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REVIEW ARTICLE

Drugs and their molecular targets: an updated overview

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ABSTRACT

About 330 targets bind approved drugs, 270 encoded by the human genome and 60 belonging to pathogenic organisms. A large number of druggable targets have been recently proposed from preclinical and first clinical data, but a huge reservoir of putative drug targets, possibly several thousands, remains to be explored. This overview considers the different types of ligands and their selectivity in the main superfamilies of drug targets, enzymes, membrane transporters and ion channels, and the various classes of membrane and nuclear receptors with their signalling pathway. Recently approved drugs such as monoclonal antibodies, tyrosine kinase and proteasome inhibitors, and major drugs under clinical studies are reviewed with their molecular target and therapeutic interest. The druggability of emerging targets is discussed, such as multidrug resistance transporters and cystic fibrosis transmembrane conductance regulator (CFTR), hyperpolarization-activated cyclic nucleotides-gated (HCN), cyclic nucleotide-gated (CNG) and transient receptor potential (TRP) ion channels, tumour necrosis factor (TNF) and receptor activator of NFκB (RANK) receptors, integrins, and orphan or recently deorphanized G-protein-coupled and nuclear receptors. Large advances have been made in the therapeutical use of recombinant cytokines and growth factors (i.e. tasonermin, TNFα-1a; becaplermin, platelet-derived growth factor (PDGF); dibotermin-alpha, bone morphogenetic proteins (BMP)2; anakinra, interleukin-1 receptor antagonist protein (IRAP), and in enzyme replacement therapy, i.e. algasidase (alpha-galactosidase) and laronidase (alpha-L-iduronidase). New receptor classes are emerging, e.g. membrane aminopeptidases, and novel concepts are stimulating drug research, e.g. epigenetic therapy, but the molecular target of some approved drugs, such as paracetamol and imidazolines, still need to be identified.

INTRODUCTION

Most drug targets are cellular proteins undergoing a selective interaction with chemicals administered to treat or diagnose a disease. These targets are human genome-derived proteins, or belong to pathogenic organisms. A limited set of drugs act through physicochemical mechanisms, or have unknown mechanisms of action.

How many drug targets for how many drugs?

Analysis of the human genome in 2002 led to the estimation of 6000–8000 targets of pharmacological interest. Only a small part of these targets relates to approved drugs. In 2003, Golden proposed that all the then-approved drugs acted through 273 proteins [1]. In 2006, Zheng et al. disclosed 268 'successful' targets in the current version of the therapeutic Targets Database [2], and Imming et al. catalogued 218 molecular targets for approved drugs [3]. A consensus number of 324 drug targets for all classes of approved therapeutic drugs was proposed by Overington et al. [4]. Of these, 266 are human genome-derived proteins, and 58 are bacterial, viral, fungal or other pathogenic organism targets.

The discrepencies between these estimations arise from the criteria chosen by each author, such as including/not including drugs under clinical trials but

not yet approved, or considering/not considering the multiple relevant targets for a unique drug. However, some interesting features can be drawn from such studies. The analysis by Overington et al. [4] identifies in excess of 21 000 drug products marketed in US corresponding to 1357 unique drugs, of which 1204 are 'small-molecule drugs' (including 192 prodrugs) and 166 are 'biological drugs'. Twenty-seven per cent of these drugs bind to G-protein-coupled receptors, 13% to nuclear receptors, 7.9% to ligand-gated ion channels and 5.5% to voltage-gated ion channels. A selected target may have a unique approved drug, or a large number of me-too molecules.

The analysis by Imming et al. [3] gives an accurate view of the different biological classes of the 218 listed targets: 66 human enzymes and 20 bacterial, viral, fungal or parasital enzymes; 20 families of G-protein-coupled receptors, each family including up to five members; 12 nuclear receptors for steroids and others; seven cytokine receptors; and about 10 ion channels; and 10 transport proteins of the plasma membrane. Altogether, these studies confirm that a very large number of putative drug targets remains to be explored.

Affinity and binding selectivity

Various chemical bonds cooperate to establish the interaction of the drug to its target: hydrophobic interactions, hydrogen and ionic bonds, charge transfer complexes and covalent bonds. These chemical interactions are related to the affinity of the drug for its target. The medium affinity of current small-molecule drugs is about 20 nM, ranging from 200 mM to 10 pM [4]. A high affinity for a therapeutically relevant target is usually considered a criterion for the selectivity of the drug, with lesser risk of binding to targets inducing undesirable or toxic effects. But the strict application of this concept would eliminate any low-affinity drugs, whereas some have proved to be of therapeutical interest. No drug can be considered specific of a single target, but only selective, according to the dose, in vivo, or the concentration, in vitro, used [5].

Various ligands for a single target

Most of the target types can be either stimulated or inhibited, depending on the ligand chosen. This leads to opposite regulations of related cellular functions. Terms used to characterize these different ligand types differ according to the biochemical nature of the targets.

Enzyme ligands most often lead to inhibition of enzyme activity, binding to the active site with competition with the substrate (competitive inhibitors) or to allosteric sites (non-competitive inhibitors). Activation of an enzyme is more difficult, unless giving or generating an excess of substrate or co-substrate.

Membrane transporters and ion channel permeability can be increased or decreased by direct binding of selected drugs termed 'openers' and 'inhibitors' (or blockers) respectively. However, such ligands are too often improperly referred to as 'agonists' and 'antagonists'.

Receptors of mediators interact with a large diversity of ligand types. Agonists mimic the effects of endogenous mediators. Some exceptions to this concept are now known, some couples of mediators acting through the binding to a single receptor with agonist or antagonist properties, respectively, i.e. interleukin-1 and interleukin-1 receptor antagonist protein (IRAP), RANK-L and osteoprotegerin (OPG), melanocortin (MSH) and agouti-related protein (AGRP). Full agonists elicit a maximal response of the organism, usually similar to that of the mediator. Partial agonists elicit a partial response of the organism, and prevent the binding of the mediator. Thus the related function of the organism is decreased. Neutral antagonists prevent the binding of the mediator and thus abolish downstream physiological responses caused by the mediator. Inverse agonists, also termed 'negative antagonists', have been found among antagonists. Similar to neutral antagonists, they prevent the binding of agonists, including mediators, but elicit a response inverse to that of agonists. Such ligands decrease the constitutive activity of receptors [6,7].

Receptors of mediators including intrinsic ion channels or enzyme activity have ligands in their receptor part (agonists and antagonists) as well as in their ion channel (openers and inhibitors or blockers) or enzyme part (inhibitors).

ENZYMES AS DRUGS AND DRUG TARGETS

At least 66 human enzymes and 20 bacterial, viral, fungal or parasital enzymes are targets of approved drugs [3], e.g. up to 40% of current targets. Note that several thousands of enzymes are coded in the human genome, opening large opportunities for developing new drugs. Some purified enzymes have also been used as drugs. Human recombinant enzymes are now produced, allowing replacement therapy in rare genetic disorders such as Fabry disease with algasidase (alpha galactosidase), and mucopolysaccharidosis I with laronidase (alpha-L-iduronidase). Enzyme replacement therapy is a growing field

with promising success in genetic diseases, for instance in glycogenosis type II (Pompe disease) with recombinant acid alpha-glucosidase [8].

The basis of using enzyme inhibitors as drugs is that inhibition of a suitable selected enzyme leads to a build up in concentration of the substrate and a corresponding decrease in concentration of the metabolite, leading to a useful clinical response. Reversible inhibition occurs when the inhibitor is bound to the enzyme through a suitable combination of Van der Vaals', electrostatic, hydrogen, and hydrophobic forces. During irreversible inhibition, covalent bonds are formed between a functional group on the enzyme and the drug.

Targeting human enzymes

The inhibitors used in therapy should possess a high selectivity towards the target enzyme, as inhibition of closely related enzymes may lead to a range of side effects. This concept has led to market isoenzyme-selective inhibitors, i.e. monoamine-oxidase inhibitors (**moclobemide** for MAO-A as an antidepressive drug, **selegiline** for MAO-B in Parkinson's disease), selective inhibitors for various cyclic nucleotide phosphodiesterases (**sildenafil** for PDE5), and selective cyclooxygenase inhibitors (celecoxib for cox2).

However, the claimed selectivity often remains quite low. For instance, imatinib, originally developed as a highly selective inhibitor of the tyrosine kinase activity of c-ABL (approved for chronic myeloid leukaemia), has subsequently been found to also inhibit tyrosine kinase activity of c-kit and PDGFR. In that case, the lack of selectivity offers the opportunity to extend the indication of the drug [9].

The inhibition of multifunctional enzymes can also have therapeutic value. The 26S proteasome is a multicatalytic intracellular protease complex expressed in eukaryotic cells. This complex hydrolyses cellular proteins that are responsible for cell proliferation, growth, regulation of apoptosis and transcription of genes. Thus, proteasome inhibition is a potential treatment option for cancer and inflammatory diseases. **Bortezomib** and PS-519 are the first proteasome inhibitors to have entered clinical trials. Bortezomib is approved for the treatment of relapsed multiple myeloma and several phase II and phase III trials in haematological malignancies and solid tumours are ongoing. PS-519 that focuses on inflammation, reperfusion injury and ischaemia is currently under evaluation for the indication of acute stroke [10].

Targeting enzymes selective of invading organisms

Interestingly, targeted enzymes of invading organisms may have no functional equivalent in human cells. For example, the unique properties of human immunodeficiency virus (HIV)-1 integrase make it an ideal target for drug design. HIV-1 integrase is essential for retroviral replication, being involved in the integration of HIV-DNA into host chromosomal DNA. HIV-1 integrase has been recently validated as a legitimate target and the data from molecules like S-1360 and JKT-303 which are under phase II/III clinical trials suggest synergistic effect with reverse transcriptase and protease inhibitors [11].

Another recent example is derived from analysing the methyl erythritol phosphate pathway for isoprenoid biosynthesis, for which the key enzyme is 1-deoxy-D-xylose 5-phosphate reductoisomerase (DXR). DXR has no functional equivalent in humans, making it an attractive target for novel antimalarial and antibacterial agents [12].

MEMBRANE TRANSPORTERS AS DRUG TARGET

Membrane transporters constitute a rather small family of drug targets. Some of them are yet to be studied for their therapeutic potential, i.e. as targets of diuretics or antidepressants, but some others are still resistant to pharmacological control. Transporter genes encode proteins generally constituted by 12 transmembrane-spanning regions. They mediate Na^+- or H^+-dependent transport of small molecules such as neurotransmitters, antibiotics, ions and cationic amino acids. Transport is performed by different mechanisms: uniport, substrate–ion symport, substrate–ion antiport, substrate–substrate or ion–ion antiport, and ATP-dependent translocation.

Established drug targets among membrane transporters

Most success stories in this field concern old drugs whose targets have been often discovered after their efficient clinical use. These include **cardiac glycosides** (sodium pump, e.g. Na^+/K^+-ATPase); **omeprazole** and analogues (proton pump, e.g. H^+/K^+ ATPase); **artemisinin** and derivatives [plasmodial sarcoplasmic and endoplasmic calcium ATPase (SERCA)]; diuretics [thiazides for the Na^+/Cl^- co-transporter (NCC); **furosemide** for the $Na^+/K^+/Cl^-$ co-transporter (NKCC)]; **reserpine**, **ephedrine** and **amphetamines** [vesicular monoamine transporter (VMAT)]; the antidepressant **paroxetine** for serotonin/Na^+ symporter (SERT); **cocaine** and the antidepressant

imipramine for the noradrenaline, dopamine and serotonin/Na^+ symporters, NET, DAT and SERT. The absence of selectivity of antidepressants for targets of a molecular and functional family, e.g. monoamine transporters of neurones is notable.

Progress in the pharmacological control of membrane transporters

ATP-binding cassette (ABC) transporters, including multidrug resistance transporters and cystic fibrosis transmembrane conductance regulator (CFTR) are putative drug targets, but progress in finding drugs of clinical interest remains very slow.

Multidrug resistance is a serious impediment to improved healthcare. Multidrug resistance is most frequently caused by active transporters, such as P-glycoprotein (ABCB-1) identified 30 years ago, that pump a broad spectrum of chemically distinct molecules out of cells, including antibiotics, antimalarials and cancer chemotherapeutic drugs in humans [13]. Around 40% of human tumours develop resistance to chemotherapeutic drugs because of the overexpression of ABC proteins. Nonetheless, success in overcoming or circumventing multidrug resistance in a clinical setting has failed. A first approach has been to modify the structure of drugs so that they are no longer substrates for ABC transporters. But any modification to a drug that substantially reduces its affinity for a transporter also tends to reduce its ability to cross the cell membrane and to bind to its target. The second approach to overcoming multidrug resistance, the development of inhibitors of ABC transporters, has also proved unsatisfactory [14].

The cystic fibrosis transmembrane conductance regulator, CFTR, discovered 20 years ago, is a cAMP-activated chloride channel, acting as an ATP-dependent pump with ATPase activity, expressed in the epithelia of the lung, intestine, pancreas, and other tissues, where it facilitates transepithelial fluid transport. Mutations in CFTR cause the hereditary disease cystic fibrosis, secretory diarrhoea and polycystic kidney disease. The most common mutation in the CFTR gene is the deletion of phenylalanine 508, ΔPhe508, which causes its retention in the endoplasmic reticulum and leads to the absence of CFTR Cl^- channels in the plasma membrane. Recently, curcumin was shown to rescue ΔPhe508–CFTR localization and function [15]. Benzothiophene, phenylglycine and sulphonamide potentiators were also identified that correct the defective gating of ΔPhe508–CFTR chloride channels, and other small molecules that correct its defective cellular processing [16]. Other mutations of CFTR, like G551D and G1349D (glycine to aspartic acid change at position 551 or 1349), cause only a gating defect. Anti-hypertensive 1,4-dihydropyridines known to block voltage-dependent calcium channels, have been identified as effective potentiators of CFTR gating, capable of correcting the defective activity of CFTR mutants [17].

VOLTAGE-GATED ION CHANNELS AS DRUG TARGETS

Ion channels are essential for a wide range of functions such as neurotransmitter secretion and muscle contraction. Ion channels mediate Na^+, Ca^{2+}, K^+ and Cl^- conductance induced by membrane potential changes. These channels propagate action potentials in excitable cells and are also involved in the regulation of membrane potential and intracellular Ca^{2+} transients in most eukaryotic cells. About 300 genes code for subunits of voltage-gated ion channels. Na^+, Ca^{2+} and K^+ channels are drug targets, but the pharmacology of Cl^- channels is not yet developed.

Voltage-gated sodium channels (Na_V channels)

These channels play a critical role in initiating the action potential. The activation of the channels allows for the inward movement of Na^+ from the extracellular space of the cell. Na_V channels from brain and striated muscles are hetero-oligomeric composed of α- and β-subunits. The α-subunit, with its 24 transmembrane helices, determines the major functional characteristics of Na_V channels. The human genome contains nine genes encoding the main α-subunit of Na_V channels and at least four genes encoding auxiliary β-subunits (one transmembrane helix), the expression of which is tissue-specific [18].

Natural toxins that block Na_V channels, such as tetrodotoxin and saxitoxin, have not found any therapeutic application. Plant toxins, such as pyrethrins and pyrethroids are currently being used as insecticides. Numerous synthetic drugs, now proposed to be inhibitors of sodium channels, have been used before the determination of channels structure and diversity. This includes local anaesthetics (**lidocaine** and analogues), class-1 antiarrythmics (**disopyramide**, **flecaine**, **quinidine**) and some antiepileptics of first (**phenytoin**, **carbamazepine**) or second generation (**lamotrigine**, **topiramate** and **felbamate**) acting through a decrease of glutamatergic neurotransmissions. The selectivity of these drugs

for different sodium channels is not yet fully determined. The chemistry of selective ligands of Na_V channels deserves to be developed.

Voltage-gated calcium channels (Ca_V channels)

Ten different genes encode different α-subunits (24 transmembrane helices) comprising the voltage-gated Ca^{2+} channels: Ca_v1 (α_{1S}, α_{1C}, α_{1D}, and α_{1F}) mediates L-type Ca^{2+} currents; Ca_v2 (α_{1A}, α_{1B}, α_{1E}) mediates P/Q-type, N-type and R-type Ca^{2+} currents, respectively; and Ca_v3 (α_{1G}, α_{1H}, α_{1I}) mediates T-type currents. The α1 subunits co-assemble with α2/δ, β, and γ subunits to form functional channels in different tissues.

Ca_V1 channels (L-type) are targets of 'calcium-channel blockers' or 'calcium antagonists' which decrease the influx of Ca^{2+} in cardiac and smooth muscle vascular cells: **nifedipine** and analogues, **verapamil** and **diltiazem**), widely used as antihypertensive, antianginal and anti-arrhythmic drugs. Ca_V1 openers, like BayK 8644, have been synthesized but have not been found to be of any therapeutic value.

$Ca_V2.2$ channels (N-type) control the release of neurotransmitters at the presynaptic level. Its selective blocker **ziconotide**, a synthetic peptide analogue of an ω-conotoxin, has recently been approved for the intrathecal treatment of severe chronic pain [19].

Ca_V3 channels (T-type) have only recently become targets of interest. Selective inhibition of T-channels may have clinical importance in cardiovascular diseases, some forms of epilepsy, sleep disorders, pain and possibly cancer [20]. Interestingly, the α2/δ subunits of Ca_V channels are the molecular targets of gabapentin and pregabalin approved for the treatment of neuropathic pain and epilepsy (see Conclusion).

Potassium channels

Potassium channels are highly heterogeneous and are thus interesting drug targets. They are classified on the basis of the structure of the α-subunit (*Figure 1*) and/or of their regulatory processes.

Voltage-gated K^+ channels

K_V1 to K_V9, the α-subunit of which contains six transmembrane helices with a single pore, includes the slow delayed rectifiers $K_{V(s)}$ (or K_VLTQ), and the rapid delayed rectifiers $K_{V(r)}$ (or K_VEAG-like). The old experimental blocker of K_V channels, **4-aminopyridine**, is in phase III for the treatment of multiple sclerosis [21].

Voltage and G-protein-gated K^+ channels

K_{Ach} and K_M (six helices) are quite similar to $K_{V(s)}$ and $K_{V(r)}$, but interact with Gi proteins coupled to M2 muscarinic acetylcholine receptors or with Gq proteins coupled to M3 receptors, respectively [22]. Stimulation of M2 or M3 muscarinic receptors by acetylcholine in pacemaker cardiac cells activates K^+ currents. Heart rate is thus slowed down by hyperpolarization of the pacemaker's depolarization potential as well as by blocking of tonic β-adrenergic stimulation of depolarizing pacemaker channels (see below, under KCN channels).

Voltage and calcium-activated K^+ channels

BK_{Ca} (seven helices) are voltage-sensitive and activated by direct binding of intracellular calcium, IK_{Ca} and SK_{Ca}

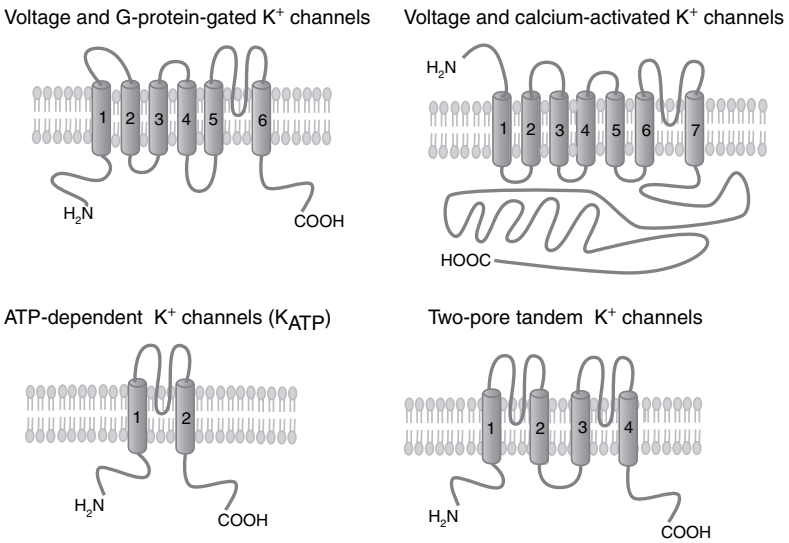

Figure 1 Structure of the α subunits of potassium channels.

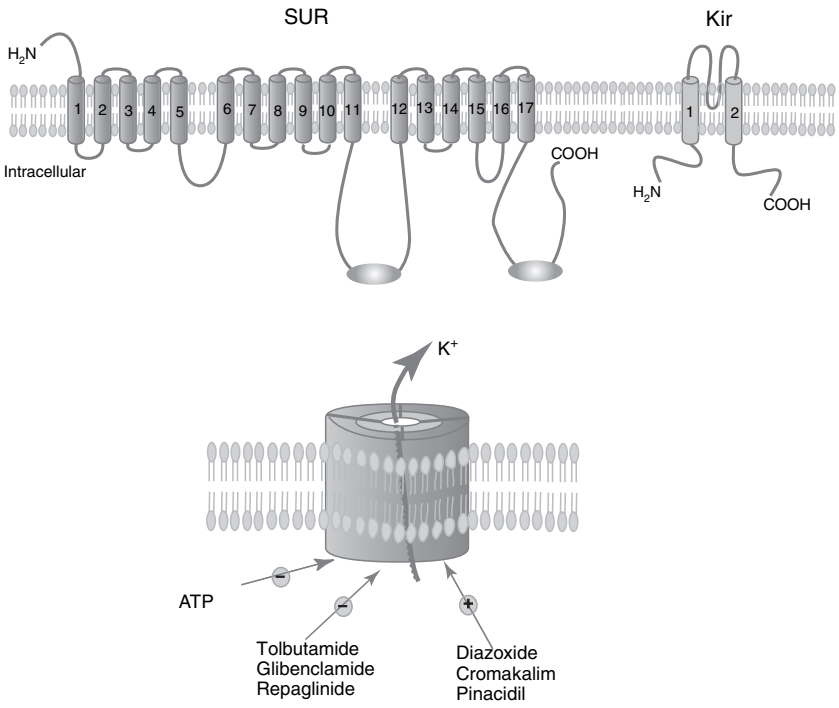

Figure 2 Structure of the ATP-dependent K^+ channels (K_{ATP}). The ion channel is formed by 4 Kir subunits surrounded by 4 SUR proteins including the ATP-binding site.

(six helices) are poorly sensitive to voltage and activated by calcium through calmoduline. BK and IK channel openers are studied in view of the prevention and treatment of cardiovascular disorders [23].

ATP-dependent K^+ channels (K_{ATP}) are composed of four inwardly rectifying K^+ channel subunits (K_{ir} subunits) and four regulatory sulphonylurea receptors (SUR) (*Figure 2*). These channels are inhibited by binding of intracellular ATP and by sulphonylureas. Sulfonylureas (**tolbutamide**, **glibenclamide)** and glinides (**repaglinide**) block the pancreatic β-cell K_{ATP} channels, thus inducing depolarization of the cell membrane which stimulates the opening of Ca_V channels and leads to insulin secretion. Vasodilators such as **cromakalim**, **pinacidil** and **diazoxide** are openers, directly activating K_{ATP} channels. The associated membrane hyperpolarization closes Ca_V channels, leading to a reduction of intracellular Ca^{2+}.

Two-pore tandem K^+ channels (*Figure 1*) are responsible for the background K^+ conductance in the cell at rest. Fifteen mammalian genes encode these channels including TASK1-3 (involved in chemoreception in respiratory motor neurones), TREK1-2 (expressed in neurones involved in thermoregulation), TWIK1-2 and others. They are controlled by several stimuli such as oxygen tension, pH, or mechanical stretch. Their druggability is not fully explored.

Non-selective cation channels

These channels have six transmembrane helices and are considered non-selective for Na^+, K^+ and Ca^{2+}, although their opening often corresponds to membrane depolarization with Na^+ and Ca^{2+} influx.

Hyperpolarization-activated cyclic nucleotide-gated cation channels (HCN channels) are present in the heart (HCN-1, -2 and -4) and brain (HCN-1 to -4). They are activated by hyperpolarization with potentiation induced by direct binding of cAMP to their intracellular C-terminus. The opening of HCN channels of pacemaker cells increases cardiac rate (I_f current) due to cAMP generated through the activation of β-adrenergic receptors (see above the inverse effect of K_{Ach} and K_M channels). The I_f blocker ivabradine has recently been approved to slow heart rate in angina [24].

Cyclic nucleotide-gated ion channels (CNG channels) are activated by the binding of cGMP. They mediate sensory signal transduction in photoreceptors and olfactory cells. Some human visual disorders are caused by mutations in retinal rod or cone CNG genes [25].

Transient receptor potential ion channels (TRP channels) are poorly voltage-sensitive. The 28 mammalian TRP channels include six main subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), and TRPA (ankyrin). TRP channels are expressed in almost every tissue and

cell type and play an important role in the regulation of vascular tone, thermosensation, irritant stimuli sensing and flow sensing in the kidney [26]. Recent data concerning TRP vanilloid (TRPV) type 6, TRP melastatin (TRPM) type 1 and eight channels indicate their relevance for common human cancer types [27]. Numerous ligands of TRP channels have been recently proposed, such as analogues of **capsaicin** for TRPV with a view to developing new peripheral analgesics [28].

DIRECT LIGAND-GATED ION CHANNELS (RECEPTORS WITH INTRINSIC ION CHANNEL)

Direct ligand-gated ion channels are homomultimeric or heteromeric proteins that span the cell membrane and include both a binding site for neurotransmitters and an ion-conducting pore (*Figure 3*). These receptors control the fastest synaptic events in the nervous system by increasing transient permeabilities.

Excitatory neurotransmitters such as acetylcholine and glutamate, induce an opening of cation channels. These channels are relatively unselective for cations, but this results in a net Na^+ inward current, which depolarizes the cell and increases the generation of action potentials occuring in milliseconds.

Inhibitory neurotransmitters such as γ-aminobutyric acid (GABA) and glycine, decrease the firing of the action potential by opening of anionic channels which results in an inwards flux of Cl^- with slight hyperpolarization. This relatively small group of drug targets is especially important because, besides agonists and competitive antagonists, both positive and negative allosteric effectors have been developed with therapeutic relevancies. Moreover, each type of these ion channels with receptor property exists in multiple molecular forms, offering large opportunities for selective ligands. They are divided into three main families according to the number of transmembrane helices present in each subunit (*Figure 3*).

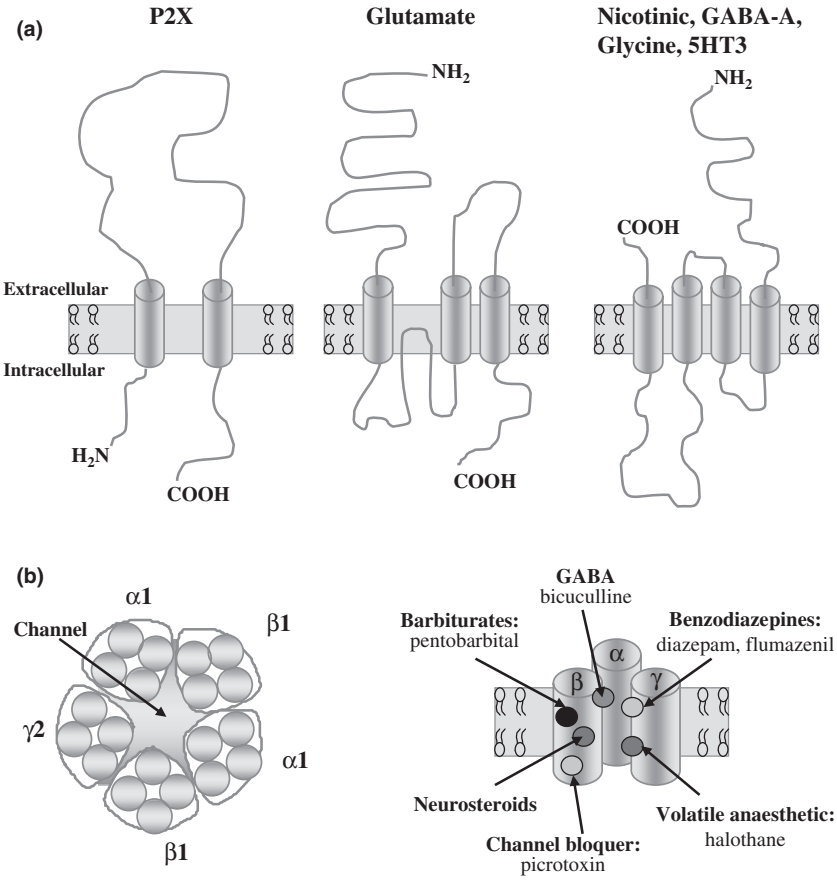

Figure 3 Structure of ligand-gated ion channels. (a) Alpha subunits of the three families of ligand-gated ion channels. (b) Proposed topography of the γ-aminobutyric acid receptor (GABA-A). Left, a cross-section in the plane of the membrane. Right, putative binding sites for allosteric ligands.

P2X-ATP receptors

P2X receptors for ATP are formed from the homotrimeric or heterotrimeric assembly of seven different receptor subunits (P2X1–7) to give a range of phenotypes. P2X are present in most cells like neurones and smooth muscle cells, and are putative drug targets [29]. Other purinergic receptors (ADP-P2Y receptors and adenosine-A receptors) belong to the GPCR superfamily.

Glutamate-activated receptors

These cationic channels (also referred to as 'ionotropic receptors' in contrast to 'metabotropic receptors' which are GPCR activated by glutamate), include *N*-methyl-D-aspartate receptors (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic-acid receptors (AMPAR) and kainate receptors. They are widely expressed in the central nervous system where they play key roles in excitatory synaptic transmission, neuronal plasticity and long-term potentiation involved in memory processes.

N-methyl-D-aspartate receptors are tetrameric complexes incorporating different subunits within a repertoire of three subtypes: NR1, NR2 and NR3. There are eight different NR1 subunits generated by alternative splicing from a single gene, four different NR2 subunits (A, B, C and D) and two NR3 subunits (A and B). Several approved drugs are non-competitive antagonists (channel blockers) of NMDAR: **phencyclidine** and **ketamin** (general anaesthetics), **amantadine** (Parkinson) and **memantine** (Alzheimer). Given the growing body of evidence that diverse brain disorders implicate different NMDAR subtypes, such as NR2B in pain or NR3A in white matter injury, there is a growing interest for the development of novel NMDAR subtype-selective compounds [30].

AMPAR and kainate receptors are also multimeric. In recent years several classes of AMPA receptor potentiators have been reported including **piracetam**, **aniracetam** and **cyclothiazide**. Clinical and preclinical data have suggested that positive modulation of AMPAR may be therapeutically effective in the treatment of cognitive deficits [31].

The 'Cys-loop receptor superfamily'

This family is called so because of a conserved cysteine loop in their extracellular domain. These allosteric proteins are heteropentameric, each subunit made up of an extracellular amino-terminal domain and four transmembrane segments. This superfamily includes the following receptors.

Nicotinic acetylcholine receptors, nAchR (cationic), transduce acetylcholine effects beside acetylcholine muscarinic receptors (M1 to M5) belonging to GPCRs. Nicotinic receptors of skeletal muscles are pentamers composed of four distinct subunits (α, β, γ or ϵ, and δ) in the stoichiometric ratio of 2 :1 :1 : 1. The neuronal type is a pentameric combination of several α- and β-subunits. Nine distinct α-subunits and four β-subunits have been cloned, making the existence of a great number of nicotinic receptors possible. But their physiological significance still needs to be completely understood. Only ligands showing selectivity between α4β2 and α7 receptors have been obtained [32]. The α4β2 partial agonists cytisine and varenicline are approved for the treatment of smoking addiction. Moreover, altenicline and ispronicline are α4β2 agonists entered in phase II for Parkinson and age-associated memory impairment treatment respectively.

Serotoninergic 5-HT3 receptors (cationic) transduce the effects of serotonin [5-hydroxy tryptamine (5-HT)], together with other 5-HT receptors which are GPCR-selective 5-HT3 antagonists, such as ondansetron and analogues, are antiemetics used during cancer therapy.

Gamma-aminobutyric acid (GABA-A) receptors, (anionic) mediate most synaptic inhibition in the central nervous system (CNS) (GABA-B receptors are GPCRs). The pentameric structure of GABA-A (*Figure 3*) is homologous with the nAChR. Binding of different ligands to the 'benzodiazepine site' can either potentiate the opening of the Cl^- channel elicited by GABA ('agonist' benzodiazepines such as **diazepam** with anxiolytic, anticonvulsivant and sedative effects), or decrease this opening (experimental inverse agonists with anxiogenic and convulsant properties), or block the benzodiazepine effect ('antagonist' benzodiazepines like flumazenil). Other allosteric sites bind **barbiturates**, **etomidate**, ***n*-octanol**, **ethanol**, **propofol**, **halothane,** and **neuroactive steroids**, with increase of GABAergic neurotransmissions.

Strychnine-sensitive glycine receptors (GlyRs) (anionic) mediate synaptic inhibition, beside GABA, in spinal cord, brainstem and other regions of the CNS. (Glycine is also a strychnine-insensitive co-agonist at NMDAR, with a binding site distinct to that of glutamate.) GlyRs regulate not only the excitability of motor and sensory neurones, but are also essential for the processing of photoreceptor signals, neuronal development and inflammatory pain sensitization. GlyRs, which are subtype-selective compounds, are expected to emerge that will allow dissection of specific GlyR isoform functions and druggability [33].

RECEPTORS WITH INTRINSIC ENZYME ACTIVITY

These membrane receptors are glycoproteins spanning the membrane only once with an intrinsic enzymatic activity (guanylate cyclase, serine/threonine kinase or tyrosine kinase), are located intracellularly, and are activated following the extracellular agonist–receptor interaction. Their dimerization is usually considered in their active state. Drug targeting concerns both the agonist binding site and the enzyme entity.

Receptors with guanylate cyclase activity

The cyclase catalytic domain converts GTP to cGMP. These membrane receptors mediate the action of the atrial natriuretic peptide (ANP) and its structural analogues, i.e. the other natriopeptides BNP and CNP, guanyline peptides and the heat-stable enterotoxin of *Escherichia coli.* (A second family of guanylate cyclases, activated by NO, is found in the cytosol.) This small, putative, drug target family has not yet received enough attention as far as progress in the therapy of cardiovascular, renal and intestinal diseases is concerned. Currently, the only natriuretic peptide available commercially to treat congestive heart failure is BNP, i.e. **nesiritide** [34].

Receptors with serine/threonine kinase activity

Over 35 distinct transforming growth factor (TGF)-β members have been identified in the human genome, including TGF-βs, growth differentiation factors, bone morphogenetic proteins (BMP), activins, inhibins, and glial cell line-derived neurotrophic factor. All members of this family have profound effects on developmental processes ranging from the development of soft tissues, including angiogenesis, to the development of the skeleton.

These mediators exert their effect by binding to specific serine/threonine kinase type I and type II receptor complexes. Seven type I receptors, also termed activin receptor-like kinase (ALK) 1 to 7, and five type II receptors have been identified. TGF-β has high affinity for the TGF-β type II receptor (TβRII), and on binding a specific TGF-β type I receptor (TβRI) is recruited and transphosphorylated. This results in the phosphorylation of Smad proteins which translocate to the nucleus and modulate gene expression.

Active studies concerning this family are going on for finding drug targets in the fields of cancer, angiogenesis and bone therapy. **Dibotermin-alpha** (rhBMP2), a recombinant form of BMP, is accepted for the local treatment of acute tibial fractures in adults, as an adjunct to standard care using open fracture reduction and intramedullary nail fixation. Three platforms of TGF-β inhibitors have recently evolved: antisense oligonucleotides, monoclonal antibodies and small molecules. Some of them are in phase I or II trials [35].

Receptors with tyrosine kinase activity

These receptors mediate the actions of many growth factors such as insulin, insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), nerve growth factor (NGF), platelet-derived growth factor (PDGF), and the stem cell factor (cKit ligand). Their cytoplasmic domain includes the tyrosine kinase activity as well as tyrosine sites of autophosphorylation. While most of these receptors possess a single polypeptide chain, the IGF and insulin receptors have two chains, α and β, arising from a single gene, linked by disulphide bonds. In this case, the α-chains possess the ligand-binding site and the β-chains, the tyrosine kinase activity.

The tyrosine kinase domain seems to be similar among these receptors while the ligand-binding domain shows very little sequence homology between the members of this family. The autophosphorylation on tyrosine residues allows the association of various proteins characterized by SH2 domains: phospholipase C-γ (PLC-γ), and adaptor proteins such as Grb2. These first events initiate cascades of reactions including small G-proteins, PI3 kinases, cytosolic tyrosine kinases and mitogen-activated protein kinases (MAP kinases). MAP kinases phosphorylate one or more transcription factors that initiate gene expression, resulting in a variety of cellular responses, including cell division and proliferation.

This large family of drug targets has received much attention in the last decade. The mediators themselves are of therapeutical interest, like **insulin**, and more recently, **becaplermin**, a recombinant form of PDGF approved to treat ulcers of the foot, ankle or leg in patients with diabetes.

Several inhibitors of their tyrosine kinase activity (non-selective from one receptor to the other) are approved for cancer therapy (**imatinib**, **erlotinib**, **sorafenib**, **sunitinib**) and many others are clinically studied (**dasatinib**, **nilotinib**, **pazopanib**, **vatalanib**, **vandetanib**, etc.). Another strategy is the therapeutic use of, either monoclonal antibodies directed against the receptors with blocking, antagonist-like effect, approved as anticancer drugs (**trastuzumab** and **cetuximab**), anti-EGFR

(HER2), or of monoclonal antibodies directed against the mediator itself (**bevacizumab**, anti-VEGF). Another antagonist-like effect is that of **pegaptanib**, a pegylated modified oligonucleotide which directly interacts with VEGF, preventing its binding to VEGFR (approved for the treatment of neovascular, wet, age-related macular degeneration). These families of drugs related to tyrosine kinase receptors are still growing.

RECEPTORS COUPLED TO VARIOUS CYTOSOLIC PROTEINS

These membrane receptors are glycoproteins spanning the membrane only once, but often occuring as homodimers or heterodimers, and coupled to cytosolic proteins (enzymes or transcription factors), either directly, or via various adaptor proteins.

Receptors coupled to the cytosolic tyrosine kinase JAK

These JAK/STAT-coupled receptors (*Figure 4*) concern the effects of cytokines: the growth hormone somatotropin (GH), erythropoietin (EPO), prolactin (PRL), granulocyte-colony-stimulating factor (G-CSF), granulocyte and macrophage-colony-stimulating factor (GM-CSF), interferon-α, -β and -γ, leptin, thrombopoietin, and interleukin-2, -3, -4, -5, -6, -7, -9, -10 and -15.

These receptors do not have intrinsic kinase activity but associate, when activated by ligand binding, with JAK tyrosine kinases (JAK1 to JAK5 and TYK2). JAKs constitute a family of the very large superfamily of cytosolic tyrosine kinase proteins. Activated JAKs phosphorylate the cytoplasmic domain of the receptor, thereby creating recruitment sites for latent cytoplasmic transcription factors, signal transducers and activators of transcription (STATs).

The recombinant forms of some of these cytokines are marketed: GH, EPO, interferons, **aldesleukine** (interleukin-2), **filgrastime** (G-CSF) and **molgramostime** (GM-CSF). **Pegvisomant** is an analogue of human GH with antagonist properties. Overproduction of GH leads to abnormally high levels of insulin-like growth factors (IGF-I), which then cause acromegaly-like symptoms. Thus, pegvisomant is approved for treatment of acromegaly. Moreover, the JAK/STAT pathway represents an excellent opportunity for targeted cancer therapy and active research concerns its pharmacological control at the intracellular level [36,37].

Receptors coupled to the cytosolic Src, Zap70/Syk and Btk tyrosine kinases (immunoreceptors)

These receptors are heteromultimeric including antigen receptors of T lymphocytes, TCR, B lymphocytes, BCR, and natural killer (NK) cells NCR, and receptors for the Fc portion of immunoglobulins located on various haemopoietic cell types, FcεRI (IgE receptor), FcαR (IgA receptor), FcγR (IgG receptor), FcδR (IgD receptor) and FcμR (IgM receptors). CD20, present on lympho-

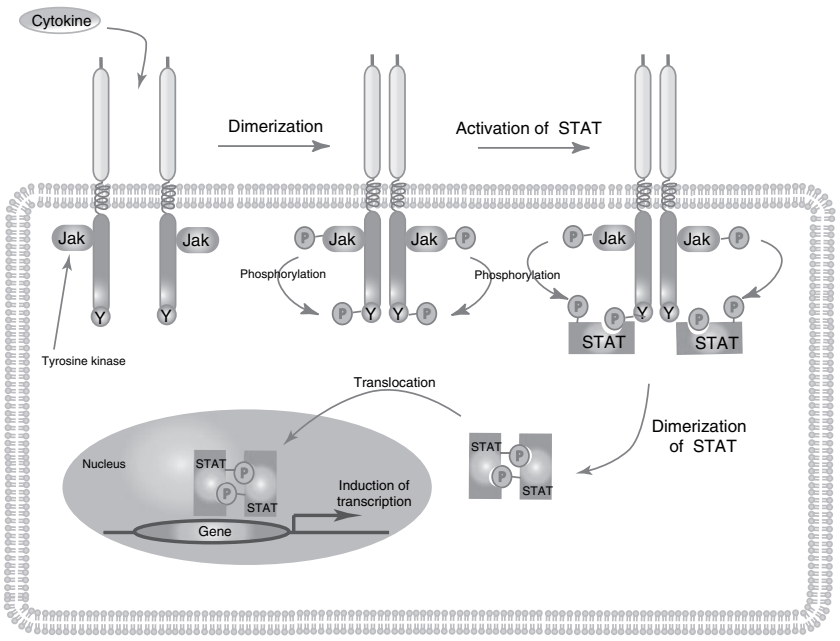

Figure 4 Receptors coupled to the cytosolic tyrosine kinase JAK.

cytes B, is also coupled to Src, but its ligand remains unknown.

Immunoreceptors bear immunoreceptor tyrosine-based activation motifs (ITAMs) in their intracellular domain. ITAMs initiate cellular activation by modulating three families of tyrosine kinases: Src, ZapP70/Syk and Btk. This signal predicates all subsequent outcomes of cell activation, including PI3 kinase and MAP kinase activation, leading to the activation of transcription factors, and to secretion of mediators (histamine, cytokines, arachidonic acid derivatives) involved in inflammatory, allergic and other immunological processes.

This knowledge has not yet led to significant therapeutic advance in the respective fields. **Omalizumab** is a recombinant DNA-derived humanized IgG1k monoclonal antibody that selectively binds to human IgE, used mainly in allergy-related asthma therapy, with the purpose of reducing allergic hypersensitivity. **Rituximab** and **ibritumomab** are anti-CD20 monoclonal antibodies. Rituximab is considered as the single first-line therapy for patients with follicular lymphoma, and ibritumomab for various non-Hodkin's lymphoma patients. Other membrane receptors, SLAM, SAP and CD31, are also involved in cytokine secretion during the development of innate and adaptive immune responses. The increasing number of studies on these new receptors might lead us to consider them as potential focal targets for novel therapeutic approaches [38].

Receptors coupled to the cytosolic serine/threonine kinase, IRAK

This receptor family mediates the effects of interleukin-1, a major pro-inflammatory cytokine, and interleukin-18, and includes toll-like receptors (TLR) of macrophages like TLR2 which recognize peptigoglycans of Gram-positive bacteria. These receptors are coupled to the cytosolic serine/threonine kinase interleukin receptor-associated kinase (IRAK). Downstream signals include the activation of transcription factors like nuclear factor (NF)κB, or others via MAP kinases.

Interestingly, an endogenous structural analogue of interleukin-1, IRAP, plays the role of antagonist of interleukin 1. **Anakinra** is a recombinant nonglycosylated form of IRAP indicated for the reduction in signs and symptoms of moderately to severely acute rheumatoid arthritis. Another approach is the inhibition of the interleukin-1-converting enzyme (ICE) which converts pro-interleukin-1 into its mature, proinflammatory form, and the inhibition of the p38-MAP kinase which controls interleukin-1 and TNFα production [39,40].

Receptors coupled to caspases and to NFκB

These receptors mediate the effects of TNFα (TNF receptors), and of the RANK ligand, RANK-L or receptor activator of NFκB, and its endogenous antagonist, OPG (RANK receptors). Tumour necrosis factor-α is the founding member of 19 different proteins identified within this cytokine family, including the Fas ligand and the TNF apoptosis ligand (TRAIL). TNF family members exert their biological effects through the TNF receptors (TNFR1, TNFR2 and Fas) that share a stretch of 80 amino acids within their cytoplasmic region, the death domain (DD), critical for recruiting the death machinery. This includes the p38-MAP kinase and the transcription factor NFκB controlling inflammation processes, and the caspase cascades which convey activation and an apoptotic signal in a proteolytic pathway that degrades cellular proteins leading to cell death. **Tasonermin** is a recombinant form of human TNFα-1a approved for cancer therapy. **Infliximab** and **adalizumab** are anti-TNFα monoclonal antibodies with immunosuppressive effects indicated in rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis and Crohn's disease. **Etanercept** is a recombinant fusion protein acting as an antagonist of TNF receptors, also approved for its immunosuppressive effects.

Another approach is the inhibition of the TNF-converting enzyme (TACE), which converts pro-TNF into its mature, proinflammatory form [39,40]. Moreover, numerous inhibitors of p38 and Erk MAP kinases have been synthesized and some have reached the clinical trial stage. MAP kinase p38 occupies a central role in the signalling network responsible for the upregulation of proinflammatory cytokines like interleukin-1 and TNFα [41]. The pathway of Erk MAP kinase is often upregulated in human tumours and as such represents an attractive target for the development of anticancer drugs [42].

Diverse drug targets in this field are at an early stage of development, such as endogenous 'inhibitors of apoptosis proteins' (IAP), a family of caspase inhibitors that selectively bind and inhibit caspases-3, -7, and -9. The inhibition of these IAP might stimulate apoptosis of cancer cells with potential for treatment of malignancy [43].

RANK-L, the endogenous agonist of RANK receptors, and OPG, their endogenous antagonist, directly regulate osteolysis and osteoclast differentiation. RANK-L is a powerful inducer of bone resorption and OPG acts as a strong inhibitor of osteoclastic differentiation. RANK-L also induces bone morphogenetic protein (BMP-2)

expression in chondrocytes. Furthermore, recent data demonstrate that the OPG–RANK–RANK-L system modulates cancer cell migration. RANK-L promotes the activation of several intracellular signalling pathways, including stimulation of MAP kinase/NFκB pathways, and the Akt/protein kinase B pathway. **Denosumab**, studied in phase III, is a humanized monoclonal antibody directed against RANK-L with anti-osteoclast activity, resulting in inhibition of osteoclast activity, a decrease in bone resorption, and an increase in bone mineral density. Denosumab also decreases bone turnover in advanced cancer [44].

Receptors of cellular adhesion

Cellular adhesion is performed by a large set of transmembrane proteins: integrins, cadherins, selectins and Ig superfamily members (Ig-N-Cam, ICAM, etc.). Integrins are heterodimer transmembrane receptors of the extracellular matrix composed of α- and β-subunits (24 known integrin heterodimers). Natural integrin ligands include laminin, fibronectin, vitronectin, fibrinogen and fibrin, thrombospondin and fibroblast growth factor. **Natalizumab** is an anti-α_4 integrin (VLA4) monoclonal antibody targeting immune cells and approved for the treatment of multiple sclerosis. Abciximab is an anti-$\alpha_{IIb}\beta_3$ integrin (also termed anti-IIb/IIIa glycoprotein) monoclonal antibody. Its binding to the receptor prevents fibrinogen, von Willebrand factor, vitronectin, and other adhesive molecules from binding to the receptor, thereby inhibiting platelet aggregation. It is indicated as an adjunct to aspirin and heparin for the prevention of acute cardiac ischaemic complications. **Eptifibatide**, a synthetic heptapeptide, and **tirofiban**, a piperidinyl derivative of tyrosine, also bind to the $\alpha_{IIb}\beta_3$ integrin with antagonist effect inhibiting platelet aggregation.

Integrin $\alpha_V\beta_3$, the vitronectin receptor, has been identified as a potential target for the treatment of osteoporosis, diabetic retinopathy and cancer. Several classes of integrin antagonists are currently in preclinical and clinical stages of development: monoclonal antibodies targeting the extracellular domain of the heterodimer, **vitaxin**, **cilengitide** and several peptidomimetics [45].

G-PROTEIN-COUPLED RECEPTORS

G-protein-coupled receptors are the largest class of receptors mediating the effects of small neurotransmitters, all known neuropeptides, many peptide hormones and inflammatory mediators, some lipids and even calcium for the control of its blood concentration.

G-protein-coupled receptors are formed by a single polypeptide chain of 350–1200 residues, hydropathy plots revealing seven hydrophobic regions which are likely to correspond to transmembrane α-helices (*Figure* 5). Thus GPCRs are also termed 7-TM receptors or heptahelical receptors. The amino-terminal extracellular domain contains potential N-linked glycosylation sites in most receptors that might be involved in ligand affinity [46]. The carboxy-terminal cytoplasmic end is involved in the coupling to G-proteins.

Almost 30% of all marketed drugs act on GPCRs. The most familiar GPCRs as historical drug targets are the muscarinic acetylcholine receptors, the alpha and beta adrenergic, dopaminergic, histaminergic and opioid receptors. Some other GPCR ligands have been developed as drugs during the last three decades, i.e. serotonin 5-HT, prostaglandins, leucotrienes, ADP or calcium receptor ligands. The actual top-selling GPCRs ligands are **clopidogrel** (ADP-P2Y12 antagonist, platelet antiaggregant), **olanzapin** (mixed serotonin-5HT2/dopamine-D2 antagonist, neuroleptic), **valsartan** (angiotensin-AT1 antagonist, antihypertensive), **fexofenadine** (histamine-H1 antagonist, antiallergic), **sumatriptan** (serotonin-5HT1D antagonist, antimigrainous), **leuprorelin** (GnRH/LH-RH peptidic agonist, antihormone-dependent cancer). Only a small proportion of known GPCRs are currently targeted by therapeutic agents. This provides a great number of promising targets for the development of new medicines.

How many druggable GPCRs?

According to recent analysis of the human genome, about 780 to more than 860 genes encode GPCRs. More than 50% of GPCRs are activated by sensory stimuli. The full repertoire of receptors for endogenous ligands is likely to include 367 members. Among the latter, about 180 GPCRs are activated by well-characterized endogenous ligands [47]. Note that one endogenous mediator may activate several GPCRs: 13 for serotonin, nine for adrenaline and noradrenaline, eight for glutamate, five for dopamine, five for acetylcholine, four for histamine, and two for GABA.

Since 1995, 60 neuropeptides activating GPCRs have been discovered (nociceptin/orphanin, orexins/hypocretins, prolactin-releasing peptide apelin, ghrelin, melanin-concentrating hormone, urotensin II, neuromedin U, metastatin, prokineticin 1/2, relaxin 3, neuropeptide B/W, neuropeptide S, relaxin 3, obestatin, etc.) [48].

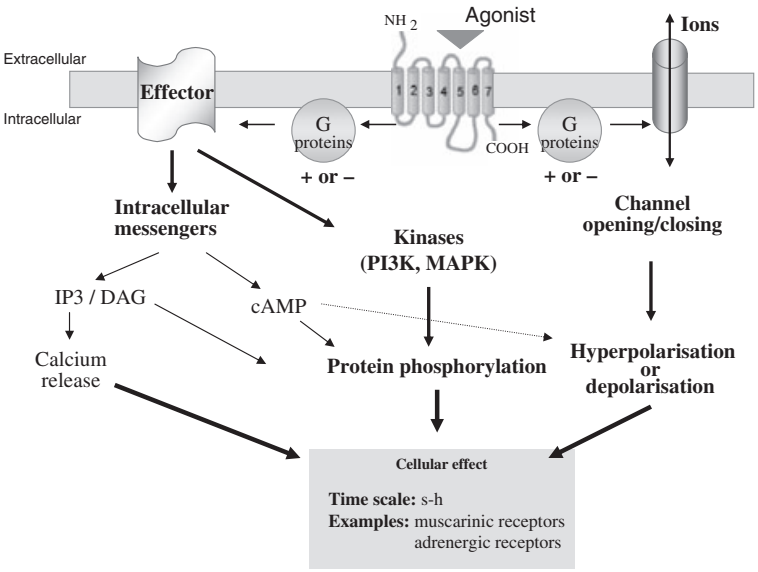

Figure 5 G-protein-coupled receptors and main signalling pathways.

Their receptors immediately became new putative drug targets. However, there are still more than 140 orphan GPCRs, and deciphering their function remains a priority for fundamental and clinical research. Research into orphan GPCRs has concentrated mainly on the identification of their natural ligands, whereas recent data suggest additional ligand-independent functions for these receptors [49]. This emerging concept is associated with the observation that orphan GPCRs can heterodimerize with GPCRs that have identified ligands, and by so doing regulate the function of the latter.

Some non-heptahelical receptors might also activate heterotrimeric G-proteins, but the relationship between these receptors and trimeric G-proteins remains controversial [47]. The pentaspanin integrin-associated protein (IAP or CD47), a receptor for thrombospondins associated with integrins, mimicks heptahelical receptors. Its coupling to Gi proteins is well demonstrated [47,50]. CD47 is considered as a valuable drug target [51,52].

Diversity of G-proteins

G-proteins are located on the inner side of the plasma membrane. They are heterotrimeric with α, βand γ subunits, in contrast to small G-proteins which are monomeric [53]. The diversity of heterotrimeric G-proteins has been demonstrated, around 1980, with the purification of Gs (s = stimulatory for adenylate cyclase), Gi (i = inhibitory for adenylate cyclase), and Gt (t = transducine), which activates a cGMP phosphodiesterase in retinal cells. The number of subunit variants identified includes 27 Gα (39 to 52 kDa in size), 5 Gβ (36 kDa) and 14 Gγ subunits (7–8 kDa). This leads to a high diversity of heterotrimers, questioning the selectivity of receptor–G-protein interactions. The usual classification of G-proteins remains based on their α-subunits with four families (Gs, Gi, Gq and G12), each α-subunit modulating selective effectors [53]. Numerous chemicals are able to bind purified G-proteins, suggesting that G-proteins may be considered as putative drug targets [54].

Diversity of GPCR-elicited signalling and related drug targets

Effector enzymes have multiple subtypes that differ in tissue distribution. Thus, targeting such molecules may lead to organ-specific pharmacological regulation. However, most GPCR-elicited pathways (PLCs, PI3 kinases, small G-proteins and MAP kinases) are also actors in signalling of other receptor families, decreasing their druggability.

Adenylate cyclases are intrinsic proteins of the plasma membrane transforming ATP to cAMP. Adenylate, or adenylyl, cyclases are activated through stimulation of Gs-coupled receptors, and some of them are inhibited through stimulation of Gi-coupled receptors. The plant terpenoid **forskolin** stimulates cAMP formation by acting directly on the inner side of adenylate cyclases. Water-soluble forskolin derivatives with high selectivity for type 5 (cardiac) adenylate cyclase have been proposed in the treatment of acute heart failure. Adenine analogues or P-site inhibitors are utilized to develop isoform-specific inhibitors as well [55].

Cyclic AMP is broken down by phosphodiesterases (PDEs). The PDE superfamily currently includes 20 different genes subgrouped into different PDE families [56]. Subtype-specific phosphodiesterase inhibitors, such as sildenafil, a PDE5 inhibitor, and milrinone, a PDE3 inhibitor, are now widely used in the treatment of erectile dysfunction and heart failure respectively. The search for selective PDE inhibitors remains active [56].

Phospholipases C (PLCs) are cytosolic enzymes transforming the membranous lipid phosphatidylinositol-4, 5-bisphosphate to diacylglycerol, which remains a membrane component regulating protein kinases C, and to cytosolic inositol-1,4,5-triphosphate (IP_3). IP_3 binds to endoplasmic membrane receptors and liberates calcium from endoplasmic reticulum stores. PLC subtypes (four β-, two γ-, four δ-isoforms) have been characterized with multiple spliced variants. PLCβ is regulated by Gαq and by the Gβγ dimer. PLCγ has a SH2 domain allowing its interaction with phosphorylated tyrosine residues. The interest of PLCs as drug targets is poorly considered.

Phosphoinositide 3 kinases (PI3Ks) are a large family of intracellular signal transducers that have attracted much attention over the past 10 years. PI3Ks phosphorylate inositol lipids at the 3′-position of the inositol ring to generate the 3-phosphoinositide PI(3,4,5)P3 (PIP3). PI3Ks of class 1B are directly activated by Gβγ. Other PI3Ks also belong to pathways of receptors with tyrosine kinase activity, or coupled to cytosolic tyrosine kinases. PIP3 recruits to the membrane various protein kinases like protein kinase B (PKB or Akt), PLCγ and cytosolic tyrosine kinases such as Btk.

The main involvement of PI3K may be in the control of cell development and differentiation. The central role of PI3K signalling in allowing cancer cells to bypass normal growth-limiting controls has led to the development of PI3K inhibitors [57]. PI3K is also involved in the pathogenesis of other diseases including heart failure, autoimmune and inflammatory disorders. The tissue selectivity of PI3K isoforms has to be closely considered in the development of PI3K inhibitors. For instance, inhibitors of PI3K pathways, including tyrosine kinase inhibitors, used to treat cancer, may induce cardiopathies [58]. Interestingly, an inhibitor of the mammalian target of **rapamycin** (m-TOR), a downstream effector of PI3K, did not have adverse effects on the heart [59], showing possible alternative targeting downstream effectors. Conversely, isoform-selective PI3K inhibitors are now proposed as novel therapeutic agents for the treatment of acute MI [60]. Interestingly, the polyphenol **resveratrol**, which has chemopreventive and chemotherapeutic properties thought to be related to histone deacetylase HDAC3 (sirtuin) inhibition [61], also inhibits PI3K [62]. This points out the difficulties in correlating the therapeutic properties of drugs to their known targets and the complementarity of new research strategies associating molecular and integrative processes.

NUCLEAR RECEPTORS (LIGAND-GATED TRANSCRIPTION FACTORS)

These receptors modulate gene expression, acting as homodimers or heterodimers (*Figure 6*). Their ligands are lipophilic molecules that bypass the plasma membrane to reach their intracellular targets. Nuclear receptors are subdivided into three classes. The steroid receptor family, and the thyroid/retinoid family include targets of largely developed drugs. The orphan receptor family includes half the members of the nuclear receptor superfamily. Their druggability has recently attracted much attention.

The steroid receptor family

This well-known family includes androgen receptors (AR), oestrogen receptors (ER, α and β), progesterone receptors (PR, A and B), glucocorticoid receptors (GR), and mineralocorticoid receptors (MR) [5]. Previously used models propose that, upon binding their hormonal ligand, the receptors release heat shock proteins like hsp90, translocate into the nucleus, and bind as homodimers to upstream promoter sites. Their ligands are well known and widely used. The more recent

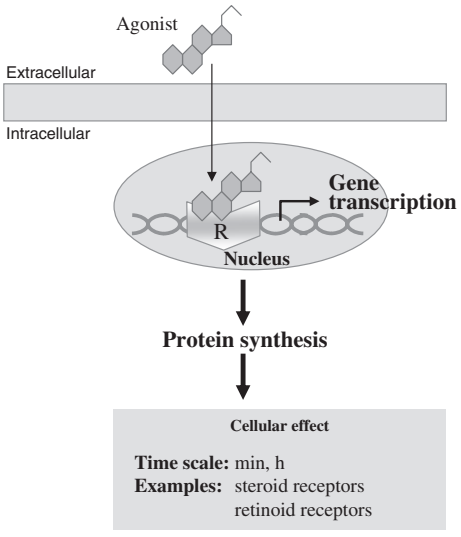

Figure 6 Nuclear receptors (ligand-gated transcription factors).

advances in this field concern the so-called selective ER modulators (SERMs), like tamoxifen and raloxifene. SERMs are used for the prevention and treatment of diseases such as osteoporosis and breast cancer. Ideally, it is presumed that SERMs should selectively act as an agonist in the bone and brain while simultaneously acting as an antagonist in the breast and uterus [63].

The thyroid/retinoid receptor family

This family includes the thyroid receptors (TR α and β), vitamin D receptors (VDR), retinoic acid receptors (RAR α, β and γ), retinoic X receptors (RXR, α, β and γ) and peroxisome proliferator-activated receptors (PPAR, α, β/δ and γ). These receptors typically function as heterodimers, often including RXR, which tend to stay bound to their response elements regardless of whether agonist ligands are present. In the absence of ligand, gene activation is prevented by corepressor interactions with the DNA-bound heterodimer. Upon binding ligand, corepressor proteins are released and coactivators are recruited, leading to transcriptional activation.

Thyroid receptors, VDR and RAR are targets for current medicines. PPARs regulate the expression of genes involved in the control of lipid and lipoprotein metabolism, glucose homeostasis and inflammatory processes. Their wide range of potential therapeutic actions make them attractive targets for the development of oral agents targeting risk factors associated with the metabolic syndrome, type 2 diabetes and cardiovascular diseases. PPARα agonists belong to the fibrate class (**clofibrate**, **fenofibrate**, **bezafibrate**, **ciprofibrate**, **gemfibrozil**), widely prescribed to reduce triglycerides while increasing plasma HDL-cholesterol. PPARγ agonists (**pioglitazone**, **rosiglitazone**) have beneficial effects on glucose homeostasis by increasing insulin sensitivity and glucose disposal, but are under critical discussion due to increased cardiovascular risk [64]. To date, no PPARβ/δ agonist has been fully developed and the clinical potential of targeting this isotype remains to be clearly determined.

PPAR agonists remain interesting drugs but they display side effects which limit their therapeutic use. Current strategies aim at reducing side effects by identifying selective PPAR modulators (SPPARMs) and the optimization of the selectivity ratio between the different PPAR isoforms [65].

The orphan receptor family

This family gathers nuclear receptor which endogenous ligand, if any, is unknown, but most of them belong to the steroid or the thyroid/retinoid family. This includes half the members of the nuclear receptor superfamily, a large reserve of drug targets. Some examples of these nuclear orphan, or deorphanized, receptors are RXR, ROR, LXR, FXR and ERR.

Retinoic X receptors (RXR, α, β and γ), also termed rexinoid receptors, belong to the thyroid/retinoid family. RXR is an obligatory partner dimerizing with other thyroid/retinoid receptors [66]. RXR selectively bind the 9-*cis* isomer of retinoic acid, whereas RAR bind all-*trans* retinoic acid as well as its 9-*cis* isomer. RXR-selective agonists are termed rexinoids, like **bexarotene** used as an antineoplastic in the treatment of cutaneous T-cell lymphoma and the cutaneous lesions of T-cell lymphomas and Kaposi's sarcoma.

Retinoic acid receptor-related orphan receptor α (RORα) has been recently deorphanized, cholesterol being identified as its ligand. RORα is expressed in many tissues and is therefore a regulator of multiple biological processes. A beneficial modulatory role of RORα is proposed in the pathogenesis of dyslipidaemia, inflammation and atherosclerosis [67].

Liver X receptors (LXR α and β) are oxysterol receptors that regulate multiple target genes involved in cholesterol homeostasis. Recent studies also suggest that they may also be involved in glucose metabolism, inflammation and Alzheimer's disease. Although the prototypic LXR agonists induce liver triglyceride accumulation by regulating the hepatic lipogenesis pathway, it is hoped that a subtype-specific agonist or selective modulators would provide the desired cardioprotection without the undesirable induction of lipogenesis [68].

The *farnesoid X receptor (FXR)* is activated by the bile acids, chenodeoxycholic acid, lithocholic acid and deoxycholic acid. Upon activation, FXR heterodimerizes with RXR and regulates a cohort of genes involved in cholesterol catabolism and bile acid biosynthesis. Thus, development of potent FXR agonists might represent a new approach for the treatment of cholestastic disorders [69]. Interestingly, bile acids also activate TGR5, a Gs-protein-coupled receptor. Selective ligands are now available to differentiate genomic and non-genomic effects mediated by bile acids [70].

The *oestrogen-related receptors (ERR, α, β and γ)* is structurally most related to the canonical oestrogen receptor and has been shown to modulate oestrogen signalling. These observations have heightened interest in ERR as a therapeutic target in both breast and ovarian cancer and in other oestrogenopathies [71].

CONCLUSION

This review shows some of the recent advances in the knowledge of druggable targets proposed from preclinical and first clinical data. Drug research in progress follows well-established avenues, for instance the therapeutical use of recombinant proteins and of monoclonal antibodies, as well as a large set of promising, but more risky, paths. A major starting point in drug research remains the discovery of unknown mediators, including current biological products the mediator properties of which were previously unknown, based on the finding of orphan receptors (inverse pharmacology).

Novel concepts may lead to a full reappraisal of well-established regulatory processes. This is the case, for instance, of the renin–angiotensin system with the observation that angiotensin IV, previously considered as an inactive product of angiotensin II degradation, plays an active role through its own receptor AT4. Curiously, AT4 was identified as the insulin-regulated aminopeptidase (IRAP, EC 3.4.11.3), a membrane-spanning protein with an extracellular catalytic site able to hydrolyse numerous neuropeptides [72]. Angiotensin IV inhibits the peptidase activity, leading to extension of the half-life of endogenous neuropeptides, regulates GLUT4 and therefore glucose uptake, and initiates signalling pathways, including NFκB, through the intracellular domain of AT4. Thus AT4 is a putative drug target in the cardiovascular, metabolic and cognition fields (73,74). Moreover, AT4 might be the first member of a new receptor class, indicating that the classification of receptors that we propose in the present review is not restrictive. Many other new biological concepts are feeding drug research, for instance the epigenetic cell memory, a base for the development of an epigenetic drug therapy of cancer, neuro-degenerative and inflammatory diseases (61,75–77).

The future of drug research would also benefit of the discovery of the mechanism of action of some established drugs, such as acetaminophen (paracetamol) or imidazolines whose molecular targets still need to be identified. In this line, a recent evolution in the knowledge of drug targets of drugs yet to be approved concerns the false friends of GABA, gabapentin and pregabalin, which exert their therapeutic effects independently of GABA, but through their binding to $\alpha 2/\delta$ subunits of Ca_V channels [78,79].

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