receptors, and such receptors are often named after these compounds (Chapter 12). Morphine is a classical example of a natural product used for receptor characterization. Radiolabeled morphine was shown to bind with high affinity to receptors in the nervous system, and these receptors are known as opiate receptors. More than three decades ago, the physiological relevance of these receptors was documented by the findings that endogenous peptides, notably enkephalins and endorphins, served as receptor ligands (agonists). Analogs of morphine have been useful tools for the demonstration of heterogeneity of opiate receptors (Chapter 19) (Figure 1.3).

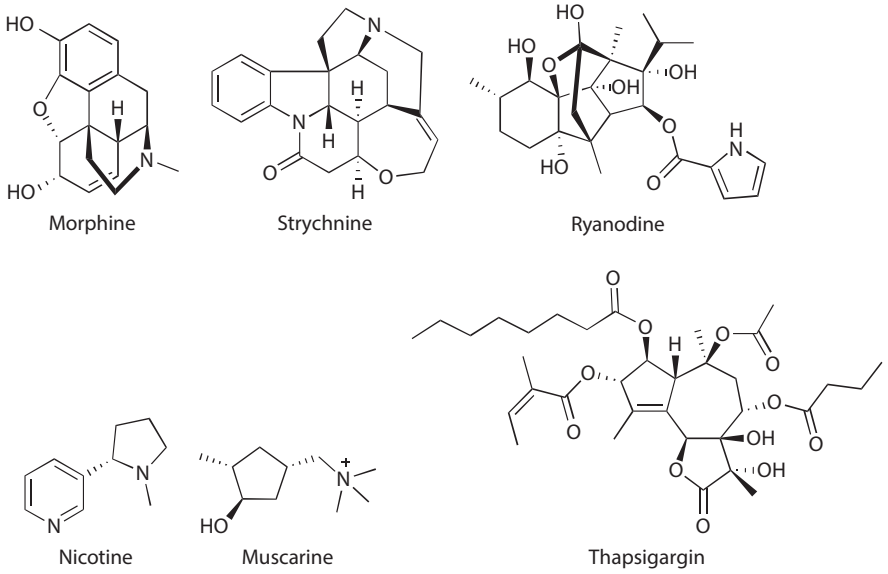


FIGURE 1.3 Chemical structures of morphine, strychnine, ryanodine, nicotine, muscarine, and thapsigargin.

The very toxic and convulsive alkaloid, strychnine, has been extensively studied pharmacologically. Using electrophysiological techniques and tritiated strychnine for binding studies, strychnine was shown to be an antagonist for the neuroreceptor mediating the inhibitory effect of glycine, through the $glycine_A$ receptor located primarily in the spinal cord.

Acetylcholine is a key transmitter in the central and the peripheral nervous system. Acetylcholine operates through multiple receptors, and the original demonstration of receptor heterogeneity was achieved using the naturally occurring compounds, nicotine and muscarine. Whereas the ionotropic class of acetylcholine receptors binds nicotine with high affinity and selectivity, muscarine specifically and potently activates the metabotropic class of these receptors. Using molecular biological techniques, a number of subtypes of both nicotinic and muscarinic acetylcholine receptors have been identified and characterized (Chapters 12 and 16).

The ryanodine receptor is named after the insecticidal naturally occurring compound, ryanodine. Extensive studies have disclosed that ryanodine interacts with high affinity and in a calcium-dependent manner with its receptor which functions as a calcium release channel. There are three genetically distinct isoforms of the ryanodine receptor which play a role in the skeletal muscle disorder, central core disease.

The sesquiterpene lactone, thapsigargin which is structurally unrelated to ryanodine, also interacts with an intracellular calcium mechanism. Thapsigargin has become the key pharmacological tool for the characterization of the sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA). Thapsigargin effectively inhibits this ATPase, causing a rise in the cytosolic calcium level which eventually leads to cell death. Although the SERCA pump is essential for all cell types, attempts to target thapsigargin toward prostate cancer cells have been made based on a prodrug approach (see Chapter 10).

1.4.2 Natural Products as Lead Structures

Although a number of biologically active natural products have been indispensable as tools for identification and characterization of pharmacological and potential therapeutic targets, these compounds normally do not satisfy the demands on drugs for therapeutic use (Chapter 7).

Thus, although morphine is used therapeutically, it is not an ideal drug and has, to some extent, been replaced by a number of analogs showing lower side effects and higher degrees of selectivity for subtypes of opiate receptors (Chapter 19). Prominent examples are the μ-selective opiate agonist fentanyl and the experimental tool U50,488 which selectively activates the κ-subtype of opiate receptors (Figure 1.4).

The main psychoactive constituent of *Cannabis sativa*, the highly lipophilic tetrahydrocannabinol (THC), has been a useful tool for the identification of the two cannabinoid receptors, CB1 and CB2 receptors, operated by endocannabinoids. Since different preparations of *C. sativa* have psychoactive effects, health authorities in most countries have been reluctant to accept THC and analogs as therapeutic agents for the treatment of pain and other disease-related conditions. This may change with time, as medicinal chemists have synthesized a number of cannabinoid receptor ligands, including the receptor agonist CP55,940 which is markedly less lipophilic than THC (Chapter 19).

The nicotine acetylcholine receptors (nAChRs) have become important targets for therapeutic approaches to treat pain, cognition disorders, depression, schizophrenia, and nicotine dependence. For several reasons, nicotine has limited utility as a therapeutic agent, and a wide variety of nAChR agonists have been synthesized and characterized (Chapter 16). (–)-Cytisine is a naturally occurring toxin acting as a partial nAChR agonist. Using (–)-cytisine as a lead structure, varenicline was developed as a partial nAChR agonist showing a balanced agonist/antagonist profile for smoking cessation. Muscimol is another example of a naturally occurring toxin which has been extensively used as a lead for the design of specific GABA receptor agonists and GABA uptake inhibitors (Chapter 15). Muscimol which is a 3-isoxazolol bioisostere (see Section 1.4.3.1) of GABA, is a

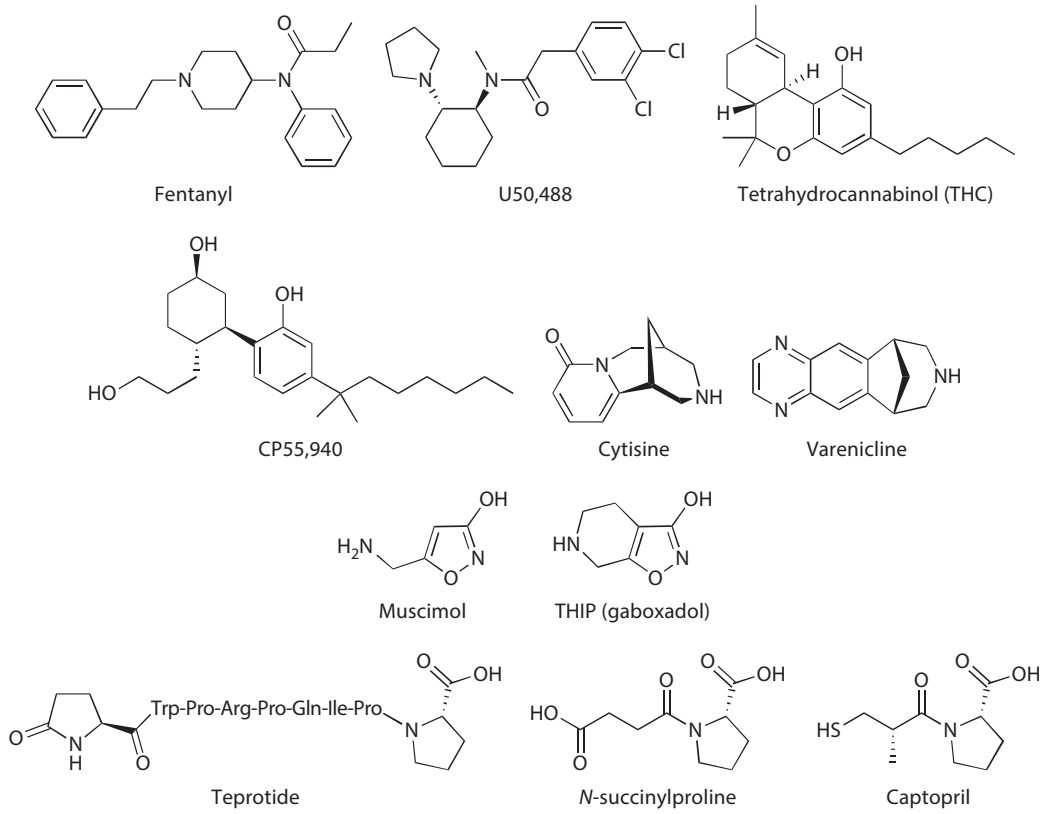


FIGURE 1.4 Chemical structures of fentanyl, U50,488, tetrahydrocannabinol (THC), CP55,940, cytisine, varenicline, muscimol, THIP (gaboxadol), teprotide, *N*-succinylproline, and captopril.

constituent of the mushroom *Amanita muscaria*. Muscimol is toxic, it is metabolically unstable, and it interacts with the different GABA synaptic mechanisms and with a broad range of $GABA_A$ receptor subtypes. The cyclic analog of muscimol, THIP (gaboxadol), is highly selective for the therapeutically interesting extrasynaptic $GABA_A$ receptors. Gaboxadol is a clinically active nonopioid analgesic and a nonbenzodiazepine hypnotic which at present is in clinical trials (see also Chapter 15).

The angiotensin-converting enzyme (ACE) is a zinc carboxypeptidase centrally involved in the regulation of blood pressure and is an important target for therapeutic intervention. Peptide toxins from the Brazilian pit viper, *Bothrops jararaca*, and the synthetic peptide analog, teprotide, are inhibitors of ACE (Figure 1.4), but are not suitable for therapeutic use. Systematic molecular dissection of teprotide led to the nonpeptide ACE inhibitor, *N*-succinylproline which was converted into the structurally related and much more potent analog, captopril, that is now marketed as an effective antihypertensive drug.

1.4.3 Basic Principles in Lead Development and Optimization

Potency, efficacy, and selectivity are essential but certainly not the only parameters to fulfill for a pharmacologically active compound to become a therapeutic drug. A large number of additional requirements have to be met, and the most important ones have been summarized in the acronym, ADME or ADME-Tox (ADME and toxicity). Obviously, the drug must reach the site of action in a timely manner and in sufficient concentration to produce the desired therapeutic effect.

After oral administration, one of many routes of administration, the drug must survive the acidic environment of the stomach. In the small intestine, the bulk of absorption takes place. Here, the pH is neutral to slightly acidic. In the gastrointestinal system metabolism can take place. The presence of digestive enzymes creates particular problems for polypeptide drugs which may call for other routes of administration, as the gut wall is rich in oxidative enzymes.

Unless the drug acts as a substrate for active energy-requiring uptake mechanisms which normally facilitate uptake of, for example, amino acids and glucose, it must be significantly unionized to penetrate cell membranes in order to enter the blood stream. Following absorption, the blood rapidly presents the drug to the liver, where Class I metabolic transformations (oxidation, hydrolysis, reduction, etc.) and in some cases phase II transformations (glucuronidation, sulfation, etc.) take place. The polar reaction products from these reactions are typically excreted in the urine or feces.

The rate of absorption of drugs, their degree of metabolic transformation, their distribution in the body, and their rate of excretion are collectively named pharmacokinetics. This is in effect the influence of the body on a drug as a function of time. The interaction of the drug with its targets, and the consequences of this interaction as a function of time are pharmacodynamics.

Both of these characteristics are alone governed by the drug's chemical structure. Thus, the medicinal chemist is expected to remedy any shortcomings by structural modification. In addition to ADME-Tox, a number of other characteristics must also be satisfactory, such as

- Freedom from mutagenesis
- Freedom from teratogenicity
- Chemical stability—shelf stability
- Synthetic or biological accessibility
- Acceptable cost
- Ability to patent
- Clinical efficacy
- Solubility
- Satisfactory taste (per oral administration)
- Ability to formulate satisfactorily for administration
- Freedom from idiosyncratic problems

A number of strategies are used by the medicinal chemists in order to optimize lead compounds in order to fulfill all these requirements related to optimization of desired activities and minimization of undesired effects:

- Variation of substituents—change of size, shape, and polarity
- Extension/contraction of structure—change chain size or ring size
- Ring closure/ring variation/ring fusion
- Simplification of structure
- Rigidification of structure

Examples of such modification are presented especially in Chapters 15 through 19, and generally these efforts aim toward optimizing the active conformation and physicochemical properties of the drugs with the essential and necessary pharmacophoric groups present. A very versatile principle for variation of molecules, functional groups, and substituents with focus on optimizing biological activity is the use of bioisosteres (see Section 1.4.3.1). Furthermore, stereochemical control of drug interactions with the chiral environment is essential as described in Section 1.4.3.2 and subsequent sections.

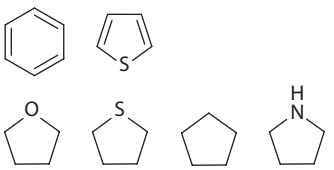
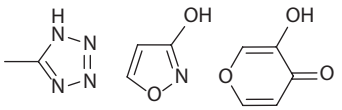
These challenges emphasize the key importance of scientists trained in interdisciplinary medicinal chemistry in drug discovery projects.

1.4.3.1 Bioisosteres

Bioisosteric replacement, also named molecular mimicry, is one of the most widely used principles for optimization of drug molecules. Bioisosteres are molecules in which atoms or functional groups are modified in order to obtain new molecules with a biological activity related to the parent molecule. The purpose is to obtain drug molecules with improved biological properties. Bioisosteric replacement can change a number of physicochemical properties of the resulting molecules compared to the parent molecule: size, shape, electronic distribution, solubility, pK_a, and chemical reactivity. These changes may lead to changes in the pharmacodynamics as well as pharmacokinetic properties, e.g., changes in potency, selectivity, bioavailability, and metabolism.

Bioisosteres have been classified as either classical or nonclassical. In classical bioisosterism, similarities in certain physicochemical properties have enabled investigators to successfully exploit several monovalent isosteres. These can be divided into the following groups: (1) fluorine versus hydrogen replacements; (2) amino-hydroxyl interchanges; (3) thiol-hydroxyl interchanges; and (4) fluorine, hydroxyl, amino, and methyl group interchanges (Grimm's hydride displacement law, referring to the different number of hydrogen atoms in the isosteric groups to compensate for valence differences). The nonclassical bioisosteres include all those replacements that are not defined by the classical definition of bioisosteres. These isosteres are capable of maintaining similar biological activity by mimicking the spatial arrangement, electronic properties, or some other physicochemical properties of the molecule or functional group that are of critical importance. A number of classical and nonclassical bioisosteres are shown in Table 1.1 representing only a selection of more commonly used bioisosteres. The concept of nonclassical bioisosterism, in particular,

TABLE 1.1
Examples of Bioisosteric Replacements

Classical	
Monovalent	—F —OH —NH_2 —CH_3 —Cl —SH
atoms and groups	—Br —*i*—Pr
	—I —*t*—Bu
Bivalent	—CH_2— —O— —S— —Se— —NH—
atoms and groups	—$COCH_2R$ —CONHR —COOR —COSR
Trivalent	—N= —P= —CH= —As=
atoms and groups	
Ring equivalents	
Nonclassical	
Hydroxyl group	—OH —CH_2OH —NHCOR —$NHSO_2R$ —$NHCONH_2$
Carbonyl group	CO C=C$(CN)_2$ —SO_2NRR' CONRR′ =CHCN
Carboxylic acid	—COOH —SO_2NHR —SO_2OH —PO_3H_2 —CONHOH
	
Halogen	—X —CF_3 —CN —$N(CN)_2$ —$C(CN)_3$
Spacer group	—$(CH_2)_3$— 

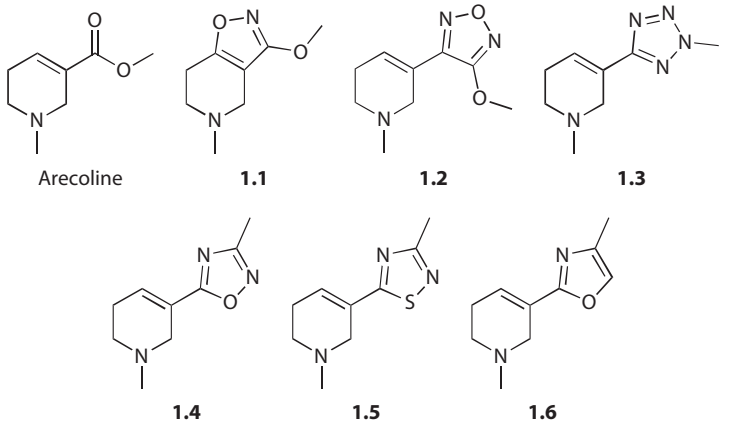


FIGURE 1.5 Chemical structures of arecoline and analogs.

is often considered to be qualitative and intuitive, and there are numerous and a constantly growing number of such bioisosteres used effectively in drug design projects (see, e.g., Chapters 15 and 16).

The conversion of the muscarinic acetylcholine receptor agonist arecoline, containing a hydrolysable ester group, into different hydrolysis-resistant heterocyclic bioisosteres is illustrated in Figure 1.5. The annulated (**1.1**) and nonannulated (**1.2** and **1.3**) bicyclic bioisosteres are potent muscarinic agonists. Similarly, compounds **1.4** and **1.5** interact potently with muscarinic receptors as agonists, whereas **1.6**, in which the 1,2,4-oxadiazole ester bioisosteric group of **1.4** is replaced by an oxazole group, shows reduced muscarinic agonist effects. Thus, the electronic effects associated with these heterocyclic rings appear to be essential for muscarinic activity.

It must be emphasized that a bioisosteric replacement strategy which has been successful for a particular group of pharmacologically active compounds, may not necessarily be effectively used in other groups of compounds active at other pharmacological targets.

1.4.3.2 Stereochemistry

Receptors, enzymes, and other pharmacological targets which by nature are protein constructs, are highly chiral. Other targets like DNA and other macromolecules in the human body are all build up of chiral building blocks. Thus, it is not surprising that chirality in the drug structures generally plays an important role in pharmacological responses. In racemic drug candidates, the desired pharmacological effect typically resides in one enantiomer, whereas the other stereoisomer(s) are pharmacologically inactive or possess different pharmacological effects. Thus, chiral drugs should preferentially be resolved into stereochemically pure isomers prior to pharmacological examination. Since many, especially of older date, synthetically prepared chiral biologically active compounds have been described pharmacologically as racemates, much of the older pharmacological literature should be read and interpreted with great care. At best the nonactive stereoisomer in a racemate is inactive and can be looked upon as chemical waste. However, with the introduction of an "inactive enantiomer" by therapeutic application of a racemic drug, one always runs the risk of introducing undesired and unknown activities and side effects. The most significant example of undesired effects of a racemic drug is the tragic case of thalidomide (Figure 1.6a). Racemic thalidomide was developed in the 1950s and was used as a mild sleeping agent and to treat morning sickness in pregnant women. Unfortunately, the drug was teratogenic and gave serious fetal abnormalities. Later it was discovered that the (*S*)-enantiomer possessed the teratogenic effect and the (*R*)-enantiomer possessed the desired activity. However, the studies also revealed that the enantiomers of thalidomide racemize under physiological conditions; thus the use of pure (*R*)-form was not a solution. This caused major changes for the legislative requirements for the introduction of chiral

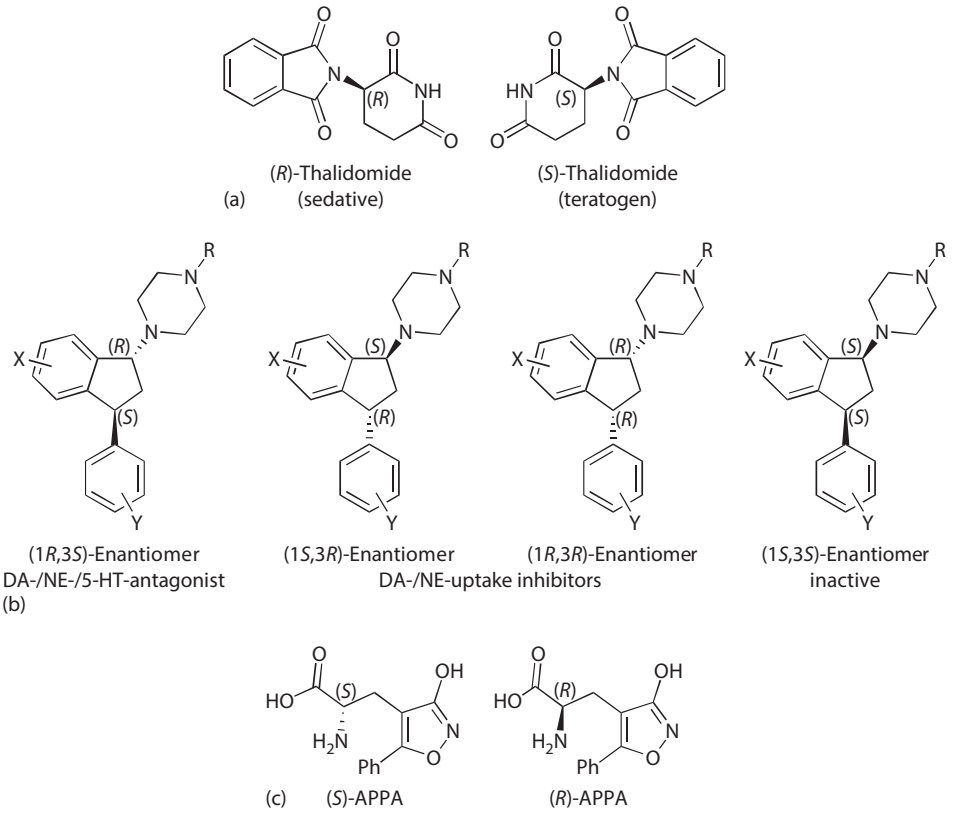


FIGURE 1.6 Chemical structures of (a) (*R*)- and (*S*)-thalidomide, (b) the four stereoisomers of 1-piperazino-3-phenylindans, and (c) the two enantiomers of the phenyl analog of AMPA (APPA).

drugs subsequently and mandatory test for teratogenic activity. Thalidomide was off the market for many years, but has been introduced again for treatment of leprosy and other diagnoses, but under very strict guidelines.

Figure 1.6 exemplifies the importance of stereochemistry in studies of the relationship between structure and pharmacological activity (SAR studies). Figure 1.6b shows four stereoisomers which are two pairs of enantiomers of two diastereomeric compounds. These 1-piperazino-3-phenylindans were synthesized, resolved, structurally analyzed, and pharmacologically characterized as part of a comprehensive drug research program in the field of central biogenic amine neurotransmission. Whereas one of these stereoisomers turned out to be inactive, two of them were inhibitors of dopamine (DA) and norepinephrine (NE) uptake, and one isomer showed antagonist effects at DA, NE, and serotonin (5-HT) receptors. It is evident that a pharmacological characterization of a synthetic mixture of these compounds would be meaningless.

The 3-isoxazolol amino acid, APPA (Figure 1.6c), is an analog of the standard agonist, AMPA, for the AMPA subtype of excitatory glutamate receptors (Chapter 15). APPA was tested pharmacologically as the racemate which showed the characteristics of a partial agonist at AMPA receptors. Subsequent pharmacological characterization of the pure enantiomers quite surprisingly disclosed that (*S*)-APPA is a full AMPA receptor agonist, whereas (*R*)-APPA turned out to be an AMPA antagonist. This observation prompted intensive pharmacological studies, and as a result it was demonstrated that administration of a fixed ratio of an agonist and a competitive antagonist always provides a partial agonist response at an efficacy level dependent on the administered ratio of compounds and their relative potencies as agonist and antagonist, respectively. This phenomenon

was named "functional partial agonism." An interesting aspect of this pharmacological concept is that administration of an antagonist drug inherently establishes functional partial agonism together with the endogenous agonist at the target receptor.

1.4.3.3 Membrane Penetration: Including the Lipinski Rule of Five

In drug discovery projects, an issue of major importance is the design of drug molecules capable of penetrating different biological membranes effectively and that allow effective concentrations to build up at the therapeutic target. The structure and physiochemical properties of the drug molecule obviously are of decisive importance, and it is possible to establish the following empirical rules:

- Some small and rather water-soluble substances pass in and out of cells through water-lined transmembrane pores.
- Other polar agents are conducted into or out of cells by membrane-associated and energy-consuming proteins. Polar nutrients that the cell requires, such as glucose and many amino acids, fit into this category. More recently, drug resistance by cells has been shown to be mediated in many cases by analogous protein importers and exporters.
- The blood–brain barrier (BBB) normally is not easily permeable by neutral amino acids. However, such compounds with sufficiently small difference between the pK_a values will have a relatively low I/U ratio (the ratio between the ionized/zwitterionic form and the unionized form of the amino acid in solution). As an example, THIP (Figure 1.4) has pK_a values of 4.4 and 8.5 and a calculated I/U ratio of about 1000. Thus, 0.1% of THIP in solution is unionized, and this fraction apparently permits THIP to penetrate the BBB quite easily. Other neutral amino acids typically have I/U ratios around 500,000 and thus have much lower fractions of unionized molecules in solution, and such compounds normally do not penetrate into the brain after systemic administration.
- Molecules that are partially water soluble and partially lipid soluble can pass through cell membranes by passive diffusion and are driven in the direction of the lowest concentration.
- In cells lining the intestinal tract, it is possible for molecules with these characteristics to pass into the blood through the cell membrane alone.
- Finally, it is also possible for molecules with suitable water solubility, small size, and compact shape to pass into the blood between cells. This last route is generally not available for passage into the CNS, because the cells forming the BBB are organized much closer together and thus prevent such entry into the brain.

Whereas there are no guarantees and many exceptions, the majority of effective oral drugs obey the Lipinski rule of five:

- The substance should have a molecular weight of 500 or less.
- It should have fewer than five hydrogen-bond donors.
- It should have fewer than 10 hydrogen-bond acceptors.
- The substance should have a calculated log P (clog P) between approximately −1 and +5.

The Lipinski rule of five is thus an empirical rule, where the number five occurs several times. The rule is a helpful guide rather than a law of nature.

1.4.3.4 Structure-Based Drug Design

During the early 1980s, the possibility to rationally design drugs on the basis of structures of therapeutically relevant biomolecules was an unrealized dream for many medicinal chemists. The first projects were underway in the mid-1980s, and today, even though there are still many obstacles and

unsolved problems, structure-based drug design now is an integral part of many drug discovery programs. Major breakthroughs are represented by the publication of high-resolution 3D X-ray crystallographic structures of neurotransmitter receptors and transporters, e.g., determination of full-length $GABA_A$ receptor and full-length dopamine transporter structures, both obtained after many years of extensive research. In Chapter 4, a number of examples of this impressive drug design approach are described.

As structural genomics, bioinformatics, and computational power continue to almost explode with new advances, further successes in structure-based drug design are likely to follow. Each year, new targets are being identified, and structures of those targets are being determined at an amazing rate, and our capability to capture a qualitative picture of the interaction between macromolecules and ligands is accelerating.

1.5 INDIVIDUALIZED MEDICINE AND CONCLUDING REMARKS

The mapping of the human genome leads us to the identification of new targets for therapeutic interventions, and even allows us to dream of the possibility of correcting genetic defects, enhancing our prospects for a longer and more healthy life, and for devising drugs for specific individuals. Presuming that individual variations in therapeutic response may have a genetic origin, and thus dividing populations into subgroups with similar genetic characteristics, might allow us to prescribe drugs and even dosages within these groups. This form of individual gene typing is already possible but still very resource demanding as per day's techniques. It is likely that perplexing species differences in response to, for example, chemotherapy, that complicates drug development, may also be understood, when the individual genome mapping becomes more elaborate and cheap.

The new biological capabilities raise many new prospects and problems for drug companies and, in general, for the society, not only scientifically but also morally. Scientific knowledge by itself is morally neutral, but how it is used, is not.

In conclusion, there has never been a more exciting time to take up the study of medicinal chemistry. The technological developments and the amount of information will grow with increasing speed, and scientists may eventually risk to be drowned in this multitude of possibilities. However, the intelligent, intuitive, and skilled medicinal chemist will be able to maneuver in this ocean of multiplicity and to continue the series of brilliant achievements by the pioneers in drug discovery during the past century.

FURTHER READING

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