1 Introduction to Drug Targets and Molecular Pharmacology

1.1 Introduction to molecular pharmacology

During the past 30 years there have been significant advances and developments in the discipline of molecular pharmacology – an area of pharmacology that is concerned with the study of drugs and their targets at the molecular or chemical level. Major landmarks during this time include the cloning of the first G-protein coupled receptor (GPCR) namely the β_2-adrenergic receptor in 1986 (Dixon et al., 1986). This was quickly followed by the cloning of additional adrenergic receptor family genes and ultimately other GPCRs. The molecular biology explosion during the 1980s also resulted in the cloning of genes encoding ion channel subunits (e.g. the nicotinic acetylcholine receptor and voltage-gated Na^+ channel) and nuclear receptors. The cloning of numerous drug targets continued at a pace during the 1990s but it was not until the completion of the human genome project in 2001 that the numbers of genes for each major drug target family could be determined and fully appreciated. As would be expected, the cloning of the human genome also resulted in the identification of many potentially new drug targets. The completion of genome projects for widely used model organisms such as mouse (2002) and rat (2004) has also been of great benefit to the drug discovery process.

The capacity to clone and express genes opened up access to a wealth of information that was simply not available from traditional pharmacology-based approaches using isolated animal tissue preparations. In the case of GPCRs detailed expression pattern analysis could be performed using a range of molecular biology techniques such as *in situ* hybridisation, RT-PCR (reverse transcriptase-polymerase chain reaction) and Northern blotting. Furthermore having a cloned GPCR gene in a simple DNA plasmid made it possible for the first time to transfect and express GPCRs in cultured cell lines. This permitted detailed pharmacological and functional analysis (e.g. second messenger pathways) of specific receptor subtypes in cells not expressing related subtypes, which was often a problem when using tissue preparations. Techniques such as site-directed mutagenesis enable pharmacologists to investigate complex structure-function relationships aimed at understanding, for example, which amino acid residues are crucial for ligand binding to the receptor. As cloning and expression techniques developed further it became possible to manipulate gene expression *in vivo*. It is now common practice to explore the consequences of deleting a

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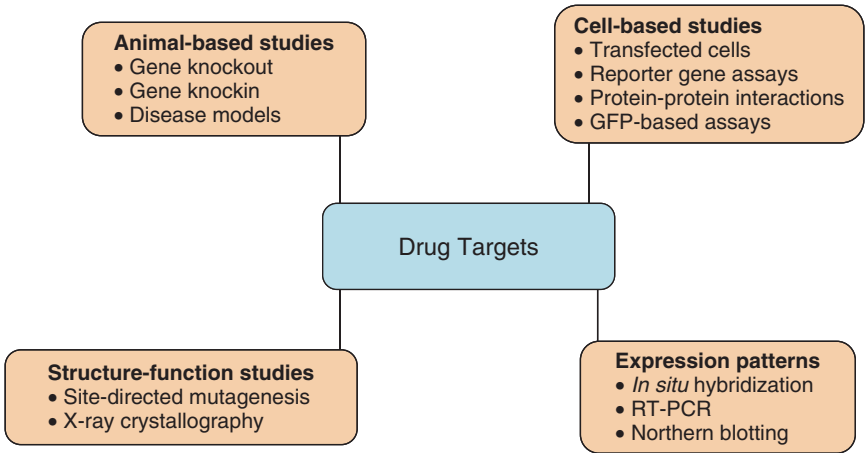


Figure 1.1 Molecular pharmacology-based methods used to interrogate drug targets.

specific gene either from an entire genome (knockout) or from a specific tissue/organ (conditional knockout). It is also possible to insert mutated forms of genes into an organism's genome using knockin technology. These transgenic approaches allow molecular pharmacologists to study developmental and physiological aspects of gene function *in vivo* and in the case of gene knockin techniques to develop disease models.

The molecular biology revolution also enabled the development of novel approaches for studying the complex signal transduction characteristics of pharmacologically important proteins such as receptors and ion channels. These include reporter gene assays, green fluorescent protein (GFP) based techniques for visualising proteins in living cells and yeast two hybrid-based assays for exploring protein-protein interactions. You will find detailed explanations of these and other current molecular-based techniques throughout this textbook. Another major breakthrough in the 2000s was the development of methods that allowed high resolution structural images of membrane-associated proteins to be obtained from X-ray crystallography. During this time the first X-ray structures of GPCRs and ion channels were reported enabling scientists to understand how such proteins function at the molecular level. Indeed crystallography is an important tool in the drug discovery process since crystal structures can be used for *in silico* drug design. More recently researchers have used NMR spectroscopy to obtain a high-resolution structural information of the β_2-adrenergic receptor (Bokoch et al., 2010). A distinct advantage of NMR-based structural studies, which are already used for structural studies of other drug targets such as kinases, would be the ability to obtain GPCR dynamics and ligand activation data which is not possible using X-ray based methods. Some of the molecular pharmacology based approaches used to interrogate drug targets are outlined in Figure 1.1.

Despite this increased knowledge of drug targets obtained during the molecular biology revolution, there has been a clear slowdown in the number of new drugs reaching the market (Betz, 2005). However, since it takes approximately 15 years to bring a new drug to market it may be too early to assess the impact of the human genome project on drug discovery. In 2009 the global pharmaceutical market was worth an estimated $815 billion. However during the next few years a major problem facing the pharmaceutical industry is the loss of drug patents on key blockbusters. The hope for the future is that the advances in molecular pharmacology witnessed during the last decade or so will start to deliver new blockbuster therapeutics for the twenty-first century.

1.2 Scope of this textbook

As briefly detailed above there have been numerous exciting developments in the field of molecular pharmacology. The scope of this textbook is to explore aspects of molecular pharmacology in greater depth than covered in traditional pharmacology textbooks (summarised in Figure 1.2). Recent advances and developments in the four major human drug target families (GPCRs, ion channels, nuclear receptors and transporters) are

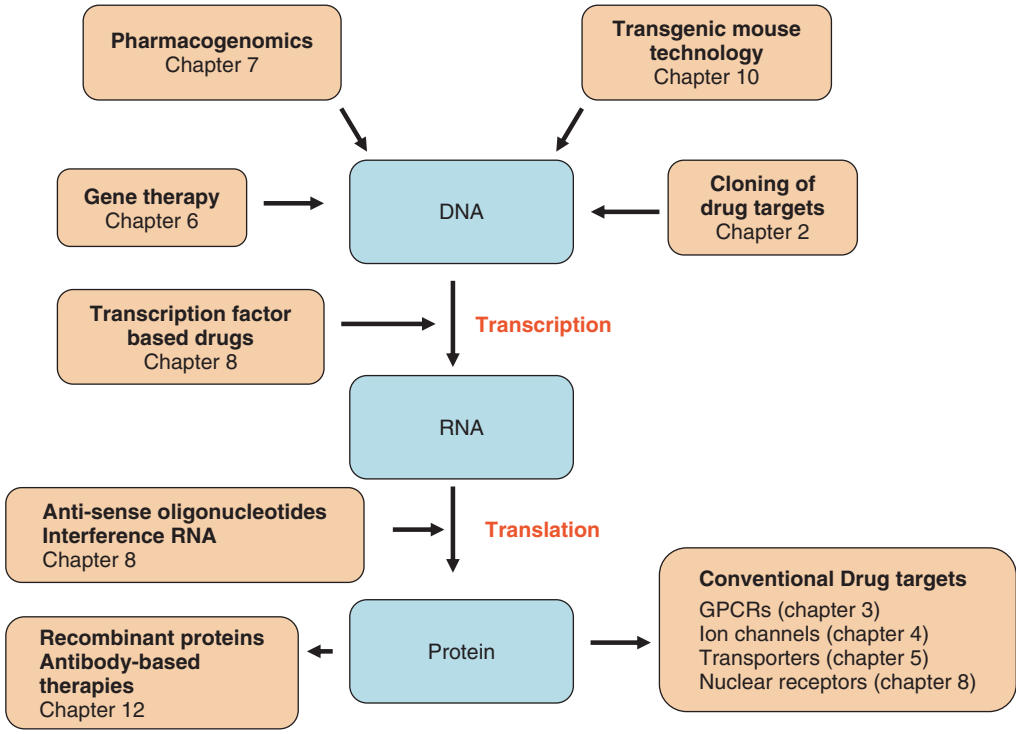


Figure 1.2 Drug targets within the central dogma of molecular biology. To date the majority of conventional therapeutics target a relatively small group of protein families that include G-protein coupled receptors, ion channels, and transporters. Novel therapeutic strategies include blocking translation of mRNA into protein using anti-sense oligonucleotide and/or RNA interference technology. Gene transcription can also be targeted via the activation/inhibition of nuclear receptor function. The chapters covering these topics are indicated.

covered in separate chapters (Chapters 3–5 and 8). The molecular targets of anti-infective drugs (anti-bacterial and anti-viral) whilst of great importance are not covered in this book. Other chapters deal with the cloning of drug targets (Chapter 2) and transgenic animal technology (Chapter 10). The concept of gene therapy is explored in a case study-based chapter which looks at current and possible future treatment strategies for cystic fibrosis, the commonest lethal genetic disease of Caucasians (Chapter 6). Another major development in molecular pharmacology has been the discipline of pharmacogenomics: the study of how an individual's genetic makeup influences their response to therapeutic drugs (Chapter 7). These naturally occurring variations in the human genome are caused predominantly by single nucleotide polymorphisms (DNA variation involving a change in a single nucleotide) and there is a major research consortium aimed at documenting all the common variants of the human genome (The International HapMap project). The information from the project, which is freely available on the internet, will enable scientists to understand how genetic variations contribute to risk of disease and drug response. Finally, we take an in depth look at the role of calcium in the cell, looking at techniques used to measure this important second messenger (Chapter 9).

1.3 The nature of drug targets

How many potential drug targets are there in the human genome? This is an important question often asked by the pharmaceutical industry since they are faced with the task of developing novel therapeutics for the future. When the draft sequence of the human genome was completed in 2001 it was estimated to contain approximately 31,000 protein-coding genes. However since its completion the number of human protein-coding genes has been continually revised with current estimates ranging

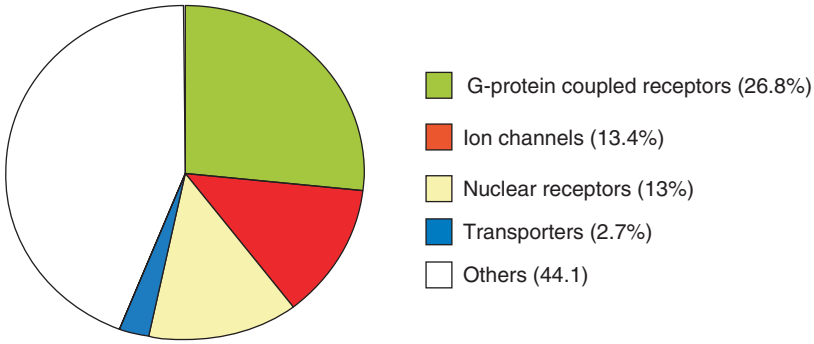


Figure 1.3 The molecular targets of prescribed drugs. The data is expressed as a percentage of all FDA approved drugs as of December 2005. Drugs that target ligand-gated ion channels and voltage-gated ion channels have been grouped together as ion channels. Drug targets grouped together as others encompass 120 different specific targets many of which are enzymes. Data taken from Overington et al. (2006).

between 20,000 and 25,000. Of these it is predicted that about 3000 are feasible protein drug targets. In 2005 it was calculated that about 100 drug targets account for all prescription drugs. On this basis there is obviously considerable scope for the development and discovery of novel drug targets to treat disease. At present the classical drug targets include GPCRs (Chapter 3), ion channels (Chapter 4), nuclear receptors (Chapter 8), transporters (Chapter 5) and enzymes. These important classical drug targets, whilst briefly covered in this Introduction, are extensively covered in later chapters. The distribution of drug targets expressed as a percentage of total products approved by the Food and Drug Administration (FDA; agency in the USA responsible for approving drugs for therapeutic use) is illustrated in Figure 1.3.

G-protein coupled receptors (GPCRs)

GPCRs represent the largest single family of pharmaceutical drug target accounting for approximately 30% of the current market. Their primary function is to detect extracellular signals and through heterotrimeric G-protein activation trigger intracellular signal transduction cascades that promote cellular responses (Figure 1.4). Whilst their share of the overall drug market is likely to fall in the future they still represent 'hot' targets for drug discovery programmes. GPCRs are conventionally targeted using small molecules (typically less than 500 Da) that are classified as agonists (receptor activating) or antagonists (inhibit receptor function by blocking the effect of an agonist). Some key examples of drugs that target GPCRs are listed in Table 1.1. Chapter 3 will explain in detail many of the recent developments in GPCR structure, function, pharmacology and signal transduction including GPCR dimerisation. Many of these exciting advances have revealed new pharmaceutical approaches for targeting GPCRs such as inverse agonists, allosteric modulators, biased agonists and bivalent ligands that target GPCR heterodimers. Since the completion of the human genome project it has emerged that the total number of human GPCRs may be as high as 865, which would account for approximately 3.4% of total predicted protein-coding genes (assuming a total of 25,000). For many cloned GPCRs the endogenous ligand(s) are unknown (so called 'orphan' GPCRs) and the identification of these orphan receptor ligands is the focus of drug discovery programmes within the pharmaceutical industry. The process of GPCR de-orphanisation is addressed in Chapter 2. In Chapter 11 the concept that GPCRs interact with a host of accessory proteins that are important in modulating many aspects in the life of a GPCR including the formation of signalling complexes will be explored. Indeed, targeting such GPCR signalling complexes with drugs that disrupt protein-protein interactions is another exciting avenue for future drug development not only in the field of GPCRs but also in other areas of signal transduction.

Ion channels

Ion channels represent important drug targets since they are involved in regulating a wide range of fundamental physiological processes. Indeed, at present they are the second largest class of drug target after GPCRs. They operate the rapid transport of ions across membranes (down their electrochemical gradients) and in doing so trigger plasma and organelle membrane hyperpolarisation or depolarisation. They are also potential drug

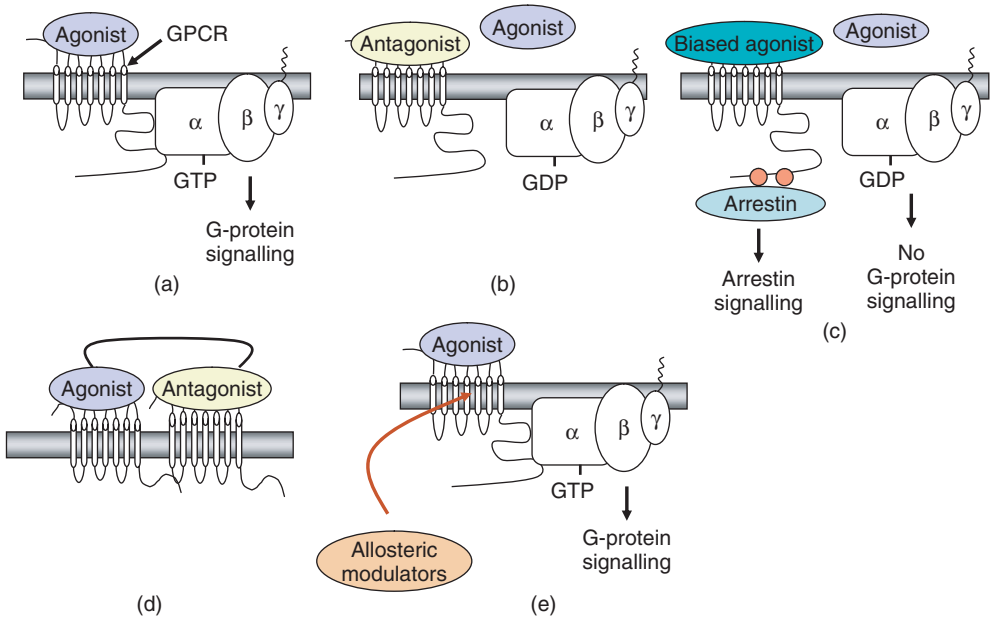


Figure 1.4 G-protein coupled receptors as drug targets. (a) GPCRs can be targeted using selective synthetic agonists which trigger receptor activation thus enabling G-protein coupling and subsequent cell signalling responses. (b) antagonists can be used to block the binding of an endogenous agonist thus preventing receptor activation. (c) GPCRs can also trigger G-protein independent cell signalling pathways which are dependent on arrestin binding to the activated receptor. Biased agonists are being developed that specifically promote the activation of arrestin-dependent signalling pathways. (d) bivalent ligands targeting specific GPCR heterodimers. (e) GPCRs can also be targeted using allosteric modulators which bind to sites on the receptor that are distinct from the agonist (orthosteric) binding site.

Table 1.1 G-protein coupled receptors as drug targets.

GPCR	Drug (brand name)	Agonist/Antagonist	Condition/use
Histamine H_2 receptor	Famotidine (Pepcidine)	Antagonist	Stomach ulcers
α_1-adrenergic receptor	Doxazosin (Cardura)	Antagonist	Hypertension
$GnRH_1$ receptor	Leuprorelin (Lupron)	Agonist	Prostate cancer
$5\text{-}HT_{1D}$ receptor	Sumatriptan (Imigran)	Agonist	Migraine
μ-opioid receptor	Fentanyl (Sublimaze)	Agonist	Analgesic

Abbreviations: GnRH, gonadotropin-releasing hormone.

targets for the treatment of rare monogenic hereditary disorders caused by mutations in genes that encode ion channel subunits. Such conditions termed 'ion channelopathies' include mutations in sodium, chloride and calcium channels that cause alterations in skeletal muscle excitability. The understanding of ion channel diversity and complexity increased significantly following the completion of the human genome project which identified over 400 genes encoding ion channel subunits. Given this number of genes it has been suggested that ion channels may rival GPCRs as drug targets in the future (Jiang et al., 2008). Other major developments include the first 3D resolution of ion channel structure by X-ray crystallography, which was reported for the voltage-gated potassium channel in 2003 (MacKinnon et al., 2003). Despite these important advances in the understanding of ion channel diversity and structure very few new ion channel drugs have reached the market during the last decade. Some key examples of ion channels as drug targets are shown in Table 1.2.

Table 1.2 Ion channels as drug targets.

Ion channel	Drug (brand name)	Condition/use
Voltage-gated Ca^{2+} channel	Amlodipine (Norvasc)	Hypertension and angina
Voltage-gated Na^{+} channel	Phenytoin (Dilantin)	Epilepsy
ATP-sensitive K^{+} channel	Glibenclamide (Glimepride)	Type II diabetes
$GABA_A$ receptor	Benzodiazepines (Diazepam)	Anxiety
$5\text{-}HT_3$ receptor	Ondansetron (Zofran)	Nausea and vomiting

Ion channels are broadly classified into two main groups (Figure 1.5). Firstly there are ligand-gated ion channels or ionotropic receptors which open when activated by an agonist binding to a specific ion channel subunit. Examples of this class include the nicotinic acetylcholine receptor, $GABA_A$ receptor, glycine receptor, $5\text{-}HT_3$ receptor, ionotropic glutamate receptors, and ATP-gated channels. The second group which includes voltage-gated or voltage-operated ion channels are opened by other mechanisms including changes in plasma membrane potential. Examples include voltage-gated Ca^{2+}, Na^{+}, and K^{+} channels. The molecular structure and classification of ion channels together with their use as drug targets will be explored in detail in Chapter 4.

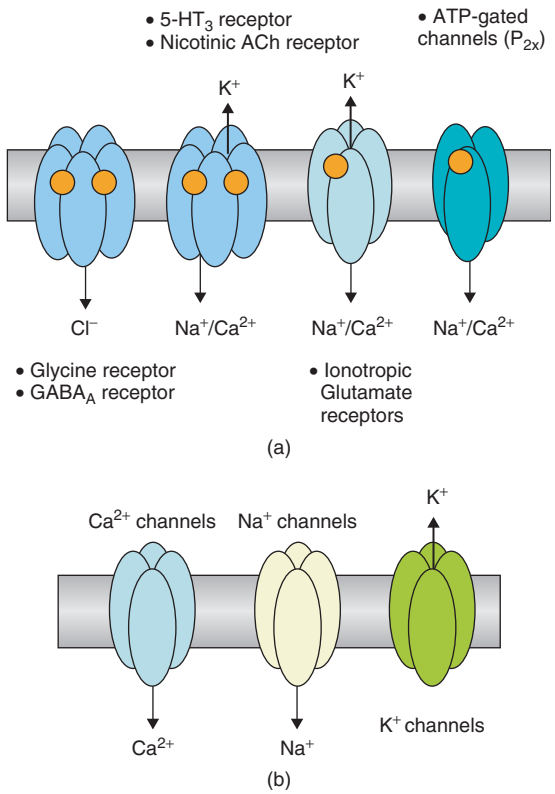


Figure 1.5 Ion channel classification. (a) Ligand-gated ion channels comprise a family of multi-subunit transmembrane proteins that are activated by a diverse set of ligands (indicated by the orange circle) that include amino acids (glycine, glutamate and GABA), 5-hydroxytryptamine (5-HT), acetylcholine (ACh), and ATP. (b) voltage-gated channel channels, which are also multi-subunit proteins, open in response to local changes in membrane potential.

Nuclear receptors

Nuclear receptors are a large family of transcription factors that play a pivotal role in endocrine function. In contrast to other families of transcription factor the activity of nuclear receptors (as their name suggests) is specifically regulated by the binding of ligands (Figure 1.6). Such ligands, which are small and lipophilic, include steroid hormones (glucocorticoids, mineralocorticoids, androgens, oestrogens and progestogens), thyroid hormones (T_3 and T_4), fat soluble vitamins D and A (retinoic acid) and various fatty acid derivatives. Since the completion of the human genome sequencing project 48 members of the human nuclear receptor family have been identified. However, for many nuclear receptors the identity of the ligand is unknown. These 'orphan' nuclear receptors are of significant interest to the pharmaceutical industry since they may lead to the discovery of novel endocrine systems with potential therapeutic use. Whilst the total number of nuclear receptors is small in comparison to GPCRs they are the target of approximately 13% of all prescribed drugs. For example, the chronic inflammation associated with asthma can be suppressed by inhaled glucocorticoids and oestrogen-sensitive breast cancer responds to treatment with the oestrogen receptor antagonist tamoxifen. The structure, classification, signal transduction mechanisms and therapeutic uses of nuclear receptor targeting drugs will be explored in detail in Chapter 8.

Neurotransmitter transporters

The concentration of some neurotransmitters within the synaptic cleft is tightly regulated by specific plasma membrane-bound transporter proteins. These transporters, which belong to the solute carrier

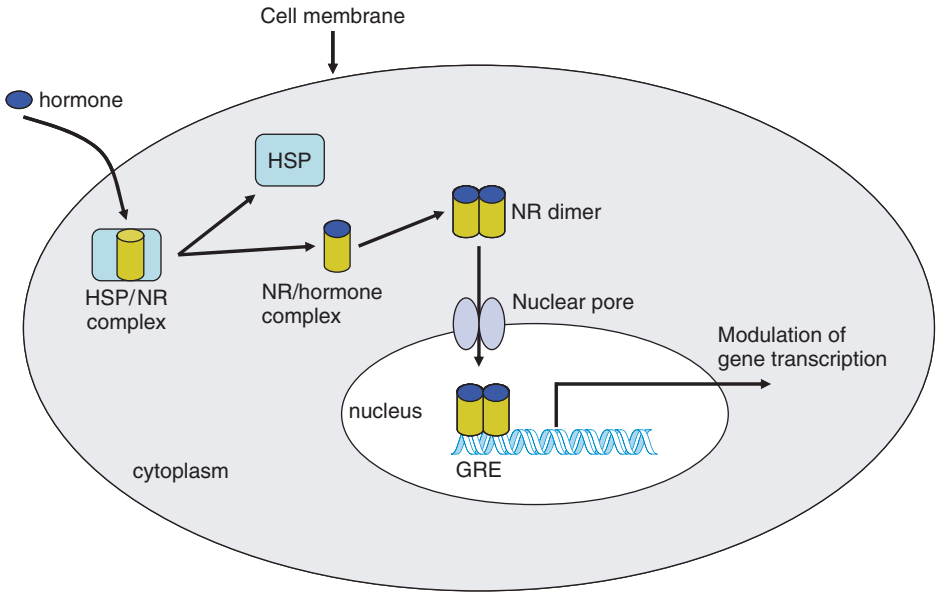


Figure 1.6 Type I nuclear receptor-mediated signal transduction. In the absence of hormone (e.g. glucocorticoid) the nuclear receptor (NR) is located in the cytoplasm bound to a heat shock protein (HSP). Hormone binding triggers dissociation of the HSP from the NR/HSP complex, dimerisation of the NR and translocation to the nucleus. Once in the nucleus the NR dimer binds to a specific DNA sequence known as glucocorticoid response element (GRE) and modulates gene transcription.

(SLC) transporter family, facilitate the movement of neurotransmitter either back into the pre-synaptic neuron or in some cases into surrounding glial cells. There are two major subclasses of plasma-membrane bound neurotransmitter transporter: the SLC1 family which transports glutamate and the larger SLC6 family which transports dopamine, 5-HT, noradrenaline, GABA and glycine (Figure 1.7). Both SLC1 and SLC6 families facilitate neurotransmitter movement across the plasma membrane by secondary active transport using extracellular Na^+ ion concentration as the driving force. As might be expected drugs that target neurotransmitter transporters have a wide range of therapeutic applications such as treatment for depression, anxiety and epilepsy. Indeed, neurotransmitter transporters are the target for approximately one-third of all psychoactive drugs (see Table 1.3). The molecular structure and classification of neurotransmitter transporters and their value as important current and future drug targets will be discussed in detail in Chapter 5.

1.4 Future drug targets

At present more than 50% of drugs target only four major gene families, namely GPCRs, nuclear receptors, ligand-gated ion channels and voltage-gated ion channels (Figure 1.3). It is likely that the market share of these classical drug targets will shrink as new drug targets and approaches are developed in the future.

Protein kinases

It is predicted that protein kinases (and lipid kinases), one of the largest gene families in eukaryotes, will become major drug targets of the twenty-first century. Protein phosphorylation is reversible and is one of the most common ways of post-translationally modifying protein function. It regulates numerous cellular functions including cell proliferation, cell death, cell survival, cell cycle progression, and cell differentiation. The enzymes that catalyse protein phosphorylation are known as protein kinases, whereas the enzymes that carry out the reverse dephosphorylation reaction are referred to as phosphatases (Figure 1.8a). The human genome encodes for 518 protein kinases and approximately 20 lipid kinases. The predominant sites of protein phosphorylation are the hydroxyl groups (−OH) in the side chains of the amino acids serine, threonine and tyrosine (Figure 1.8b). When a phosphate group is attached to a protein it introduces a strong negative charge which can alter protein conformation and thus function.

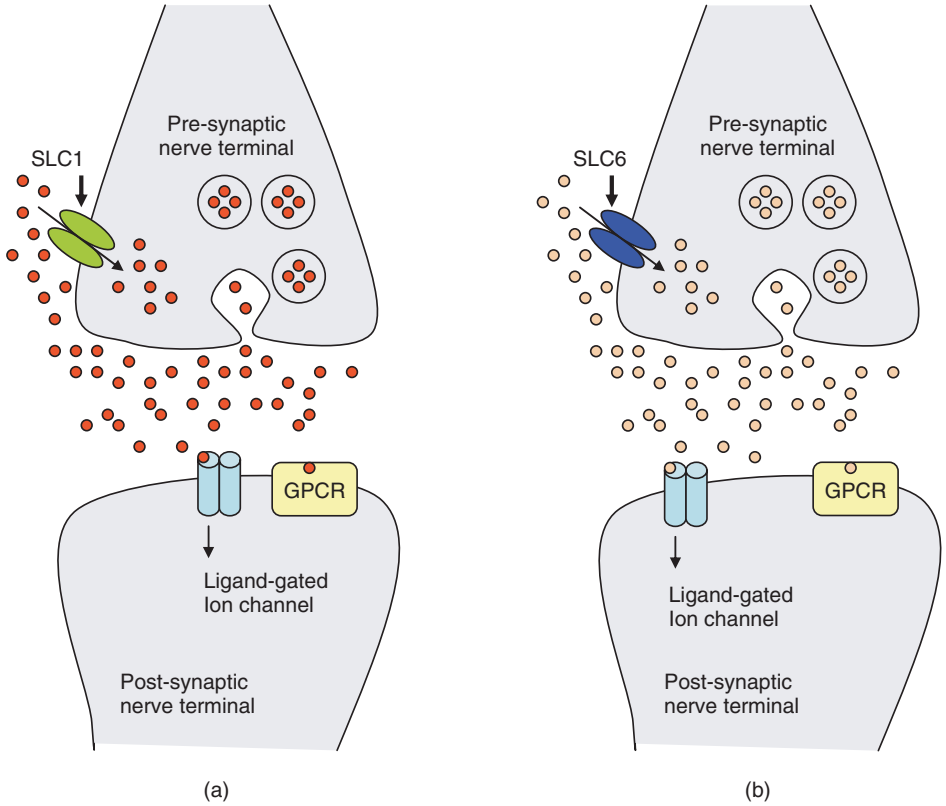


Figure 1.7 Neurotransmitter transporter classification. (a) Glutamate released into the synaptic cleft activates both ion channels (ionotropic glutamate receptors) and GPCRs (metabotropic glutamate receptors) located on the post-synaptic membrane. Released glutamate is subsequently removed from the extracellular space by SLC1 transporters located on pre-synaptic membranes. (b) Dopamine, 5-hydroxytryptamine (5-HT), γ-aminobutyric acid (GABA), noradrenaline and glycine released into the synaptic cleft activate specific ligand-gated ion channels and/or GPCRs located on the post-synaptic membrane. These released neurotransmitters are subsequently transported back into the pre-synaptic nerve terminal via SLC6 transporters. For clarity specific vesicular transporters responsible for transporting neurotransmitters from the cytoplasm into synaptic vesicles have been omitted. Figure adapted from Gether et al. (2006). Trends in Pharmacological Sciences 27: 375–383.

Table 1.3 Transporters as drug targets.

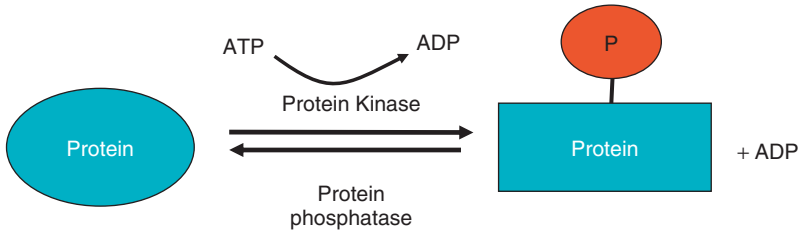
Transporter	Drug (brand name)	Condition/ use
5-HT transporter (SERT)	Sertraline (Zoloft)	Antidepressant
Dopamine transporter (DAT)	Cocaine	Drug of abuse
Noradrenaline transporter (NET)	Bupropion[a] (Welbrutin)	Antidepressant
GAT-1 (GABA)	Tiagabine	Epilepsy

[a]Affinity for DAT as well

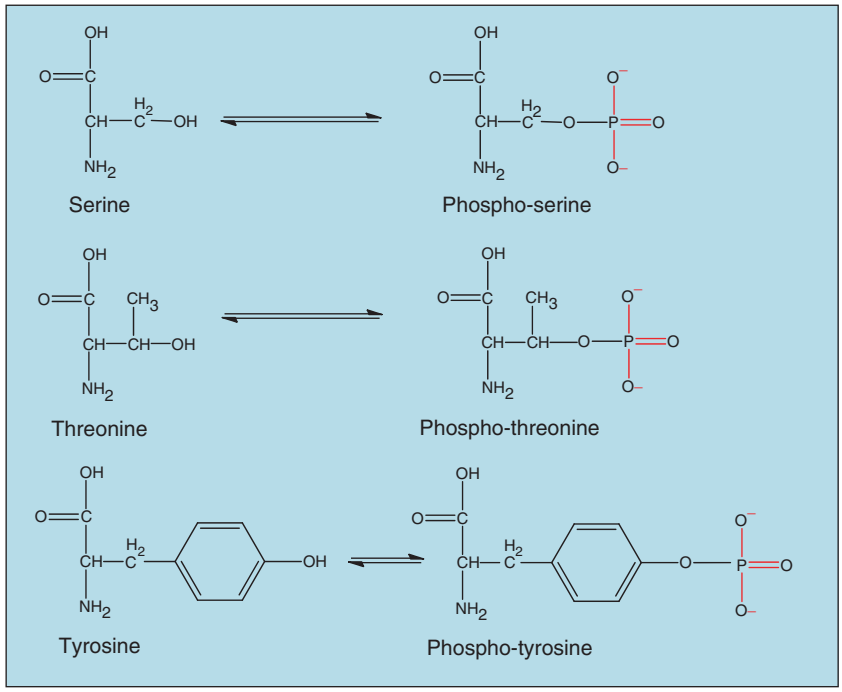
Enzymes

Enzymes are the drug target for approximately 50% of all prescribed drugs. Some key examples are listed in Table 1.4. However, because of their diverse nature they will not be the focus of a specific chapter in this book. It is also important to remember that many prescribed drugs target bacterial and viral enzymes for the treatment of infectious disease and HIV. Also many enzymes, whilst not direct drug targets, play important roles in drug metabolism for example cytochrome P450 enzymes.

Protein kinases are classified according to the amino acid they phosphorylate and are grouped into two main types: serine/threonine kinases and tyrosine kinases. In



(a)



(b)

Figure 1.8 Reversible protein phosphorylation. (a) Protein kinases transfer a phosphate group (P) from ATP to the target protein altering its biological activity. The removal of phosphate from a phosphorylated protein is catalysed by protein phosphatases. (b) Phosphate groups are transferred to the amino acids serine, threonine and tyrosine.

both cases ATP supplies the phosphate group with the third phosphoryl group (γ; gamma phosphate) being transferred to the hydroxyl group of the acceptor amino acid. Examples of serine/threonine kinases include protein kinase A (PKA; activated by the second messenger cyclic AMP) and protein kinase C (PKC; activated by the second messenger diacylglycerol). Examples of tyrosine kinases include tyrosine kinase linked receptors for insulin and epidermal growth factor and non-receptor tyrosine kinases such as Src and JAK (Janus-associated kinase). Given the prominent role of protein phosphorylation in regulating many aspects of cell physiology it is not surprising that dysfunction in the control of protein kinase signalling is associated with major diseases such as cancer, diabetes and rheumatoid arthritis. These alterations in protein kinase and in some cases lipid kinase function arise from over-activity either due to genetic mutations or over-expression of the protein. It is estimated that up to 30% of all protein targets currently under investigation by the pharmaceutical industry are protein or lipid kinases. Indeed, there are approximately 150 protein kinase inhibitors in various stages of clinical development,

Table 1.4 Enzymes as drug targets.

Enzyme	Drug (brand name)	Condition/use
HMG-CoA reductase	Statins	Used to lower blood cholesterol levels
Phosphodiesterase type V	Sildenafil (Viagra)	Erectile dysfunction and hypertension
Cyclo-oxygenease	Aspirin	Analgesic and anti-inflammatory
Angiotensin-converting enzyme	Captopril (Capoten)	Hypertension
Dihydrofolate reductase	Methotrexate	Cancer

Table 1.5 Selected small-molecule protein kinase inhibitors in clinical development.

Drug	Protein kinase target	Use
AZD 1152	Aurora B Kinase	Various cancers
NP-12	Glycogen synthase kinase 3 (GSK3)	Alzheimer's disease
Bay 613606	Spleen tyrosine kinase (Syk)	Asthma
INCB-28050	Janus-associated kinase 1/2 (JAK1/2)	Rheumatoid arthritis
BMS-582949	p38 mitogen-activated protein kinase (p38 MAPK)	Rheumatoid arthritis

some of which are highlighted in Table 1.5. Whilst protein kinases are important new human drug targets they are also present in bacteria and viruses and thus represent potential targets for infectious disease treatment.

Since the launch of imatinib in 2001 several other small-molecule protein kinase inhibitors have successfully made it to the market place as novel anti-cancer treatments (Table 1.6). The majority of these drugs are tyrosine kinase inhibitors and in some cases function as multi-kinase inhibitors (e.g. sunitinib) targeting PDGFR (proliferation) and VEGFR (angiogenesis) dependent signalling responses. Monoclonal antibodies are also used to block the increased tyrosine kinase linked receptor activity that is associated with many forms of cancer and these will be discussed in Chapter 12.

Table 1.6 Small-molecule protein kinase inhibitors approved for clinical use.

Drug (brand name)	Targets	Use
Imatinib (Gleevec®)	c-Abl-kinase, c-Kit	Chronic myeloid leukaemia
Gefitinib (Iressa®)	EGFR	Various cancers
Sunitinib (Sutent®)	PDGFR, VEGFR	Renal cell carcinoma
Dasatinib (Sprycel®)	c-Abl-kinase, Src	Various cancers
Everolimus (Afinitor®)	mTOR[a]	Various cancers

[a]Serine/threonine kinase. Abbreviations: EGFR, epidermal growth factor receptor; mTOR, mammalian target of rapamycin; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor.

A useful approach for assessing the therapeutic potential of novel drug targets is the number of approved patents for each target (Zheng et al., 2006). The level of patents gives an indication of the degree of interest in that particular target and hence likelihood of successful drugs being developed. Future targets with a high number of US-based patents include matrix metalloproteinases (MMPs) as a target for cancer treatment. MMPs are proteases which break down the extracellular matrix thus facilitating cancer cell invasion and metastasis. Other targets include phosphodiesterase 4 (PDE4), caspases and integrin receptors. Only time will tell whether any of these novel targets result in the development of effective therapeutics. For further reading on the identification and characteristics of future drug targets see the review by Zheng et al. (2006).

Therapeutic oligonucleotides

In addition to the development of small-molecule-based drugs there are several other approaches to treat human disease including the exciting prospect of therapeutic oligonucleotides (anti-sense and RNA interference based) as tools for gene silencing and the continued quest for gene therapy-based techniques. These molecular biology-based strategies for combating human disease will be addressed later in Chapter 8.

Another new class of drugs are short single-stranded oligonucleotides (DNA or RNA based) that have been selectively engineered to target specific intracellular proteins (Dausse et al., 2009). These oligonucleotides which

fold into defined three-dimensional structures are known as aptamers or 'chemical antibodies'. They are generated and repeatedly selected through a method known as SELEX (Systematic Evolution of Ligands by Exponential Enrichment). Essentially the process begins with the synthesis of a large oligonucleotide library, containing randomly generated sequences of fixed length, which is screened for binding to the target protein usually by affinity chromatography. Those that bind are repeatedly selected using stringent elution conditions that ultimately result in the identification of the tightest binding sequences. These high affinity sequences can be chemically modified to increase their affinity and effectiveness as potential therapeutic oligonucleotides. The first aptamer-based drug approved by the US Food and Drug Administration (FDA) targets the VEGFR and is used to treat age-related macular degeneration. Several other aptamer oligonucleotides are also undergoing clinical trials.

1.5 Molecular pharmacology and drug discovery

The process of drug discovery is a long and costly process with new drugs taking up to 12 years to reach the clinic. Many novel molecular pharmacology-based techniques play important roles in the process of drug discovery and development. A problem faced by many pharmaceutical companies is the huge task of screening their vast chemical libraries (in some cases this can exceed one million compounds) against an increasing number of possible drug targets. The development, in the early 1990s, of high-throughput screening (HTS) technology using 96-well microtiter plates enabled the drug screening process to be miniaturised and automated. Using such methodology it became possible to screen up to 10,000 compounds per day. However during the last decade 384-well microtiter plates and more recently 1536-well microtiter plate-based assays have been developed that allow for screening of up to 200,000 compounds a day (ultra-high-throughput screening). Since the screening of large chemical libraries is expensive several alternative strategies to increase the chances of success have been introduced in recent years. One such approach has been the introduction of fragment-based screening (FBS) or fragment-based lead discovery (FBLD). This involves screening the biological target with small libraries of chemical fragments (molecular weights around 200 Da) with the aim of identifying scaffolds or 'chemical backbones' that can be developed into lead compounds. This approach may also be combined with computer-based 'virtual screening' approaches. For example structure-based virtual screening involves the use of 3D protein structures, many of which are now widely available via public databases, to assess whether a ligand can interact or dock with the protein of interest. This can be linked with ligand-based virtual screening which involves *in silico* screening of chemical libraries for compounds that display similar structural features associated with the binding of the ligand to the target. As indicated above structure-based virtual screenings rely on the availability of accurate 3D structures of the drug target. The discipline of structural biology uses a range of biophysical techniques including X-ray crystallography, NMR spectroscopy and electron cryo-microscopy to determine protein structure. The latter is an emerging technique that can be used to determine the 3D structure of macromolecular complexes that are too large to be studied using X-ray crystallography and/or NMR spectroscopy. So far in this section we have briefly covered some of the up-and-coming techniques that can used to interrogate drug target structure and screen drug targets for lead compounds. There is also a drive towards the development novel cell-based and animal-based models that are more representative of human physiology and hence more suitable for drug screening. For example, 3D 'organotypic' cell microarrays are currently being developed that will allow drug screening in a system that is close to the *in vivo* environment of cells. In summary, we

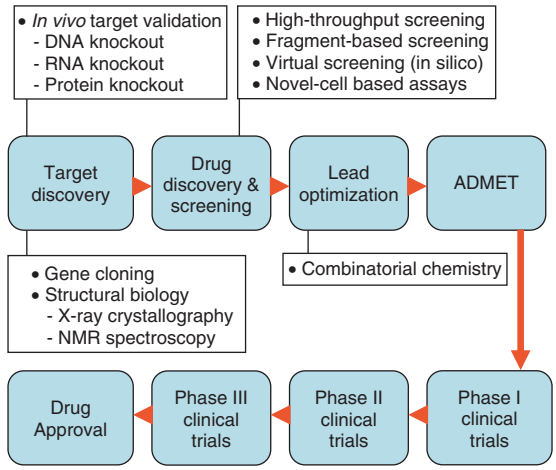


Figure 1.9 Schematic representation of the drug discovery process. ADMET (**A**dsorption, **D**istribution, **M**etabolism, **E**xcretion and **T**oxicity studies).

are witnessing exciting times in the process of drug discovery with the continued development of *in silico* and nanotechnology-based methods and the introduction of novel cell-based screening models. Has there ever been a better time to be a molecular pharmacologist? A schematic representation of the drug discovery process is shown in Figure 1.9.

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