Chapter 18

Homo and Heterodimer Ligands: the Twin Drug Approach

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There is in living organisms, human beings and societies a balance between these main two forces, between creative asymmetry, imagination, or revolution and cooperative symmetry gic or order; between Dionysios and Apollon

Jean-Pierre Changeux

I. INTRODUCTION

During the study of the structure-activity relationships (SARs) of a lead compound, the combination of two pharmacological entities in a single compound could be considered as a promising drug design strategy. Drugs containing two pharmacophoric groups covalently bounded are called twin drugs. Numerous terms have appeared recently in the literature such as "dual, dimeric, bivalent, hybrid, mixed or multiple" associated with the terms "ligands, inhibitors, activators, modulators or antagonists." Reviews discussing the interest of designing multiple ligands have been published during the last years.[1–10] In this chapter, we will use mainly the term of twin drug and will focus on the combination of only two (identical or non-identical) pharmacological entities (Figure 18.1). The multi-target approach (more than two pharmacophores targeted) will be discussed in another chapter (Chapter 28).

The association of two identical pharmacophoric entities will generate an "identical twin drug" which is equivalent to a homodimer derivative. A compound, where two different pharmacological entities are bounded, is called a "non-identical twin drug" or heterodimer. The first design strategy is equivalent to a duplication/dimerization process of an active compound or lead. The aim of this approach is the production of a more potent and/or more selective drug

compared to the single entity. The second strategy consists of an association of two different pharmacophores. In this case, the new compound will possess both initial pharmacological activities. This approach could be an advantage when the two targeted enzymes or receptors are involved in the same disease or disorder. The heterodimer drug will produce a synergic effect by modulating simultaneously the two biological targets.

The administration of twin drugs can be favorable compared to the two separated drugs. The new entity will have its own pharmacokinetic property (absorption, distribution and metabolism) and pharmacodynamic property. These properties will be more predictable compared to the administration of two separated drugs. This aspect represents the main advantage of designing dual acting drugs in addition to the beneficial therapeutic combination of the two active principles. The twin drug must express both activities in an appropriate balance: a stoichiometric association of diazepam (2–20 mg per day) with aspirin (200–2,000 mg per day) would be nonsense. A twin drug where the two different pharmacophores are released after its administration will be considered as a prodrug of the two different entities (Chapter 38). The linker (e.g. polymethylene, polyamine) or the covalent bond (e.g. amide, ester function) present in the twin drug should resist to the metabolic process.

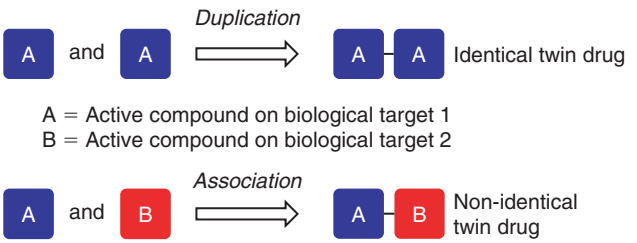


FIGURE 18.1 Identical and non-identical twin drugs.

Combination of identical or non-identical pharmacophores can be classified according to the connection modes used between the two entities. The combination could be achieved by the means a linker or not (single bond) or according to an overlap mode. The linker group can be a polymeric chain (usually a methylenic chain), an aromatic or an heteroaromatic ring and in some cases an non-aromatic cycle. Pharmacophores can be overlapped when a common structural motif (i.e. a ring or a chemical function) is identified in the two different drugs. Three examples of twin drugs are given in Figure 18.2. Duplication of aspirin led to the identical twin drug diaspirin where the connection mode is a non-linker mode. Tacrine, an acetylcholinesterase (AChE) inhibitor, was dimerized by the mean of a linker (polymethylenic chain) leading to *bis*-tacrine derivatives.[11,12] Salicyclic acid and paracetamol structure can be merged (overlap mode) to give the non-identical twin drug acetaminosalol.

Non-identical twin drugs are also named dual acting drugs or hybrids because of the different pharmacological responses targeted by the two pharmacophoric moieties. The design of dual acting drugs, called the symbiotic approach,[13] can be realized accordingly to two strategies (Figure 18.3). The first strategy combines two non-identical

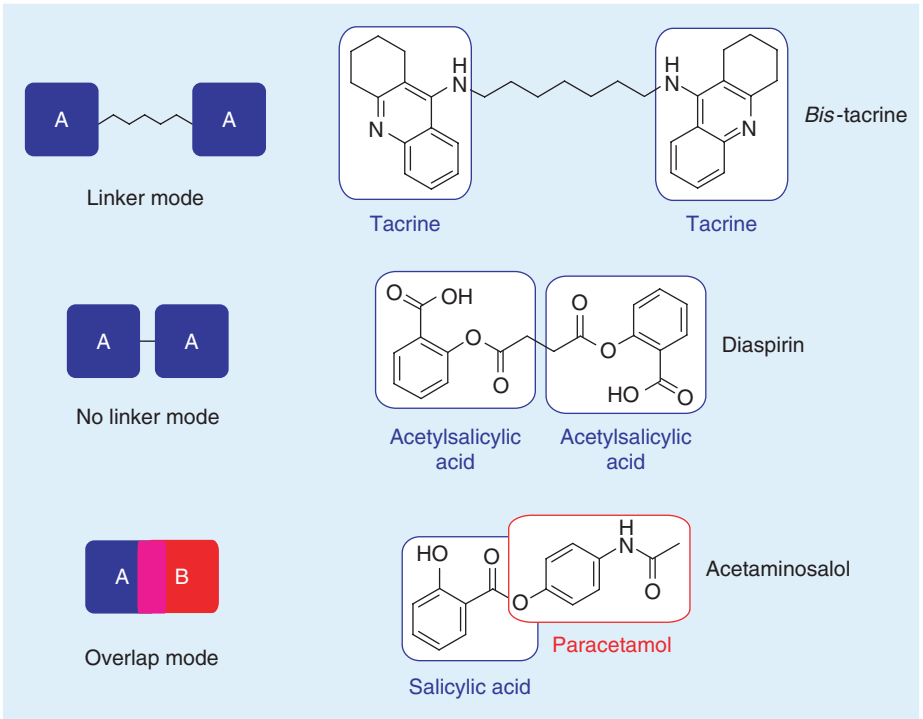


FIGURE 18.2 Combination modes for twin drugs.

selective pharmacophores into a hybrid molecule as illustrated by the sulfonamidic derivative. The associative synthesis of a chlorobenzenesulfonamide with an indole derivative through a methylenic linker generates a dual β-blocker and diuretic agent.[14] The two pharmacological entities could be easily identified in the conjugated derivative. A biphenyl motif was used to merge an angiotensin II receptor (AT_1) antagonist and an endothelin-1 receptor (ET_A) antagonist.[15] Structural elements of initial selective ligands are still recognizable in the hybrid molecule. The second strategy starts with a lead compound found to exhibit already both activities. A rational optimization will lead to an intrinsically dual acting drug such as the histamine (H_1) and platelet activating factor (PAF) antagonist benzocycloheptapyridinylene piperidine.[16] In this case, it is impossible to attribute the structural features of the molecule responsible of each biological activity. The degree of pharmacophore overlap is correlated with the molecular size of the twin drug and the structure complexity. With a low molecular weight, it would be difficult to clearly identify the structural elements necessary for both activities.

For the last 20 years drug design strategies were driven by the traditional concept: one disease–one target–one ligand approach. Identification of a biological target responsible of a disease has led to the design of potent and selective ligands or inhibitors. But in most cases, diseases involve multiple and complex systems where more than one biological target must be modulated. Some studies[17] have shown that simultaneous and moderate inhibition or activation of several targets is more efficient than the use of selective and potent drug. During the last years, combinatorial therapy (cocktail of several drugs) has been used intensively to treat diseases, such as cancer and AIDS. Thus, the development and the use of ligands that could modulate simultaneously multiple biological targets represent a promising approach for the treatment of complex disorders. This topic will be reviewed in details in Chapter 28.

In the present chapter, examples will be reported in details to illustrate the "twin drug approach" (i.e. combination of only two pharmacophores). The design of twin drugs will be classified into two differents parts (Figure 18.4).

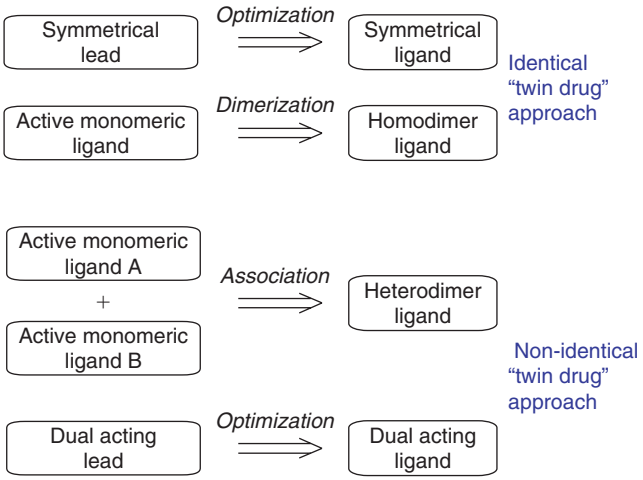


FIGURE 18.4 Twin drug design.

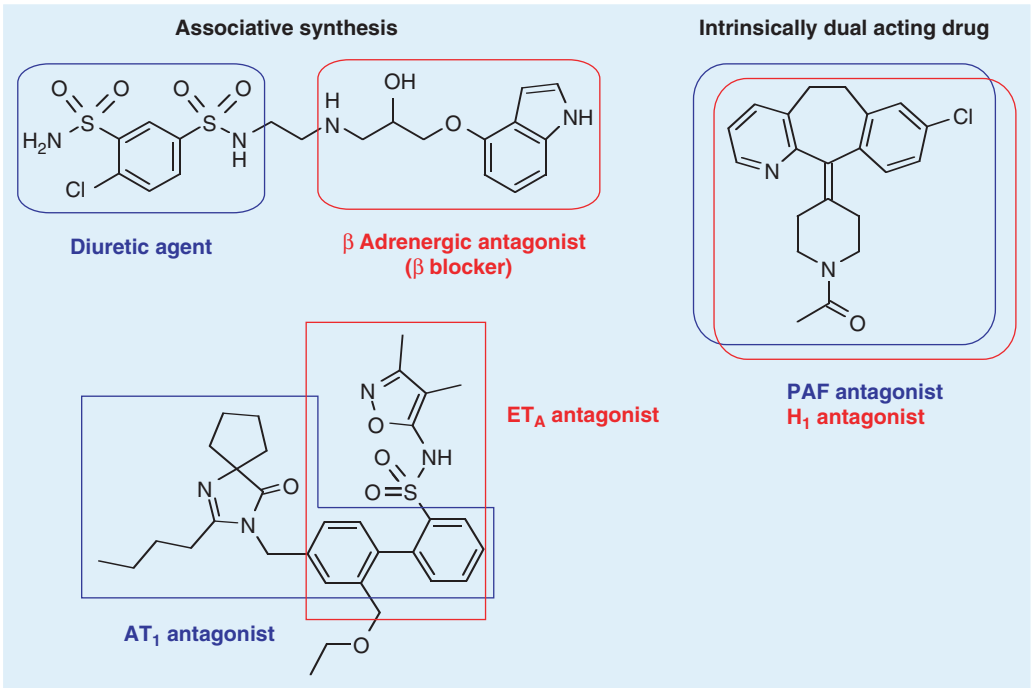


FIGURE 18.3 The symbiotic approach.

Homodimer and symmetrical ligands will be discussed in a first time. Homodimer ligands result from the dimerization of a single pharmacophoric unit whereas symmetrical drug could be obtained after an optimization process starting from an initial symmetrical active compound. The second part will focus on heterodimer ligands and dual acting drugs. Heterodimer ligands are prepared by association of two biologically active moieties for different biological targets. Dual acting drugs possess intrinsically two biological activities that could not be correlated with structural features. Such derivatives are obtained after optimization of a lead compound that possess initially both activities. The binding mode of identical and non-identical twin drugs with macromolecular structures will also be discussed in the last part. Several examples will be presented where design of twin drugs has been guided by crystallographic and molecular modeling studies.

II. HOMODIMER AND SYMMETRICAL LIGANDS

Dimerization of a biologically active molecule represents an alternative approach in the optimization process of a lead compound. Generally, the duplication of the pharmacophore leads to an equivalent or more active derivative which exhibits different selectivity profile and pharmacokinetic properties. Enzyme inhibition will be improved when using homodimer inhibitors. Antagonists and agonists of specific receptors could be transformed respectively into agonists and antagonists of another receptor subtype. Several examples will be reported and discussed below.

A. Symmetry in nature

Nature is efficient in producing compounds with a high degree of symmetry which allows to reduce information and complexity levels.[18] Natural symmetry is observed for the assembly of macromolecules (oligomers)[19] like for HIV protease, hemoglobin and insulin. The aggregation of insulin monomers to hexamers in presence of zinc affords a macromolecular complex with high degree of symmetry (C3 symmetry).[20] DNA, by means of its symmetrical double-stranded structure, determines the cell's morphology and function. These well-organized macromolecular systems constitute binding sites for smaller molecules including water and ions. Symmetrical natural compounds present generally a C2 symmetry axis (Figure 18.5) like the alkaloids lobelanine (treatment for drug addicts), sparteine (Grave's disease treatment) or isochondrodendrine, and the anticoagulant dicumarol and antispermatogenic gossypol.[21,22] Some examples of C3 symmetrical compounds are known. Valinomycin,[23] a cyclic peptide lactone antibiotic, is a highly selective K^+-carrier. It consists of a cyclic trimer containing L-valine, D-α-hydroxyisovaleric acid (Hyi), D-valine and L-lactate. The C3 symmetrical agent enterobactin is a cyclic lactonic *N*-acylated serine trimer.

B. Homodimers as receptors ligands

Identical twin drugs have shown increasing potencies and/or modified selectivity profiles as receptor ligands, when compared to their corresponding single drug. Several receptors of biogenic amines (catecholamines), quaternary ammoniums (acetylcholine, PAF-acether), and peptides (angiotensin, endothelin) belong to the well-known class of G-protein-coupled receptors (GPCR). They present one subunit with

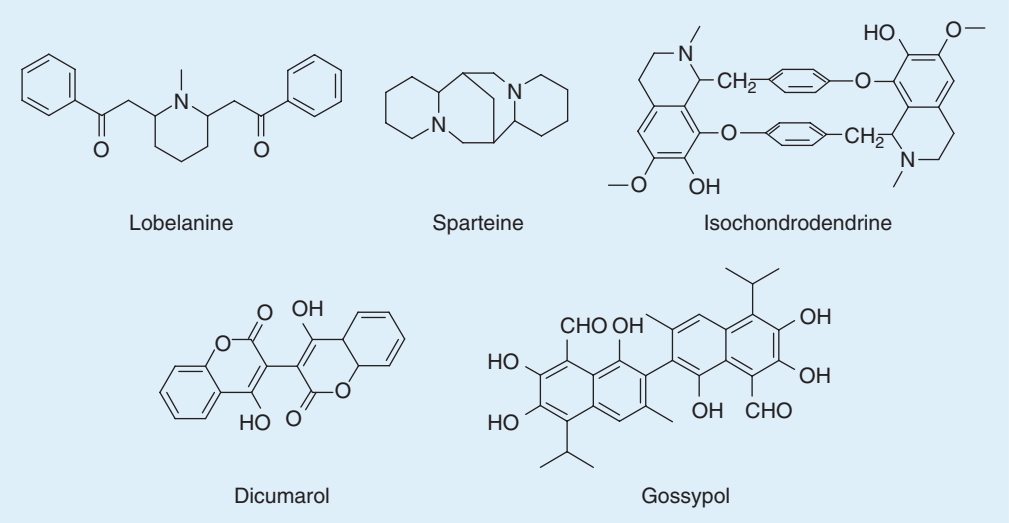


FIGURE 18.5 Natural compounds presenting a C_2 symmetry axis.

seven transmembrane spanning domains, three extracellular and three intracellular loops, which are coupled to G-proteins. Duplication of drugs within this series of ligands has been efficient in several cases.

Piperidine-based dimer ligands were prepared and evaluated[24] for their inhibiting activity for dopamine (DA) and serotonin (5-HT) transporters (Figure 18.6). Two *trans*-piperidine units were linked by a pentamethylene spacer to

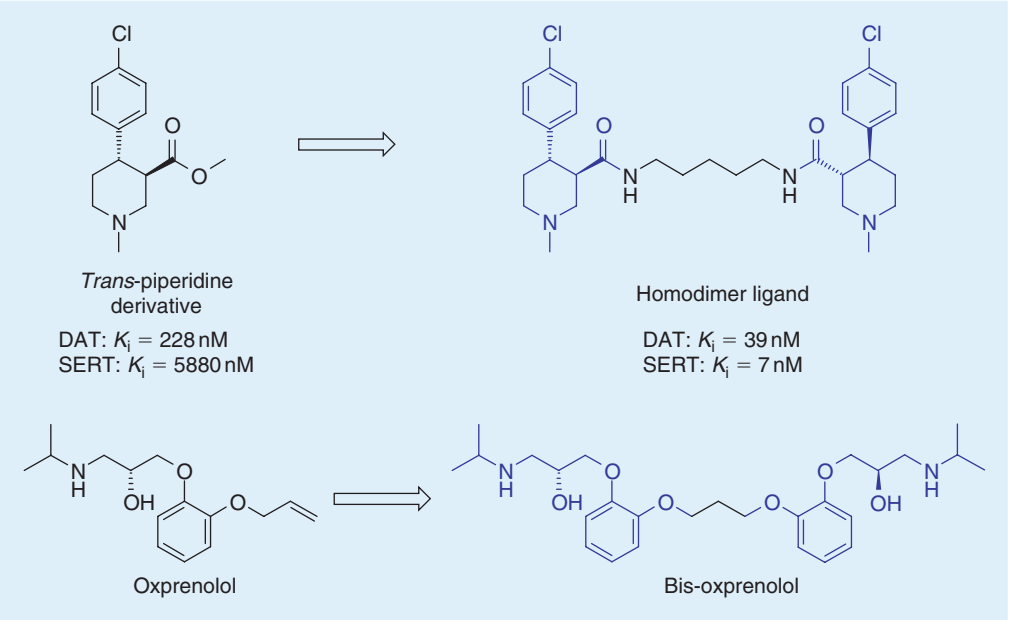


FIGURE 18.6 Duplication of monoamine receptors ligands.

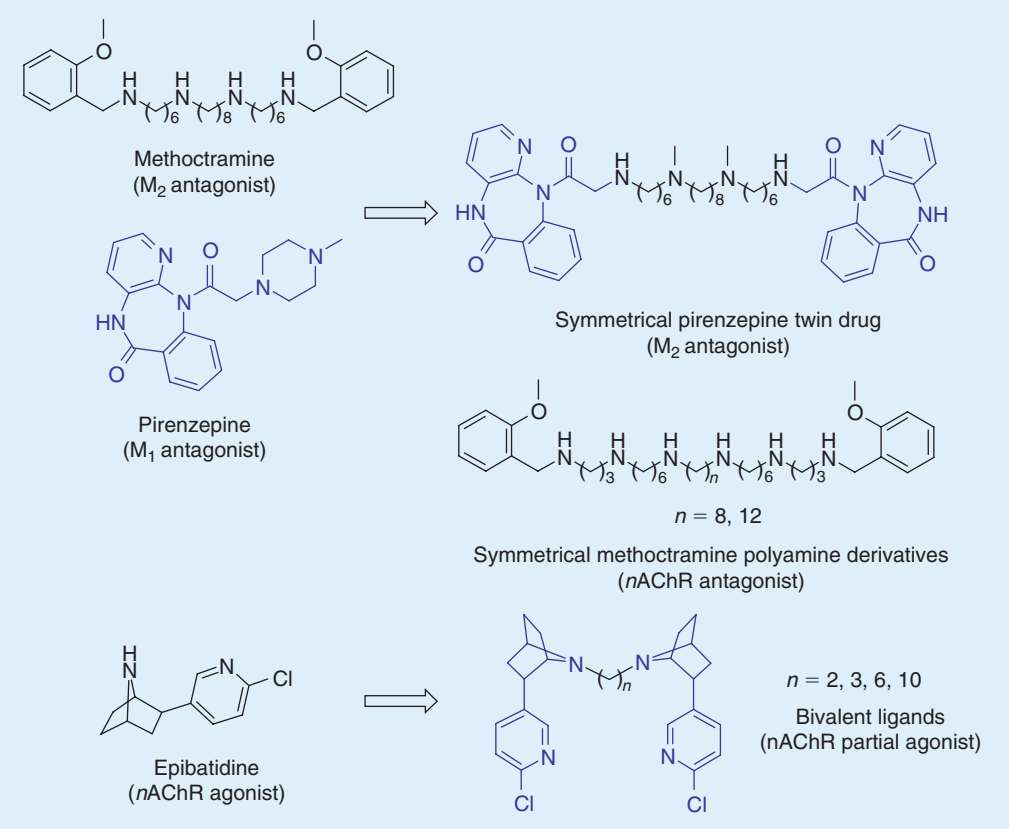


FIGURE 18.7 Ligands of acetylcholine receptor.

give a homodimer ligand. The dimerization process showed an increase of activity on both DA transporter (DAT) and 5-HT transporter (SERT). Several β_1 selective adrenoreceptor antagonists have been designed by duplication of the well-known oxprenolol.[25] The phenyloxypropanolamine pharmacophore was dimerized with a methylene linker to lead to the symmetrical *bis*-oxprenolol. According to the length of the linker, selective β_2 or β_1 antagonists could be obtained.

Methoctramine, a selective M_2 antagonist, is an useful probe for characterizing muscarinic acetylcholine receptor (mAChR) subtypes. The selectivity toward M_2 receptors was improved by modifying the terminal moiety of methoctramine.[26] The replacement of the 2-methoxybenzyl group by the hydrophobic moiety of pirenzepine leads to a very potent M2 antagonist whereas pirenzepine is known as a selective M_1 antagonist (Figure 18.7). The duplication of pirenzepine pharmacophore leads to a new antagonist with a different selectivity profile. Recently, studies[27,28] have shown that the length of the polymethylene chain of methoctramine polyamine derivatives could be important to convert muscarinic antagonist into selective nicotinic antagonist. Thus, starting from a common pattern, symmetrical twin drugs acting as antagonists at different receptors, have been designed. Epibatidine, an alkaloid known for its high affinity to nicotinic acetylcholine receptors (nAChRs) was dimerized.[29] The bivalent ligands obtained were evaluated on different nAChR subtypes and showed nanomolar binding affinities. These derivatives are weak partial agonists on a specific nAChR subtype whereas epibatidine is a full agonist.

Several studies have previously reported that dimerization of peptidergic receptor ligands can result in an increase in affinity, potency, and/or metabolic resistance.[30–32] The design of non-peptidergic antagonists of bradykinin B_2 receptor, potential therapeutic agents in the treatment of inflammation and pain, led to a symmetrical *bis*-phosphonium salt[33] (Figure 18.8). The 10 Å-distance between the two positive charges is in good agreement with that found between guanidinium cations of Arg (1) and Arg (9) of bradykinin.

In the case of enkephalines, dimerization has shown better analgetic properties when compared to their monomeric counterparts (Figure 18.9). The increase in potency and the selectivity profile depends on the length of the methylenic chain.[34] Dimerization of the oxymorphamine pharmacophore led to a bivalent ligand which showed a greater potency to opioid receptors than the monomer unit. The spacer length was important for the δ receptors selectivity.[35] In another example, the design of a bridged opioid dimer led to a κ-selective opioid receptor antagonist such as norbinaltorphimine.[36,37] In this case, the two identical morphonic units were linked by the mean of a pyrrole ring.

The potent calcium channel antagonist (blocker) nitrendipine is an effective antihypertensive agent used for the treatment of cardiovascular diseases. The design of symmetrical *bis*-1,4-dihydropyridine derivatives (BDHP) (Figure 18.10) showed an increase of the binding activity of about 10 times.[38] The length of the linker seems to have no significant contribution on the level on binding activity suggesting that the second 1,4-dihydropyridine moiety does not interact with another binding site.

Melatonin is responsible for the regulation of mammalian circadian rhythms and reproductive functions. This neurohormone acts on two different melatonin receptors (MT), namely MT_1 and MT_2. These receptors are present in different parts of the body (brain, kidney, etc.) and their physiological roles are not well known. Selective MT ligands (agonists and antagonists) are useful tools for the characterization of MT. Selective MT_1 ligands were designed (Figure 18.11) starting from the non-selective agonist agomelatine.[39] Two agomelatine moieties were linked by a polymethylenic chain with variable length. A selective MT_1 ligand was obtained which behaved as an antagonist. In this case, dimerization of a non-selective MT agonist led to a selective MT_1 antagonist.

The synthesis and design of homodimeric ligands was also achieved in the search of new peroxisome proliferator-activated receptor (PPAR) agonists. PPARs belong to the superfamily of nuclear hormone receptors (steroid, thyroid and retinoid receptors) and act as transcription factors of specific genes. PPARα activators (agonists) such as fibrates are used to lower triglycerides and to moderately raise HDL-cholesterol level. Patients with type 2 diabetes are treated with PPARγ activators (glitazones) in order to

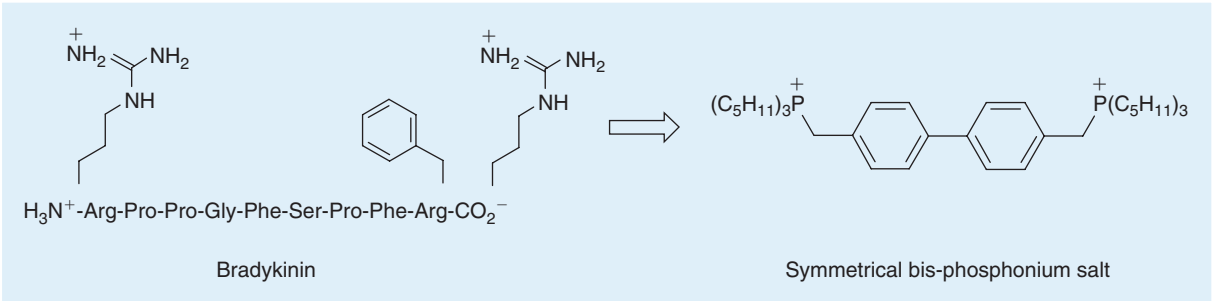


FIGURE 18.8 Bradykinin antagonists.

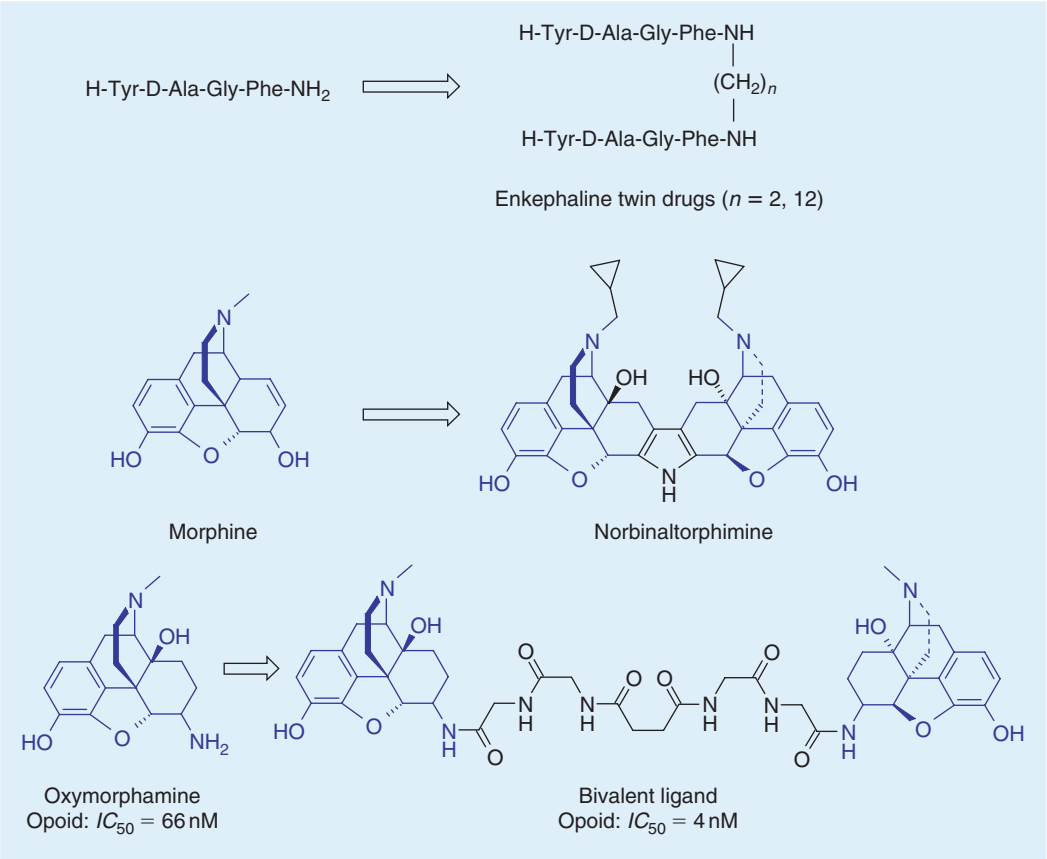


FIGURE 18.9 Twin drugs for opioid receptors.

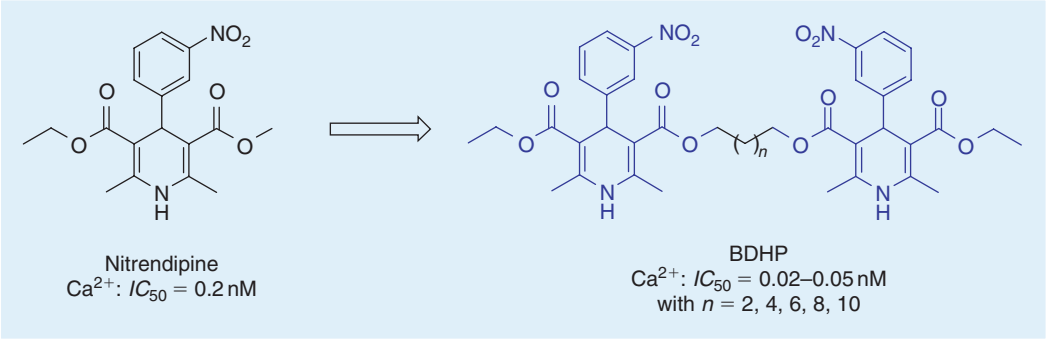


FIGURE 18.10 Other symmetrical receptor ligands.

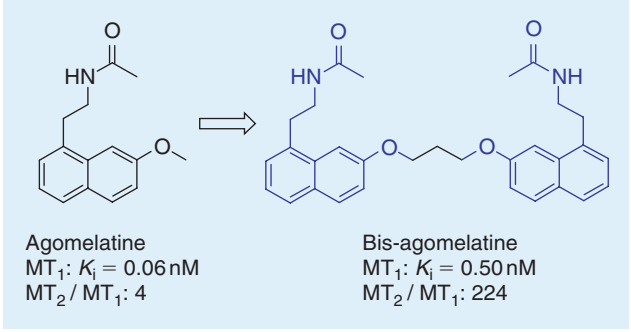


FIGURE 18.11 Melatonin receptor ligands.

decrease glucose and insulin levels. More recently, PPARδ agonists have showed an important increase of HDL-cholesterol level associated with a decrease of triglycerides. The design of combined triple agonists represent a therapeutic interest for diabetic patients. Duplication of non-selective PPAR agonists led to a PPARα, γ and δ agonist with a different profile compared to the monomer derivative.[40] The monomer was dimerized according an overlap mode by using the biphenyl moiety as the linker (Figure 18.12).

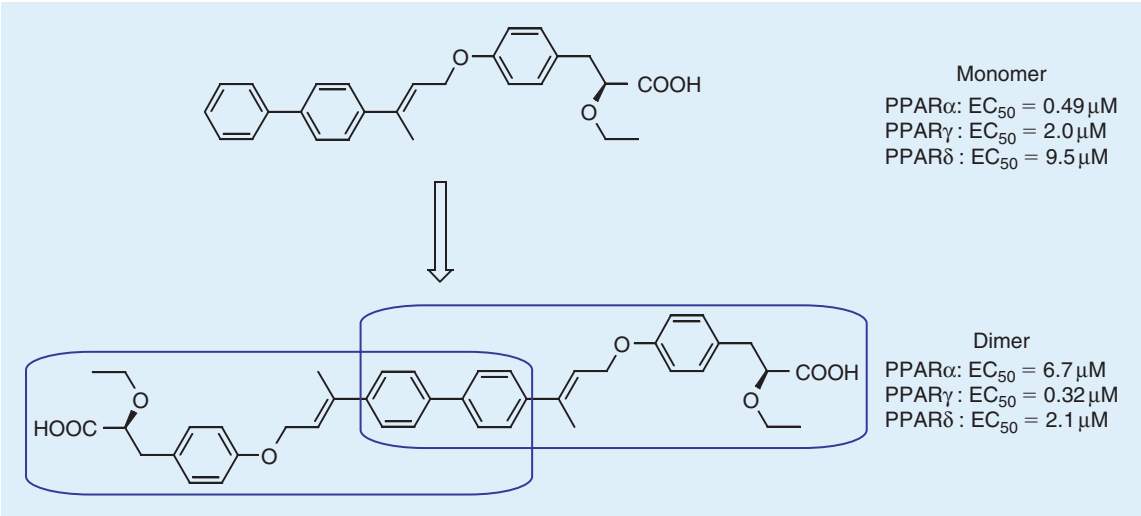


FIGURE 18.12 PPARs ligands.

C. Homodimers as enzyme inhibitors

The symmetrical arrangement of enzymes into homodimers or tetramers defines the active site of the enzyme in a highly symmetrical fashion. Thus, symmetrical inhibitors will correspond generally to the binding site of the enzyme.

HIV reverse transcriptase (HIV RT) and protease (HIV PR) are essential for the maturation and production of infectious viral particles. A combined inhibition of HIV RT and HIV PR is capable of reducing the viral load in blood patients.[41] These enzymes, which exist respectively as an heterodimer and an homodimer for HIV RT and HIV PR, are well characterized: more than 170 structures of HIV PR and its complexes with various inhibitors have been solved by protein crystallography techniques.[42,43] Thus, a dipalmitoylated derivative of 2,7-naphthalene disulfonic acid demonstrated a micromolar activity for both HIV-1 and HIV-2 RT (Figure 18.13).[44] Symmetrical nature of HIV PR was used in the search of novel anti-HIV drugs that would embody the predicted characteristic of the active site. The design of inhibitors of HIV PR has led to symmetrical compounds, which can be divided into two groups: (a) pseudosymmetrical compounds, like derivatives A 74,704[45] and L 700,417 which contain asymmetric atoms in close proximity to the inhibitor two-fold axis; (b) fully C2-symmetrical inhibitors like the cyclic urea [46] and the diol derivatives [47] (Figure 18.13).

The protein kinase C (PKC) enzyme family plays a pivotal role in the signal transduction pathways of hormones, neurotransmitters and other endogenous substances. The 11 PKC isoenzymes identified are involved in the activation of many cellular functions. PKCs possess a C-terminal catalytic region with a serine/threonine kinase function and N-terminal regulatory region. The regulatory region is subdivided into an activator binding domain C1 and a Ca^{2+} binding domain C2. Selectivity and classification of PKC isoenzyme are based on the structural and functional differences of this regulatory domain. During the search of potent activators of PKC, homodimer ligands were prepared and evaluated.[48] Dimer ligands showed a 200-fold higher affinity for PKCα than the monomer unit (Figure 18.14). The spacer optimal length was 14 carbon atoms. These bivalent ligands seem to interact with both C1a and C1b activator binding domain of PKCα or with the C1 domains of two adjacent PKCα. Dequalinium (DECA) analogs with longer and saturated linkers exhibited enhanced potency for inhibition of PKCα. The presence of a two-point contact on the enzyme by DECA analogs explain the potency and the selectivity of such compounds.[49]

Matrix metalloproteinases (MMPs) represent important targets for the development of new potential anticancer agents. MMP-2, MMP-9 and MMP-14 in particular play a significant role in metastatic tumor dispersion and angiogenesis. Potent and selective inhibitors of MMPs would be very useful in tumors study. Duplication of a monomeric MMP inhibitor was achieved (Figure 18.15) based on the observation that the Cbz aminoethyl side chain could be exposed to the solvent environment.[50] The addition of another drug entity would allow to interact with an adjacent active site or with other MMPs proteins. Two inhibitor entities were linked by the mean of a flexible spacer that could potentially interact with some enzyme regions. Whereas the new dimeric compound showed a lower activity and a similar selectivity compared to the monomer, it appeared to generate less cytotoxicity effects on cells.

Inhibitors of factor Xa (FXa), a serine protease involved in the cascade coagulation, would allow to control the coagulation process and bleeding problems. Discovery of orally

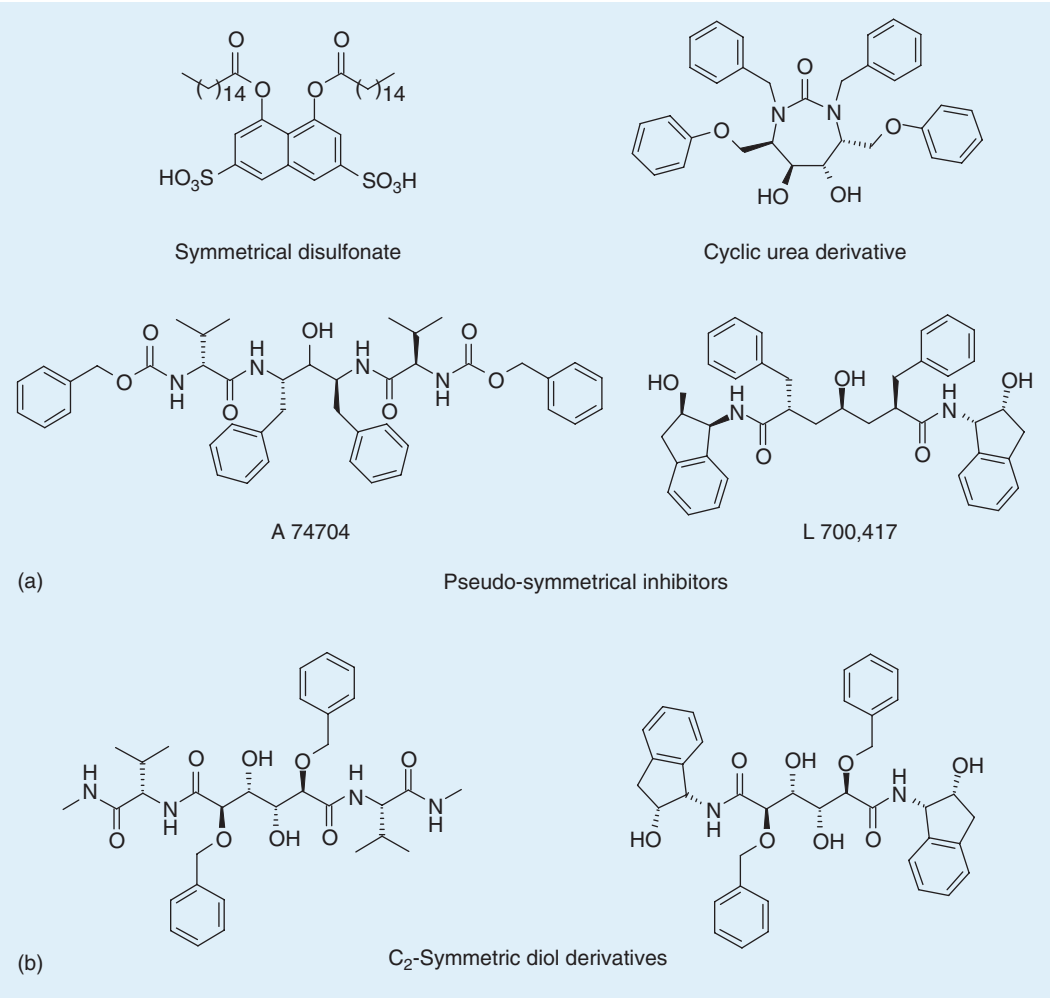


FIGURE 18.13 HIV reverse transcriptase and protease inhibitors.

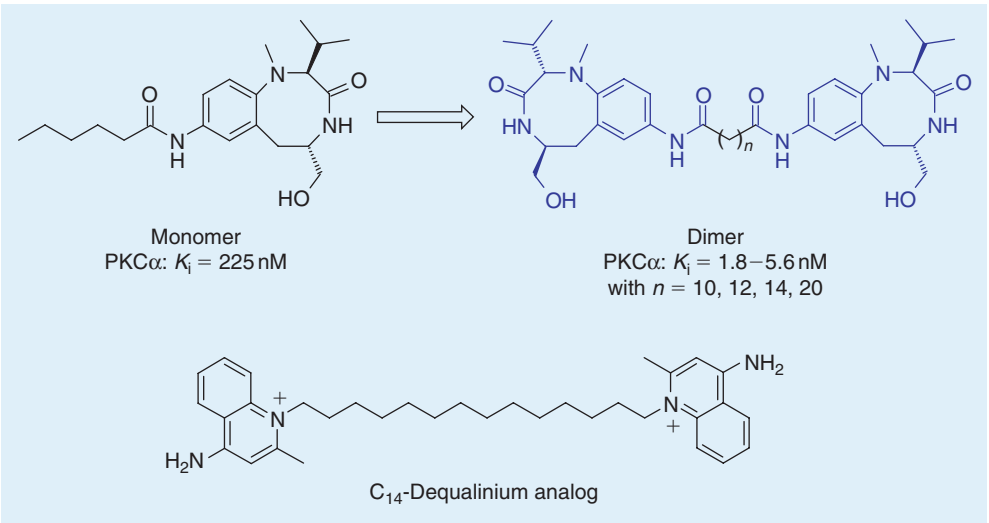


FIGURE 18.14 Protein kinase inhibitors.

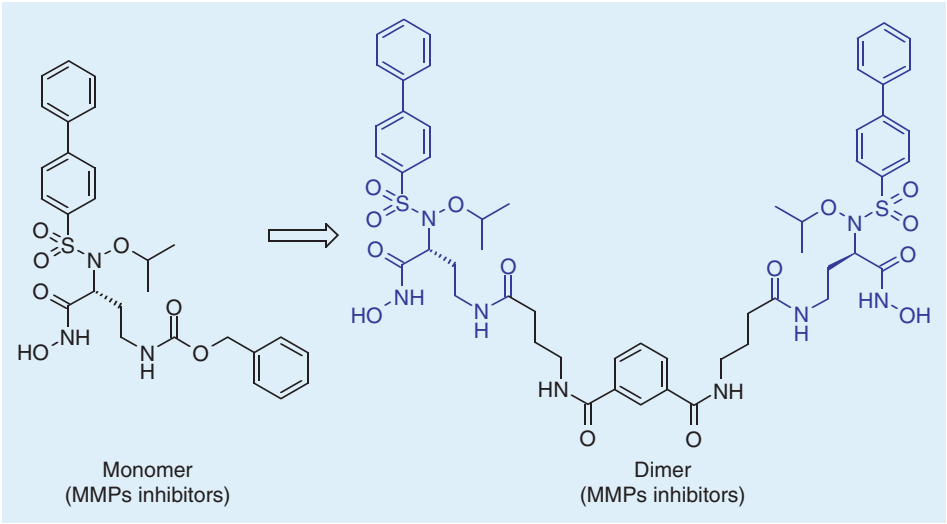


FIGURE 18.15 MMPs inhibitors.

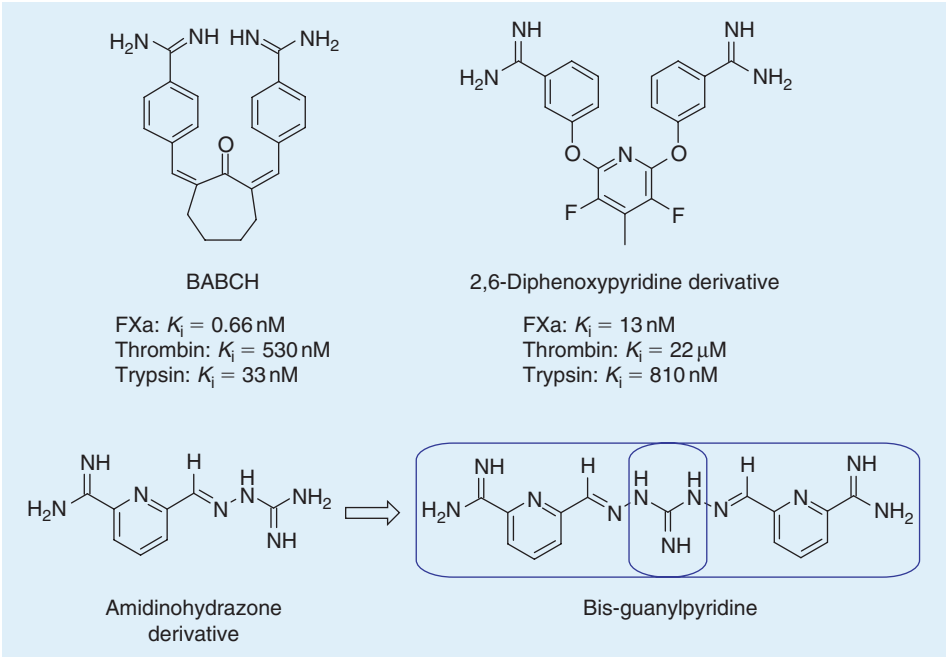


FIGURE 18.16 Various enzyme inhibitors.

active non-peptidic inhibitors of FXa present a therapeutic interest. Recently, a potent and selective FXa inhibitor (Figure 18.16) was reported, the bisamidinobenzylidenecycloheptanone (BABCH).[51] This symmetrical derivative was designed from non-peptidic inhibitors and showed a potent and selective (compared to thrombin and trypsin) inhibitory activity on FXa.[52] The replacement of the cycloheptanone central scaffold of the BABCH derivative by a substituted pyridine core led to potent and more selective FXa inhibitors. Inhibitors of enzymes involved in the polyamine metabolic pathway have been designed as potential antitumor and antiparasitic agents. S-Adenosylmethionine decarboxylase (SAMDC) is a rate-limiting enzyme of polyamine biosynthesis.[53] The *bis*-guanylpyridine analog was designed from the active amidinohydrazone moiety (Figure 18.16). The symmetrical derivative was found to be a potent and selective SAMDC inhibitor.

The design of bifunctional AChE inhibitors was achieved in order to obtain potent and selective derivatives. Tacrine, an AChE inhibitor used for the treatment of Alzheimer disease (AD) patients, has been dimerized leading to the *bis*-tacrine derivative [12] (Figure 18.17). The length of the methylene chain was optimized in order to obtain a potent and selective homodimer. *Bis*-tetrahydroaminacrine

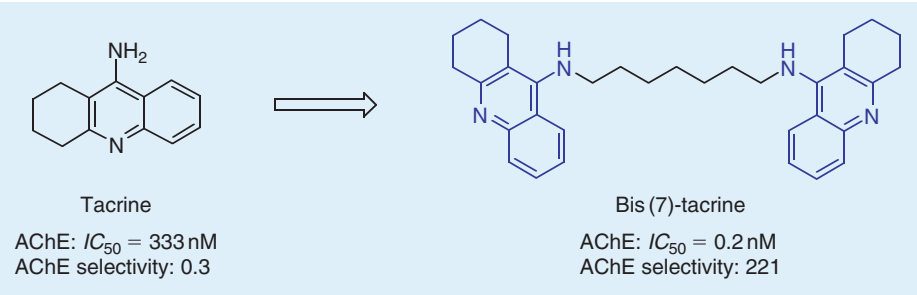


FIGURE 18.17 AChE inhibitors.

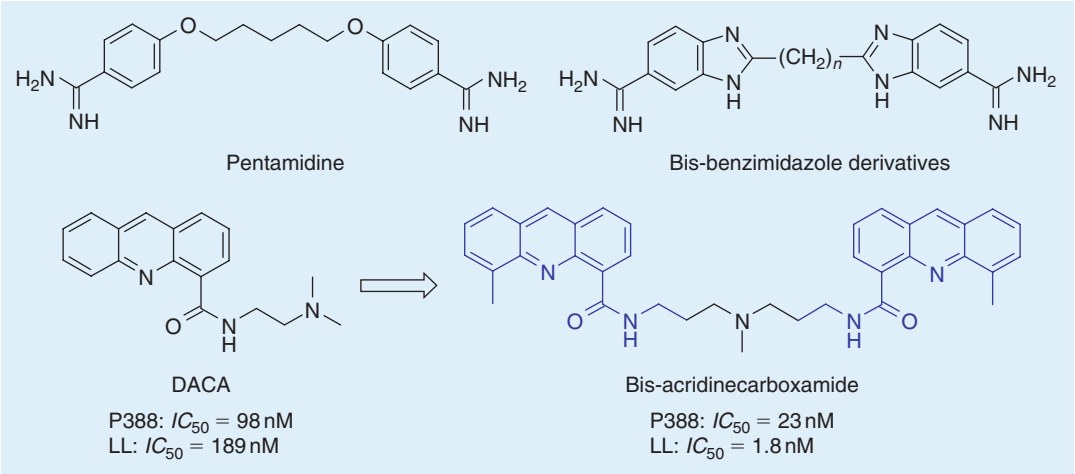


FIGURE 18.18 DNA ligands.

or bis(7)-tacrine showed a simultaneous interaction with the active site and the peripheral site[54] (allosteric site) of the enzyme resulting in an improvement of potency and selectivity.[11]

D. Homodimers as DNA ligands

The DNA molecule is the primary target of many antitumor agents. Small molecules, which bind to DNA by intercalation, require polycyclic systems in their structure for efficient binding. Because of the symmetrical arrangement of the helical double strand, symmetry is found in the structure of DNA ligands. Pentamidine and the *bis*-amidinobenzimidazoles (Figure 18.18) bind to the minor groove of DNA and show higher affinity for AT-base pairs rich regions.[55] Compounds with an even number of methylenes connecting benzimidazole rings have a higher affinity for DNA than those with an odd number of methylenes. The mono-intercalator DACA, a mixed topoisomerase I/II inhibitor with a cytotoxic activity on tumor cell lines, was dimerized using an aminopolymethylenic chain.[56] Substitution of the bis(acridine-4-carboxamides) derivatives was investigated. Analogs with substituents at 5-position showed superior potencies in a panel of cell lines compared to the monomer unit.

E. Homodimers of pharmacological interest

Treatment of malaria is less effective due to a resistance to chloroquine, the most useful antimalarial drug. Chloroquine resistance involve several mechanisms that are not completely understood. Dimerization of chloroquinoline derivatives was achieved to bypass this multidrug-resistant mechanism. Several bisquinolines such as piperaquine[57] showed activity against chloroquine-resistant malaria (Figure 18.19). In a recent study, *bis*-aminoacridine derivatives with different linker were evaluated for their antiparasitic activity.[58] The activity profile of these compounds was strongly dependent on the nature and the length of the connecting linker between the heterocyclic rings.

The search of cationic cholinergic agents has led to numerous twin drugs (Figure 18.20). The bis-quaternary ammonium salts hexamethonium and decamethonium are

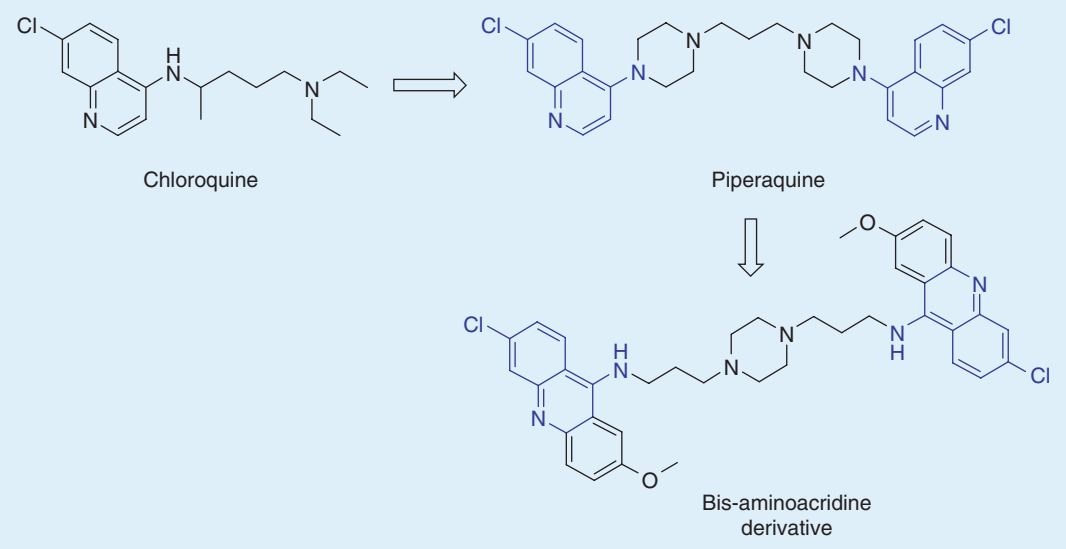


FIGURE 18.19 Aminoacridine twin drugs.

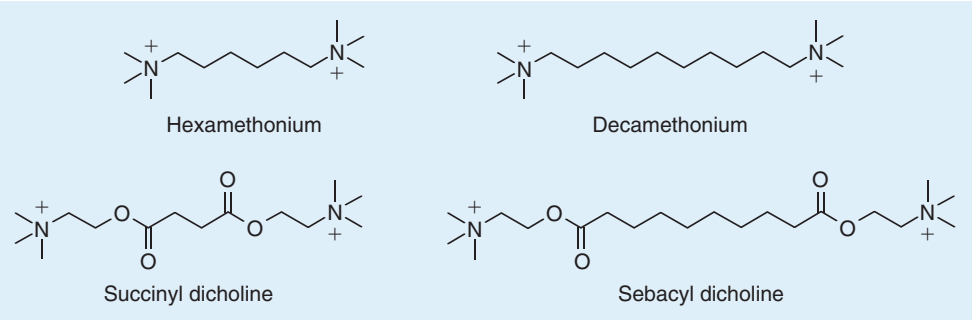


FIGURE 18.20 Cholinergic twin drugs.

potent blockers in ganglia and in neuromuscular junction, respectively. Other neuromuscular blocking agents such as succinyl and sebacyl dicholines can be regarded as pure acetylcholine twin drugs.

III. HETERODIMER AND DUAL ACTING LIGANDS

Heterodimers and dual acting drugs exert their dual action on two different biological targets. These targets could be receptors (GPCRs, receptor subtypes in the same family or different receptor families), enzymes or a combination of both of them. The association of two physiological effects is aimed to obtain a synergic response in the treatment of a disease or a disorder. Hybrid molecules could result from the association of two distinct active principles (associative synthesis) or from a compound with an intrinsic dual acting profile. In the first case, the two pharmacophoric entities are linked and recognizables whereas in the second case it is often difficult to identify the chemical part of the molecule responsible of a biological activity. In this last case, the design of dual acting drugs will be often based on structural modifications of one of the two pharmocophoric entities by incorporating SAR important elements of the other pharmacophore.

A. Hybrid molecules as ligands of two different receptors

GPCRs possess physical, biochemical and structural similarities. Thus, selectivity toward biogenic amines such as noradrenaline (NA), serotonin (5-HT), dopamine (DA) and histamine (H) depends on a typical Asp interaction (conserved amino acid localized in the third transmembranar helix) and additional binding interactions. Because pharmacophores of all these ligands are similar, the control of their selectivity constitutes an important challenge for medicinal chemists. Thus, it may be of interest to synthesize hybrid drugs that bind potently to different GPCRs as an agonist, antagonist or mixed agonist/antagonist.

During the search of antipsychotic agents without side effects (i.e. extrapyramidal side effects), 5-HT_2 receptor

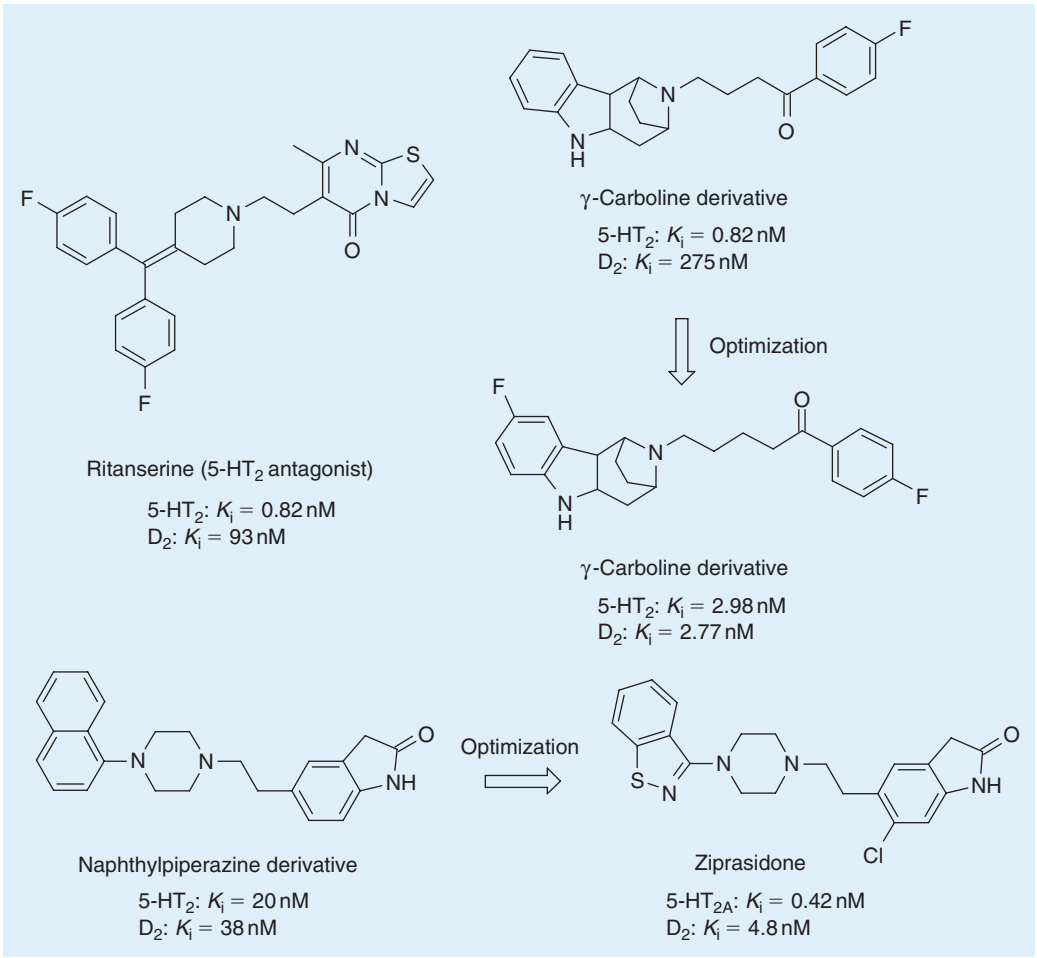


FIGURE 18.21 Dopaminergic and serotonergic dual acting drug.

antagonists such as ritanserin have shown a decrease of the negative symptoms of schizophrenia. So, it has been proposed that a combined administration of a 5-HT_2 antagonist and a D_2 antagonist could be efficient in the treatment of schizophrenic patients. SAR study on bridged γ-carbolines, reported for its potent affinity for 5-HT_2 and moderate affinity for D_2 receptors, was achieved and led to the design of a compound with equipotent and nanomolar affinity for both receptors [59] (Figure 18.21). The efficacious antipsychotic agent ziprasidone was also designed from a naphthylpiperazine derivative with moderate 5-HT_{2A} and D_2 antagonisms.[60,61] A typical SAR study allowed to optimize both DA and serotonine affinities with less extrapyramidal side effects.

Positive inotropic drugs such as arpromidine have been developed by combining in a same molecule histamine H_1 antagonistic and H_2 agonistic properties[62] (Figure 18.22). Association of a weak and partial H_2 agonist with a weak H_1 antagonist led to heterodimer which is a very potent H_2 agonist (100-fold increase) and H_1 antagonist 10 times more active than the monomeric pharmacophore. The H_1 antihistaminic drug loratadine presents a weak PAF antagonistic property. Taking into account the physiological importance of PAF in asthma, it was of therapeutic potential interest to antagonize by a single molecule the action of both mediators.[16] Optimization of this intrinsically dual acting drug was achieved and led to a derivative with a better PAF antagonistic activity and a similar H_1 binding level. Thromboxane A_2 (TXA_2) is also implicated in the pathophysiological conditions of asthma. Therefore efforts have been made to design TXA_2 receptor antagonists. The symbiotic approach concept led to the recent discovery of dibenzoxepin derivatives. The antiallergic and H_1 antagonist KW-4994 was reported to possess also a weak TXA_2 antagonizing activity. Successful modifications led to the design of dual TXA_2 and H_1 antagonists such as KF 15766.[63]

The octapeptide hormone angiotensin II is involved in vascular smooth muscle contraction and release of other endogenous substances. Since angiotensin II receptor subtypes (AT_1 and AT_2) are presents in various proportions in many tissues and organs, dual antagonists may constitute efficient pharmacological tools. Starting from losartan (Figure 18.23), a selective AT_1 antagonist, and PD 123317, a selective

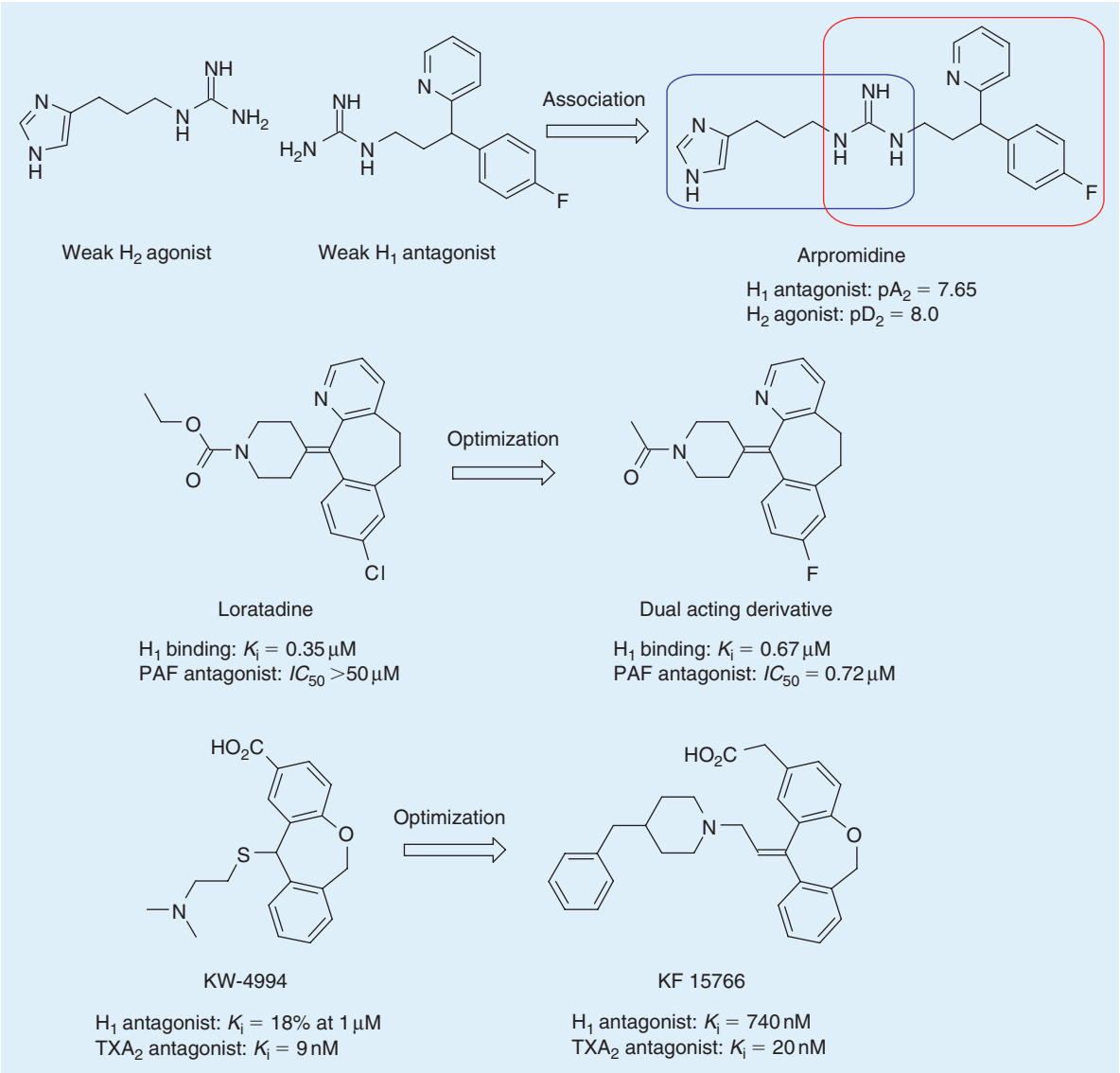


FIGURE 18.22 Histaminergic hybrid drugs.

AT_2 antagonist, dual and non-selective AT_1 and AT_2 antagonists were designed.[64] Structures of losartan and PD 123317 were merged by using common features (in blue) and the new scaffold was then optimized by a traditional SAR study leading to BIBS 222 derivative. A potent and orally active AT_1 antagonist, L-159,093, was modified to enhance binding affinity for AT_2 receptor.[65] After optimization, a potent and balanced AT_1/AT_2 antagonist (L-159,689) was obtained.

More recently, several studies in animals have reported that simultaneous blockade of AT_1 and ET_A should be very efficient in the treatment of hypertension and cardiovascular diseases such as heart failure.[66,67] The biphenylsulfonamide BMS-193884 designed as a potent and selective ET_A antagonist share the same biphenyl framework as irbesartan, a AT_1 antagonist (Figure 18.24). By merging key structural elements from these two derivatives, Bristol-Myers Squibb scientists obtained a hybrid which was optimized to give DARA 7 derivative.[15,68] DARA 7 shown a balanced activity at AT_1 and ET_A receptors and reduced blood pressure elevations in rats.

Dual acting drugs can also result from the combination of ligands belonging to completely distinct pharmacophores. As activation of substance P (SP) and adenosine (A_1) receptors produce the same effect (e.g. hypotension and analgesia), it was of therapeutic interest to combine in a single compound these properties. A xanthine derivative, an A_1 adenosine receptor antagonist, was linked with the pentapeptide terminal part of substance P to give a conjugate (Figure 18.25) with similar affinity for both A_1 and SP receptors.[69] The coupling was achieved by the means of an amino acid. The heterodimer ligand obtained will be a useful tool for SP pathways and adenosine action study.

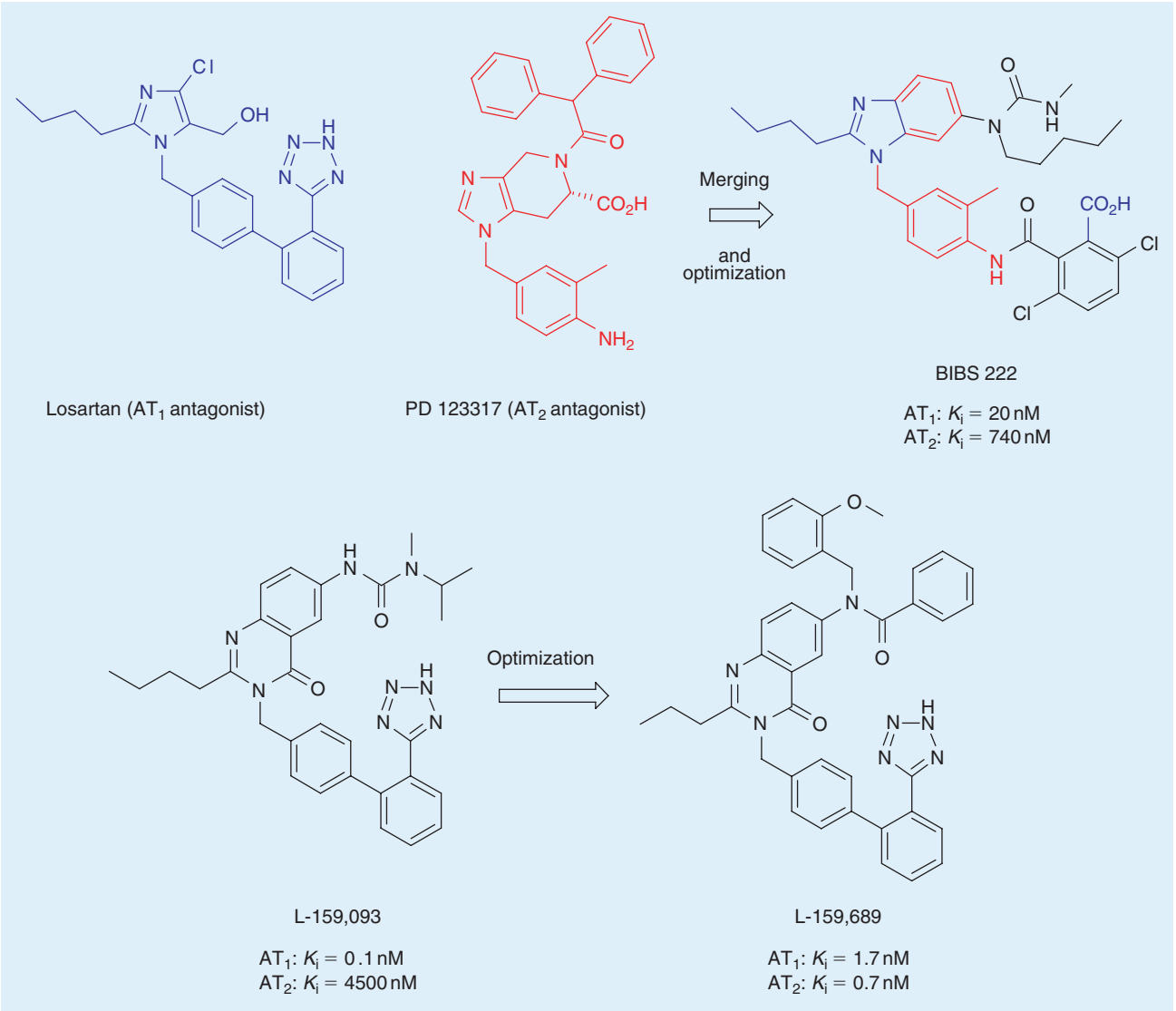


FIGURE 18.23 Angiotensine receptor ligands.

Peroxisome proliferator-activated receptors[70] (PPARs) are members of the superfamily of nuclear receptor that includes receptors for steroid, retinoid and thyroid hormones. The demonstration that PPARα and PPARγ were the receptors through which, respectively, the fibrate (lipid lowering activity) and the glitazone (insulin sensitization) drugs mediate their biological activity has led to the design of a new generation of dual acting drugs. Dual PPARα/γ agonists appear well suited for the treatment of hyperglycemia with prevention of cardiovascular disease in type 2 diabetes. The aryloxazole derivative (Figure 18.26) is a typical example of the combination of the fenofibric acid and the thiazolidinedione derivative.[71] Fenofibric acid, a PPARα agonist, was merged with a selective PPARγ thiazolidinone derivative; the hybrid derivative obtained was substituted with a phenyl group to increase potency. Further SAR studies led to the design of a more potent dual acting PPARα and PPARγ agonist such as muraglitazar. On the other hand, optimization of series of α-substituted phenylpropanoic acid derivatives [72] afforded dual PPARα/PPARδ agonists.

B. Hybrids as enzymes inhibitors

As observed with receptors, enzymatic systems can be subdivided into enzyme families, and each enzymatic type presents several isoforms. Thus, for pharmacological and therapeutic purposes, it may be of interest to combine in a same molecule structural characteristics for inhibition of two different isoenzymes, two enzymes belonging to the same family, or two enzymes for which inhibitors are showing pharmacophore similarities.

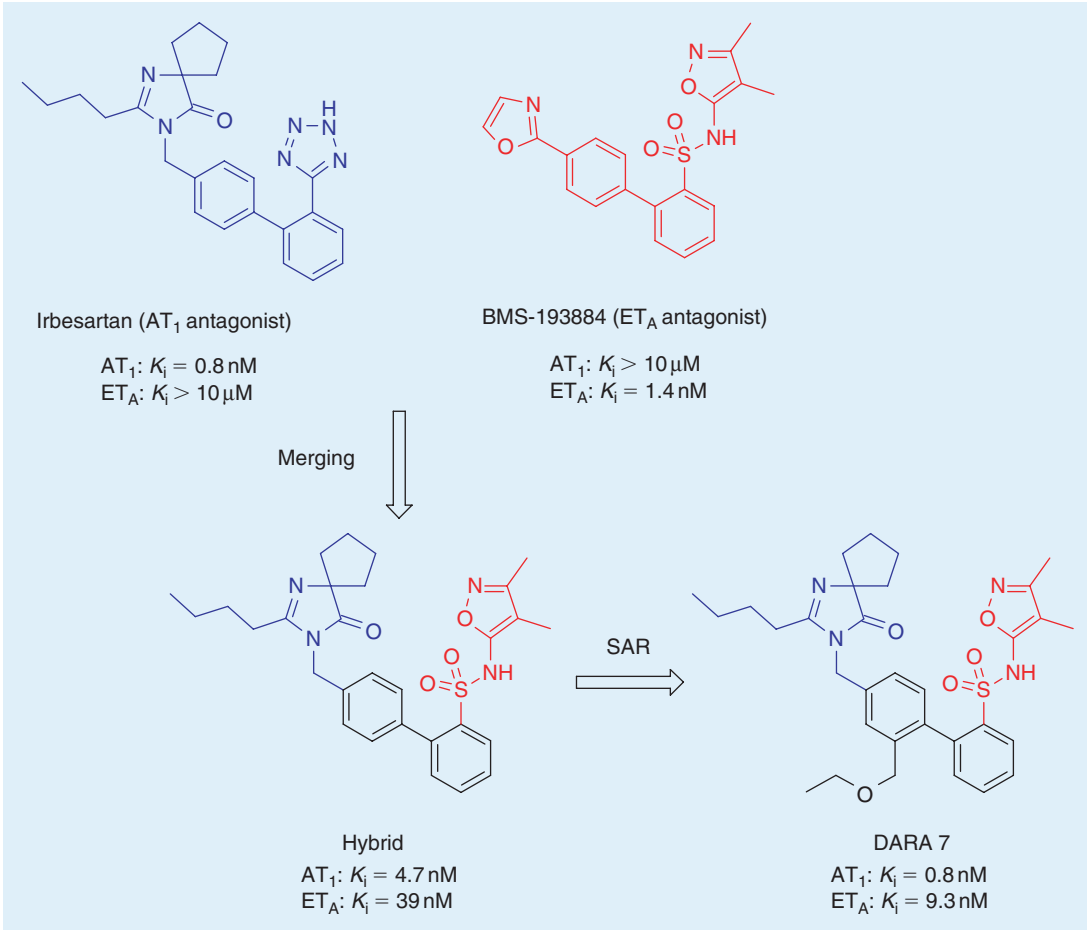


FIGURE 18.24 Dual angiotensin and ET_A ligands.

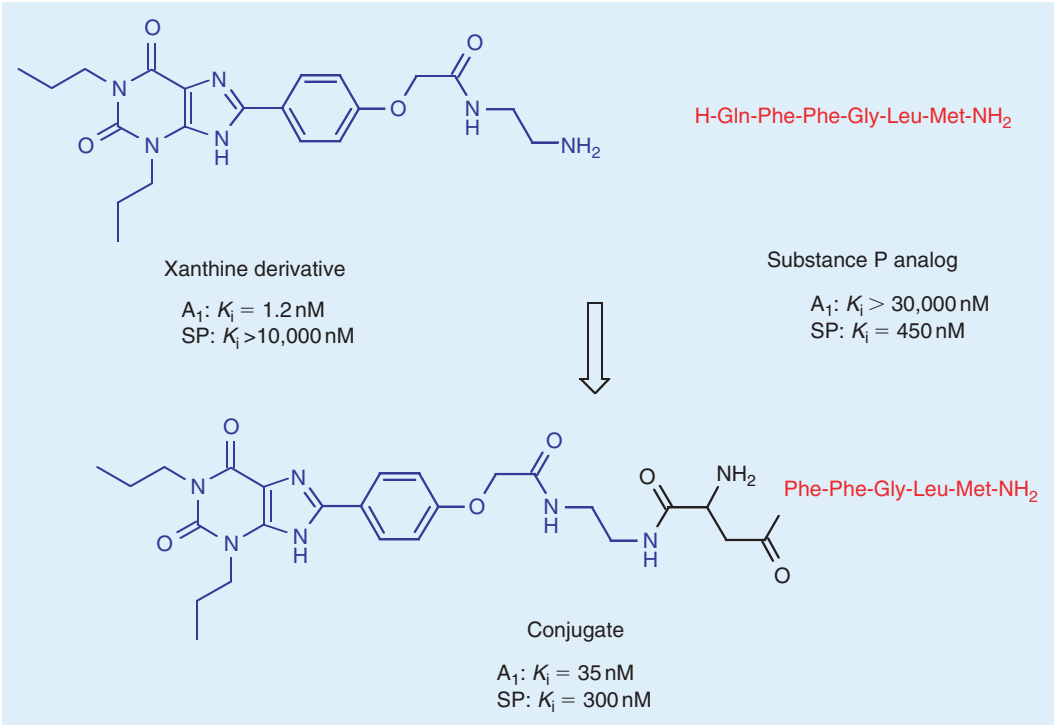


FIGURE 18.25 Substance P and adenosine hybrid ligand.

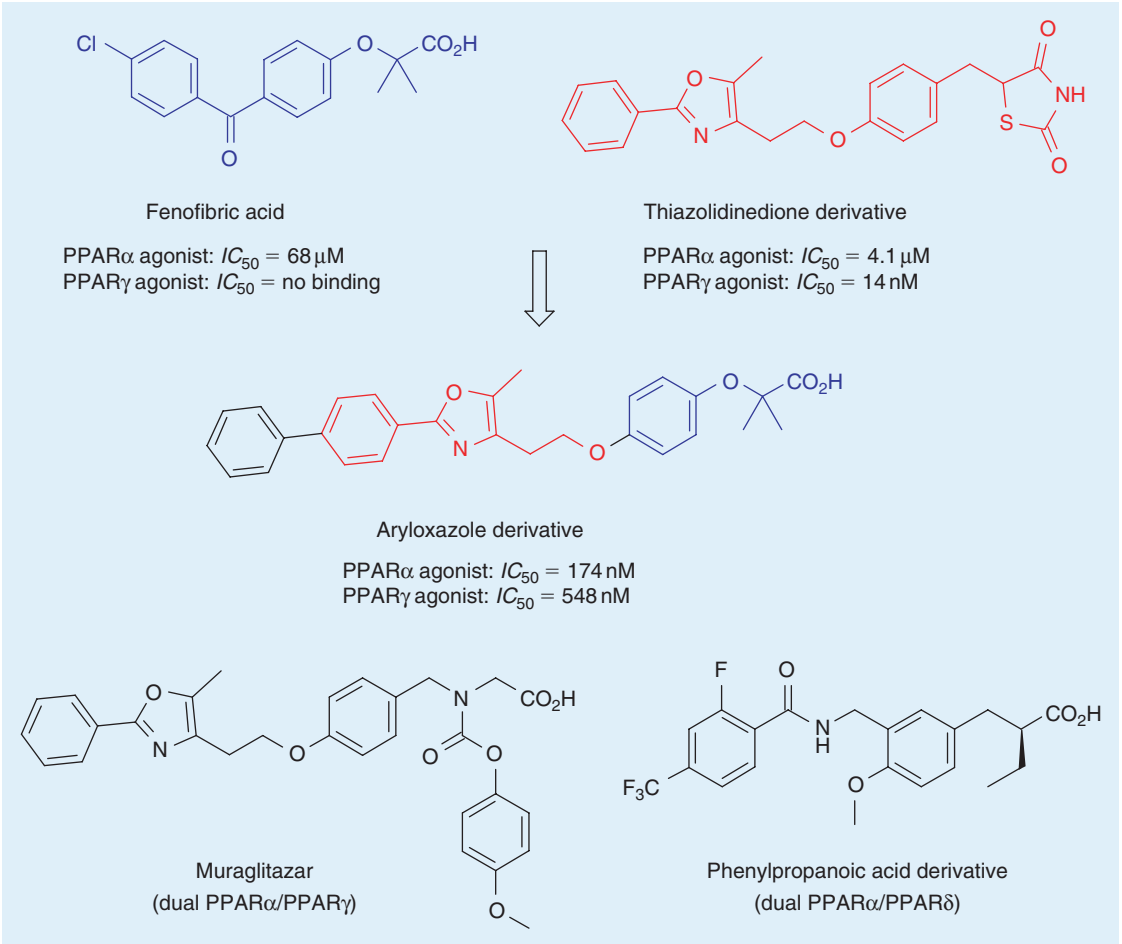


FIGURE 18.26 Dual PPAR agonists.

Inhibitors of cyclooxygenase (COX-2) and 5-lipoxygenase (5-LO), enzymes involved in the biosynthesis of prostaglandins and leukotrienes, are being studied as non-steroidal antiinflammatory agents with improved safety profile. Dual acting compounds with both inhibiting activities represent potential treatment for patients suffering from arthritis and other inflammatory disorders. The thiazolone CI-1004 (Figure 18.27) was identified as a equipotent dual COX/5-LO inhibitor.[73] Compared to KME-4 compound, this dual acting drug showed non-ulcerogenic water-soluble and orally active antiinflammatory properties. Recently, combination of the pharmacophores of selective COX-2 and 5-LO inhibitors was achieved.[74] The new conjugate derivative showed a potent COX-2/5-LO inhibition and a high COX-2 selectivity.

The inactivation of the endogenous opioid peptide enkephaline is one of the physiological role of neutral endopeptidase (NEP). It has been suggested that simultaneous inhibition of the related metallopeptidases angiotensin I-converting enzyme (ACE) and NEP might be advantageous in the treatment of congestive heart failure or hypertension. Thiorphan, the well-known inhibitor of NEP, has dual NEP–ACE inhibiting properties, but it is hundred times less potent as an ACE inhibitor than as NEP inhibitor (Figure 18.28). A rigid benzazepinone was designed as PheLeu mimetic and showed a potent dual NEP/ACE inhibition.[75] Efforts were achieved in this dual inhibitors series and led to the design of the bicyclic thiazepinone omapatrilat.[76] This compound showed equipotent and dual NEP/ACE inhibition and demonstrated an excellent blood pressure lowering in animals. Omapatrilat has been developed for the treatment of hypertension and congestive heart failure.

Nucleoside reverse transcriptase inhibitors (NRTIs) such as zidovudine (AZT) or zalcitabine (ddC) have proven highly efficacy when they are used in combination with HIV PR inhibitors. Because of increasing adverse side effects, research has been focused on non-nucleoside reverse transcriptase inhibitors (NNRTIs), inhibitors of a non-competitive binding site. NRTI–NNRTI combination therapy will exhibit a synergic activity and have a greater efficacy. Thus, conjugates containing both nucleoside analog component and non-nucleoside type inhibitor (Figure 18.29) were designed and showed micromolar anti-HIV activity.[77] The heterodimer derivative did not exhibit synergic effect

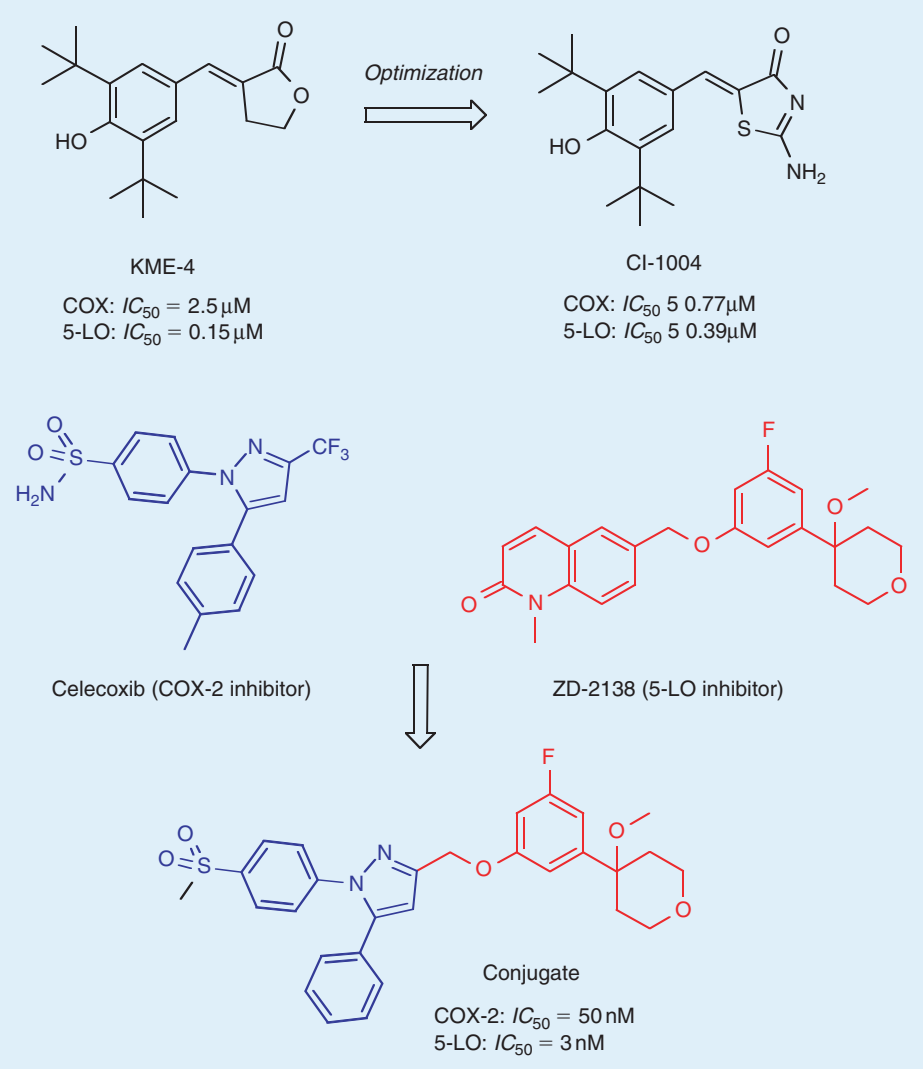


FIGURE 18.27 Dual COX/5-LO inhibitors.

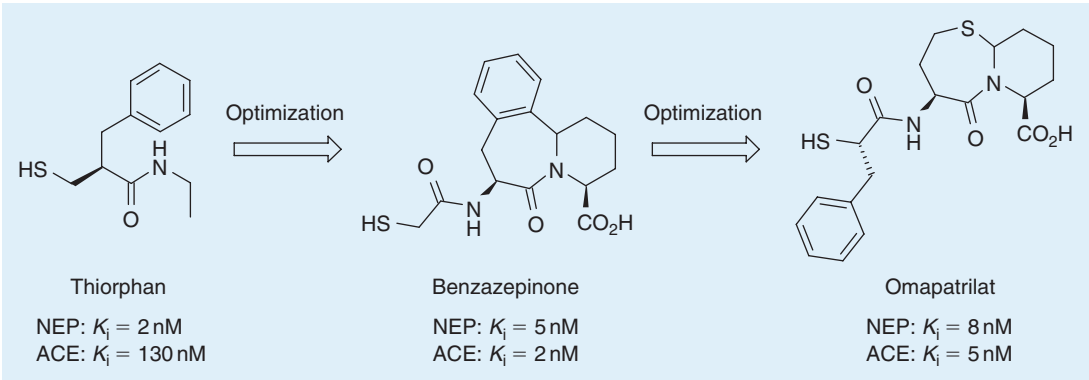


FIGURE 18.28 Dual NEP/ACE inhibitors.

suggesting that the individual pharmacophores do not bind simultaneously.

Administration of AChE inhibitors represent again a promising approach for treating Alzheimer's disease since the correlation between this enzyme and amyloid formation has been demonstrated. A potent and selective AChE inhibitor[78] was designed by merging the two different AChE inhibitors huperzine A and tacrine (Figure 18.30). The

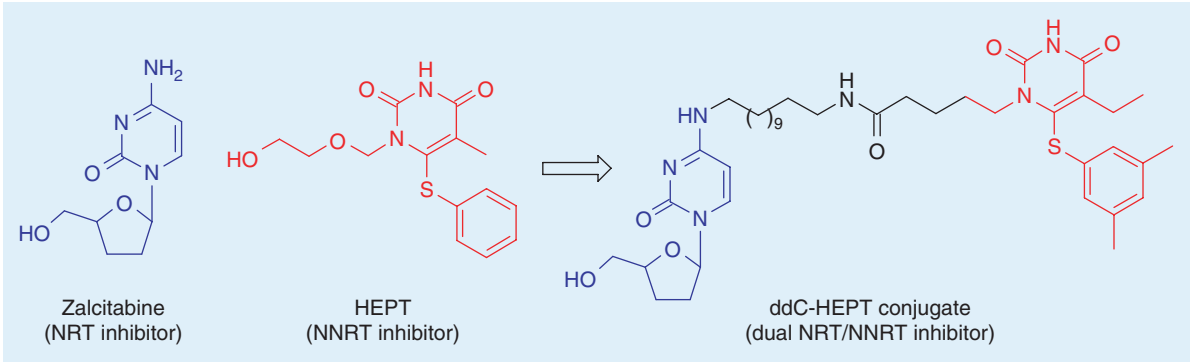


FIGURE 18.29 Heterodimer HIV inhibitors.

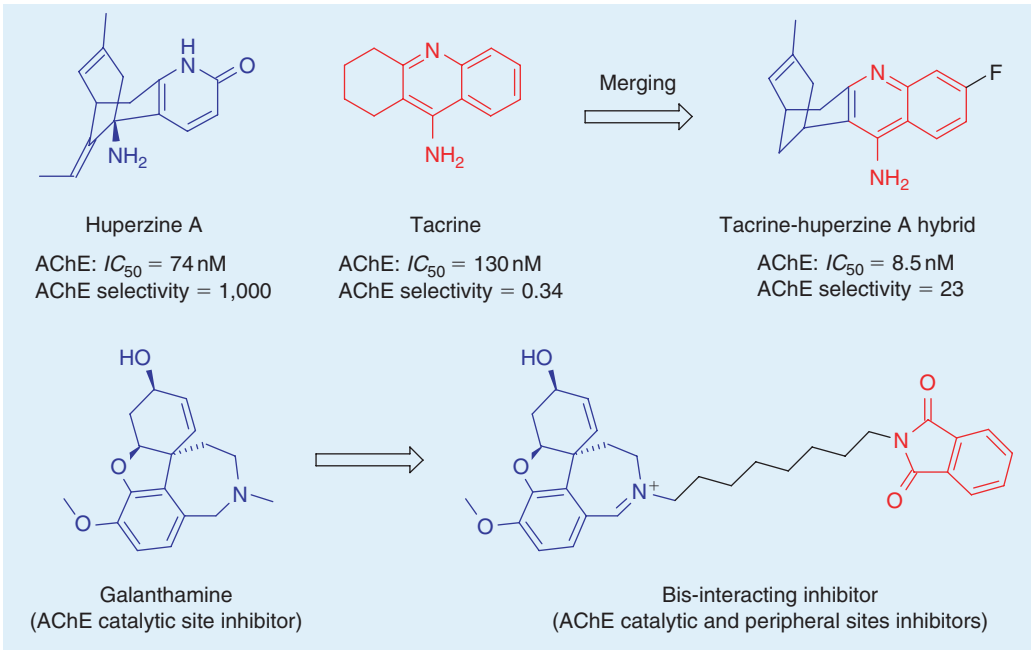


FIGURE 18.30 AChE hybrid inhibitors.

hybrid derivative showed a improved inhibiting activity with a moderate selectivity (AChE versus Butyrylcholinesterase) profile. *Bis*-interacting galanthamine ligands[79] were prepared by using different methylenic linkers with a phthalimide moiety and the centrally active inhibitor galanthamine. Crystallographic structure of AChE and biochemical studies allowed to identify clearly two binding sites on the enzyme: an active site, located at the bottom of a deep and narrow gorge, and a peripheral site, located at the opening of the gorge (for details see Section IV.A.1.). Combination of the phthalimide moiety, known to interact with the peripheral site, with galanthamine derivative (active site inhibitor) led to a conjugate that binds to both active and peripheral sites.

In addition to the deficiency of the cholinergic neurotransmission observed in AD and correlated with cognitive function disorders, AD patients also suffer from depression and anxiety. Serotonin transporters (SERT) inhibitors are used to treat these symptoms. The design of dual inhibitors (AChE and SERT) was achieved to obtain a better therapeutic effect.[80] After hybridization of rivastigmine, a marketed AChE inhibitor, and fluoxetine, a potent SERT inhibitor, a new series of dual AChE and SERT inhibitors was design (Figure 18.31).[81,82] After SAR studies, a compound ((R)-analog) with potent inhibitory effect on both enzymes was obtained.[83]

C. Hybrids acting at one receptor and one enzyme

Hybrid molecules acting simultaneously on a receptor and on an enzyme may produce potent synergistic effects. An illustration is given by the example of derivatives interfering with TXA_2, a powerful inducer of platelet aggregation

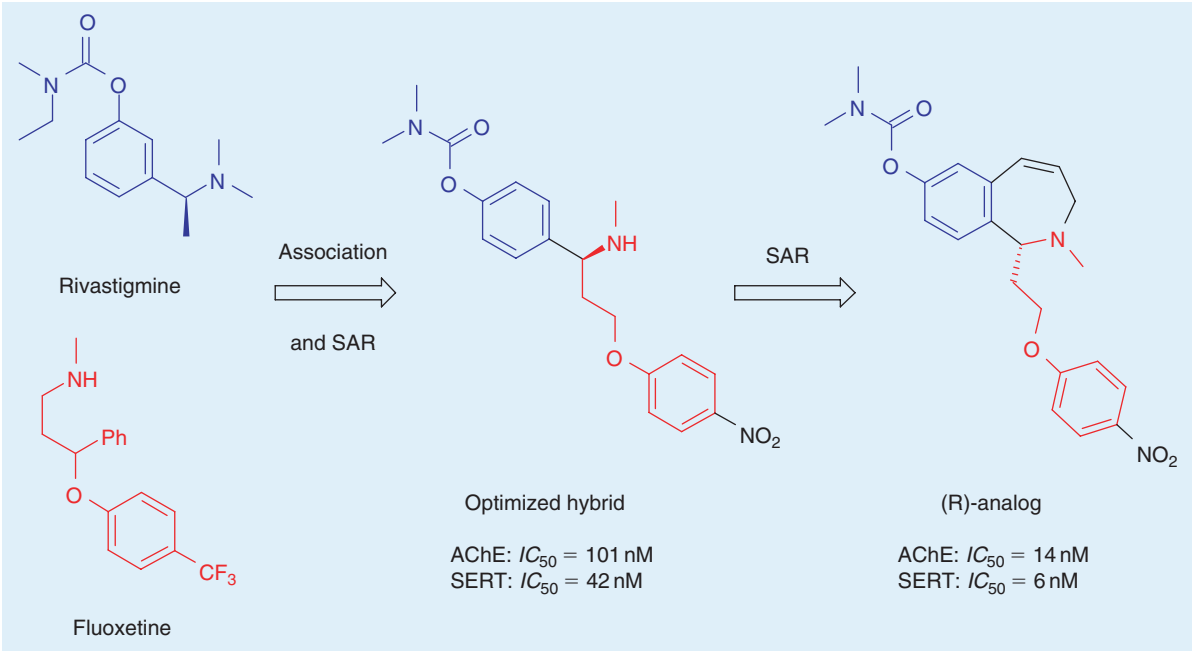


FIGURE 18.31 Dual SERT/AChE inhibitors.

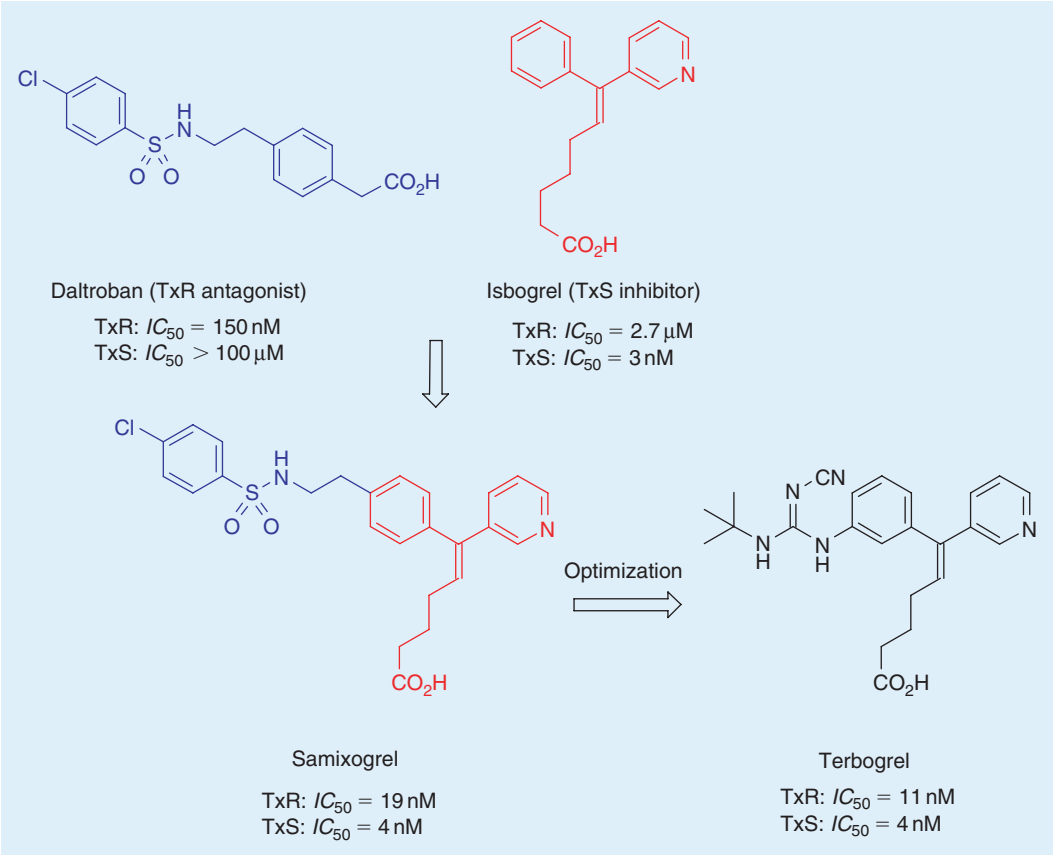


FIGURE 18.32 TXA_2 hybrid drugs.

and vascular smooth muscle contraction. The inhibition of TXA_2 synthase (TxS) and the selective blockade of TXA_2 receptors (TxR) have been pursued as alternative therapeutic strategies to prevent the thrombotic action of TXA_2. Thus, TxS inhibiting and TxR antagonistic properties have been combined in a single molecule such as samixogrel[84] starting from isbogrel, a synthase inhibitor, and daltroban, a TXA_2 receptor antagonist (Figure 18.32). Because samixogrel

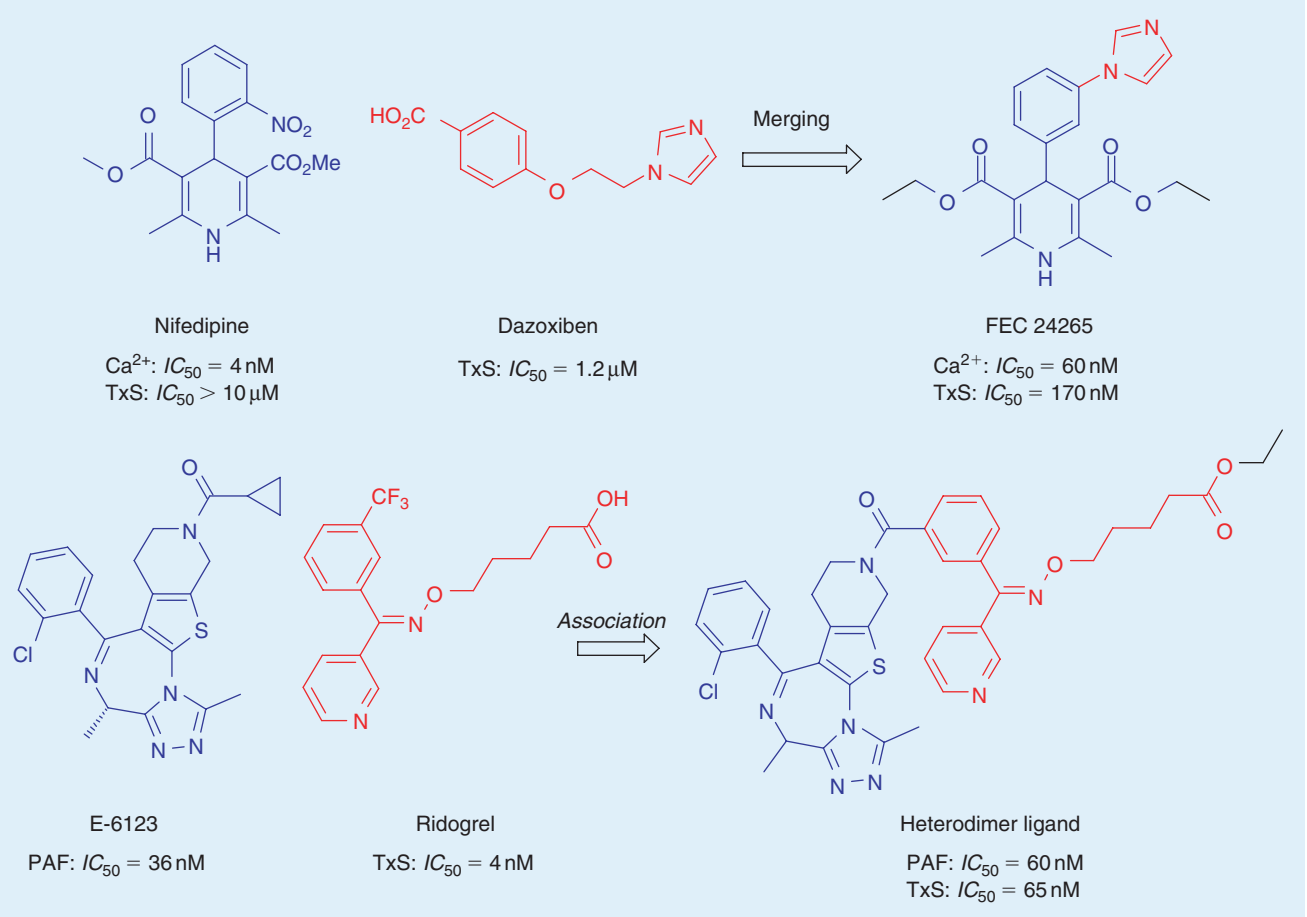


FIGURE 18.33 Hybrid drugs with synergic effects.

showed a moderate plasma levels after oral administration (low solubility in aqueous solution) it was then optimized into the guanidine derivative terbogrel which exhibits potent *in vivo* antithrombotic effects.[85]

Depending on the physiological hypothesis, both targets may belong to different systems. Dihydropyridines such as nifedipine are known to be calcium receptor (Ca^{2+}) antagonists. These drugs are commonly used for treatment of patients with cardiovascular diseases (hypertension, myocardial infarction). Combination of Ca^{2+} antagonism and TxS inhibition might induce an increase of the therapeutic efficacy for particular pathologies where both enhanced TXA_2 synthesis and cellular calcium overload are involved. The imidazol part of dazoxiben, a TxS inhibitor, was merged with the core structure of nifedipine[86] to led FEC 24265 (Figure 18.33). The hybrid derivative showed a relatively more favorable *in vivo* pharmacological profile. Platelet activating factor (PAF) is involved in inflammation process and related to pathologies such as ischemia, thrombosis and asthma. The association of PAF antagonist and TxS inhibitor would procure beneficial therapeutic treatment. Ridogrel, a potent TxS inhibitor, was directly linked to the PAF antagonist, E-6123.[87] The heterodimer ligand expressed dual and equipotent PAF affinity and TxS inhibiting activity and showed activity after oral administration.

Novel antihypertensive agents possessing both β-blocking and angiotensin-converting enzyme (ACE) inhibiting properties represent beneficial approach for the treatment of elevated blood pressure. Hybridization of the β-blocker pindolol and the ACE inhibitor enelapril led to the derivative BW-A575C which expresses both activities (Figure 18.34).[88] This dual β-blocker/ACE inhibitor drug offer therapeutic promise in hypertension and congestive heart failure.

D. Other examples of dual acting drugs

Combined treatment is necessary in the long-term treatment of essential hypertension. β-Blocker and diuretic properties in a same molecule would present a great interest for hypertension management. Few attempts to synthesize hybrid molecules by combining the structures of a β-adrenoreceptor antagonist and a diuretic were described (Figure 18.35). A hybrid sulfonamide was achieved by linking the β-blocker propranolol derivative with the 2-chlorobenzene sulfonamide moiety of mefruside.[14,89]

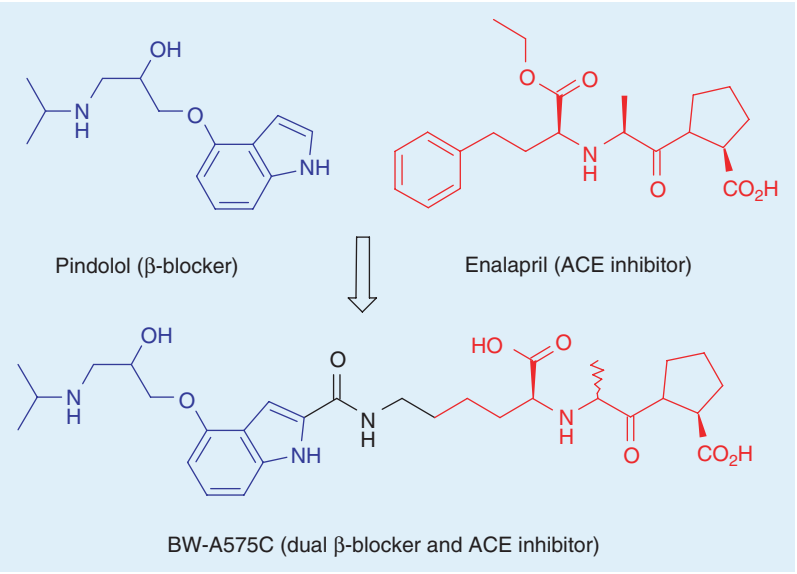


FIGURE 18.34 Dual β-blocker/ACE inhibitors.

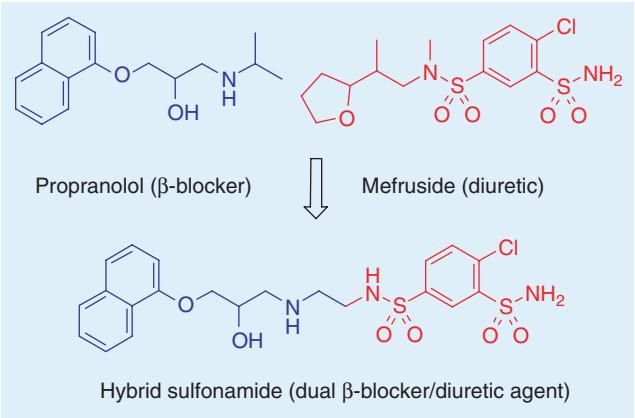


FIGURE 18.35 Hypotensive hybrid drugs.

Dual acting antibacterial drugs were designed by linking quinolones (Figure 18.36), such as ciprofloxacin, to cefotaxime. The hybrid ligand was optimized and demonstrated potent activity against a broad spectrum of Gram-positive and Gram-negative bacteria.[90]

In the search of new and efficient antidepressants, dual acting drugs with selective serotonin (5-HT) reuptake (SSR) inhibition property and 5-HT_{1A} receptor antagonism were designed (Figure 18.37). A combination of the SSR inhibitor duloxetine and an arylpiperazine derivative, a class of derivatives known to have high affinity for 5-HT_{1A} receptor (e.g. NAN-190), led to the hybrid benzothiophene piperazine, a potential class of antidepressant with a dual mechanism of action.[91] In the same manner, blockade of terminal 5-$HT_{1B/1D}$ receptors by selective antagonists would in theory prevents the initial decrease. Thus, coadministration of SSR inhibitors and 5-$HT_{1B/1D}$ antagonists would lead to a large increase of 5-HT extracellular concentration and would be efficient in the treatment of depressive disorders. Coupling of a selective inhibitor of 5-HT reuptake (e.g. the indolylpiperidine derivative) with GR 127935, the first selective 5-$HT_{1B/1D}$ antagonist reported, allowed to obtain urea derivative showing both 5-HT reuptake inhibition and 5-$HT_{1B/1D}$ antagonism *in vitro*.[92]

IV. BINDING MODE ANALYSIS OF IDENTICAL AND NON-IDENTICAL TWIN DRUGS

Bioactive molecules that affect one target only might not always affect complex biological networks in the desired way even if they are able to perfectly interact with one target protein. Single targets in complex biological networks might have alternative routes that are sometimes different enough not to respond to the same molecule, and many networks are robust and prevent major changes in their outputs. [93,94] Therefore developing molecules acting on multiple targets has become an innovative approach in drug design.[4,17]

The huge number of protein crystal structures available today and the increasing reliability of modeling approaches have improved our understanding on the structural and energetic aspects of protein–ligand interaction. Among the 45,000 (July 2007) solved protein structures stored in the Protein Data Bank, PDB (www.pdb.org/pdb) several complexes are available where identical and non-identical twin drugs have been cocrystallized together with their target proteins. These complexes shed light on the binding mode of twin drugs and give important hints how to rationally design multivalent ligands.[3]

The term twin drug or bivalent ligand refers to the phenomenon in which a biological structure that contains

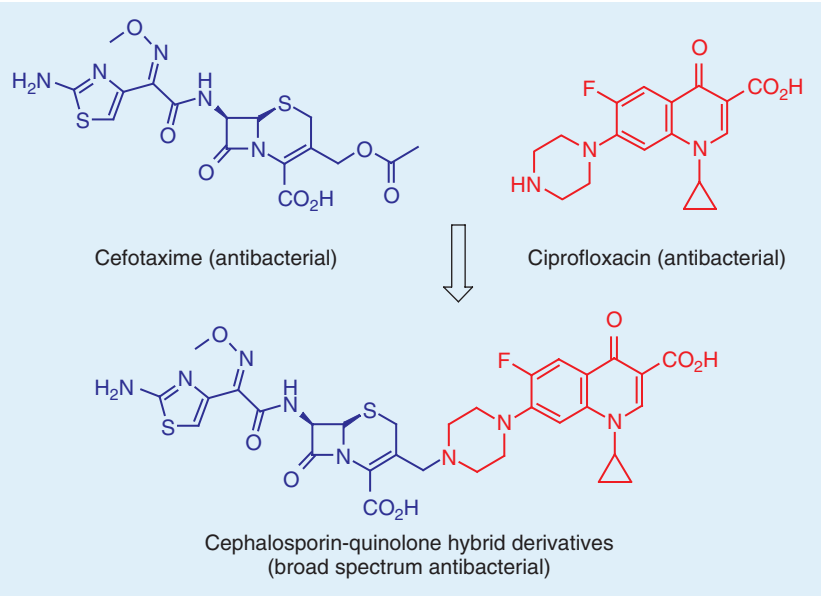


FIGURE 18.36 Antibacterial hybrid drug.

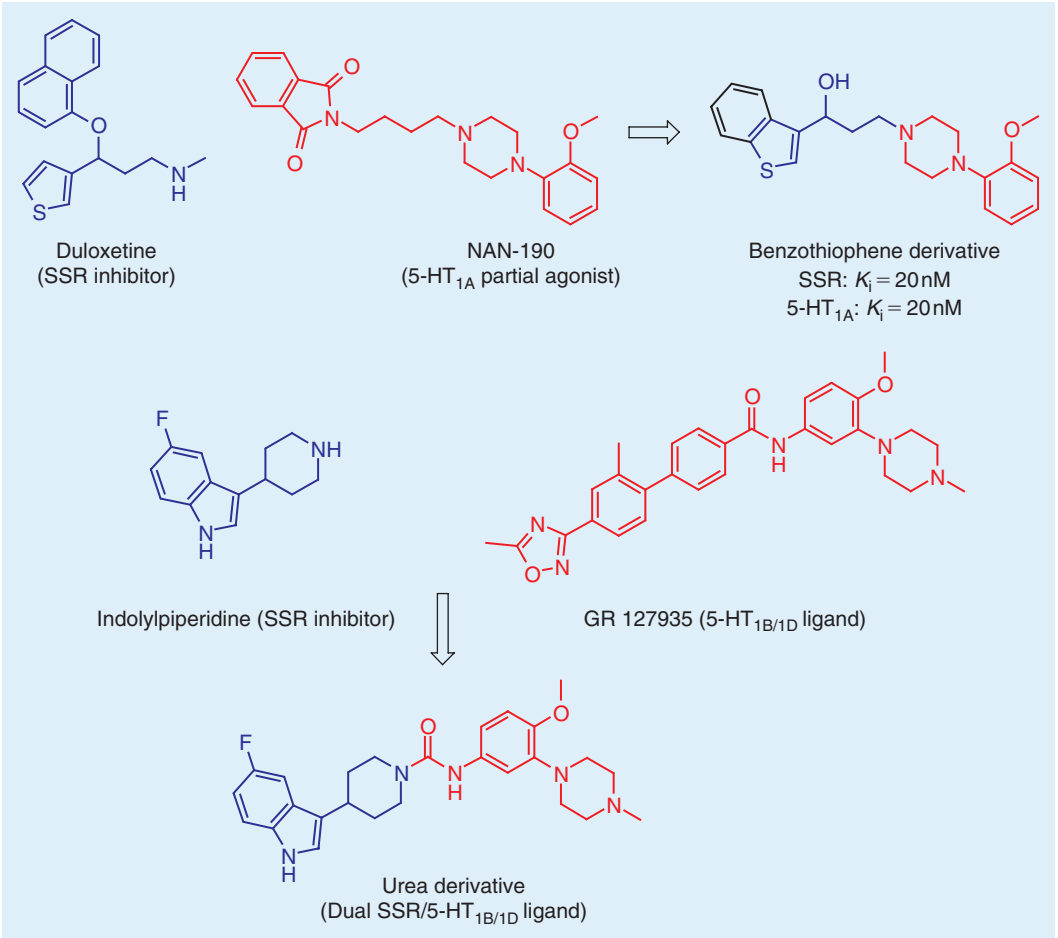


FIGURE 18.37 Antidepressant hybrid drugs.

multiple binding sites binds simultaneously to multiple ligands. This can lead to significant enhancements in binding strength for weakly binding molecules. Prominent examples of multiple ligands include, for example, binding by antibodies and lectins, and the receptor–ligand interactions involved in cell adhesion or viral attachment. The before introduced nomenclature (identical or non-identical twin drugs) is generally based on the structure of the ligands. However, classification can also be made on the basis of the target binding sites. Twin drugs, either identical or non-identical, can bind in different ways to their macromolecular target structures. Twin drug molecules which contain flexible linker regions are supposed to bind to (a) the same target protein to adjacent binding pockets; (b) to similar binding pockets located on different monomers of the same protein (i.e. a bivalent ligand is able to cross two monomers of the same target protein); or (c) to dissimilar binding pockets located on different proteins. The analysis of solved protein–ligand complexes in the PDB provides the basis for this classification. However, the question, that arises, is whether the observed binding mode represents an artefact from the crystallization process or has biological relevance.

To illustrate the above introduced classification, several examples will be presented where the development of twin drugs has been accompanied or guided by crystallographic, biophysical or molecular modeling studies.

A. Identical and non-identical twin drugs interacting with two adjacent binding sites located on the same macromolecule

In this part we discuss the binding of symmetrical and non-symmetrical twin drugs to a non-symmetrical binding site.

1. *Acetylcholinesterase inhibitors*

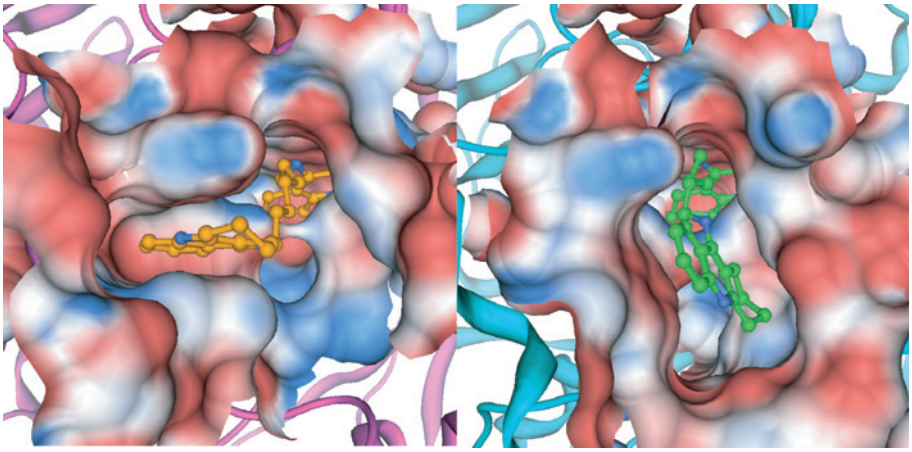
The three-dimensional structure of AChE from *Torpedo californica* revealed that the active site lies at the bottom of a deep and narrow gorge (20 Å) lined by the rings of several conserved aromatic amino acids.[95] At the "anionic subsite" of the active site, modeling suggested that the quaternary amino group of acetylcholine binds to the indole side chain of the conserved residue, Trp84, as was subsequently demonstrated for several complexes with AChE. The complexes of AChE with *bis*-quaternary ligands (decamethonium and BW284C51) led to the assignment of Trp279 as the major element of a second binding site, near the top of the active-site gorge, named "peripheral binding site," 14 Å away from the active site. These structural assignments were supported by a large body of biochemical studies, involving site-directed mutagenesis, which confirmed the importance of aromatic amino acid residues in AChE.[96] In an effort to improve drug potency and selectivity, the twin drug strategy was applied to the development of dual-site acting AChE inhibitors.[97,98] Several identical tacrine dimers, for example bis(5)-tacrine and bis(7)-tacrine, were synthesized and evaluated after computational studies predicted weak affinity of tacrine for the peripheral binding site residues (Trp279 and Tyr70) in conjunction with high affinity of tacrine for the catalytic anionic site (Trp84 and Phe330).[12,99] The increased inhibitory activity of the bivalent tacrine molecules relative to tacrine was attributed to dual-site binding.

The determination of the crystal structures of bis(5)-tacrine and bis(7)-tacrine (Figure 18.38) gave the structural basis for the observation that bis(7)-tacrine is an optimal inhibitor (*Torpedo californica* IC_{50} = 1.5 nM).[99] It forms favorable sandwich type stacking interactions in both with Trp84 (anionic site) and Trp279 (peripheral binding site) with minimal protein rearrangement. Bis(5)-tacrine which is less potent (IC_{50} = 28 nM), has only a one-sided favorable interaction in the peripheral site and induces a conformational change in the protein backbone near the acyl binding pocket. The structural changes to the native enzyme observed in the bis(5)-tacrine AChE complex showed that the selection of the optimal linker length has a dramatic influence on the inhibitory activity. These results underline the problem of current structure-based drug design approaches, where the protein structure is considered to be rigid and conformational changes are not taken into account when carrying out ligand docking.

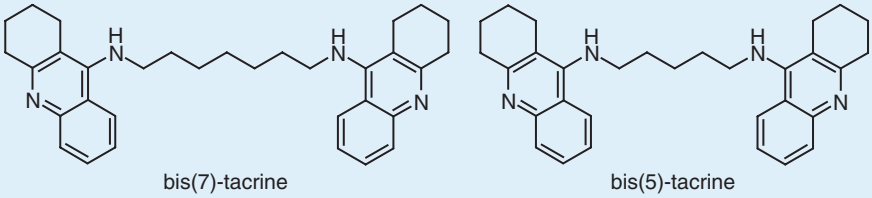
Besides tacrine also other already known "anionic site" AChE inhibitors were used to develop AChE twin drugs.[100] The development of identical and non-identical twin drugs was guided by docking studies which indicated affinity of huperzine and tacrine for both the catalytic and peripheral binding sites in AChE.[97] The modeling studies also demonstrated beneficial hydrophobic effects imparted by the alkylene linker to the peripheral site ligand.[101] Further biochemical studies of AChE revealed that the peripheral binding site at the mouth of the gorge, was implicated in promoting aggregation of the beta-amyloid (Aβ) peptide responsible for the neurodegenerative process in AD.[102] This feature of AChE initiated the development of dual-site binding inhibitors, in hopes of increasing AChE inhibition potency, and protecting neurons from Aβ toxicity.[103] The reported increased affinity for AChE of bivalent (−)-galanthamine, tacrine, hupyridone, and huperzine derivatives, and of a tacrine/propidium heterodimeric ligands, supported the design of inhibitors with dual-site binding properties (Figure 18.39).[104] The (−)-bis(10)-hupyridone inhibitors shows a inhibitory activity of 2.4 nM (*Torpedo californica* AChE) which is more than 200-fold higher compared to the monomeric AChE inhibitor huperzine A.

2. *Bisubstrate inhibitors*

Another strategy employs symmetrical and asymmetrical bivalent ligands designed to bind at the cofactor and substrate

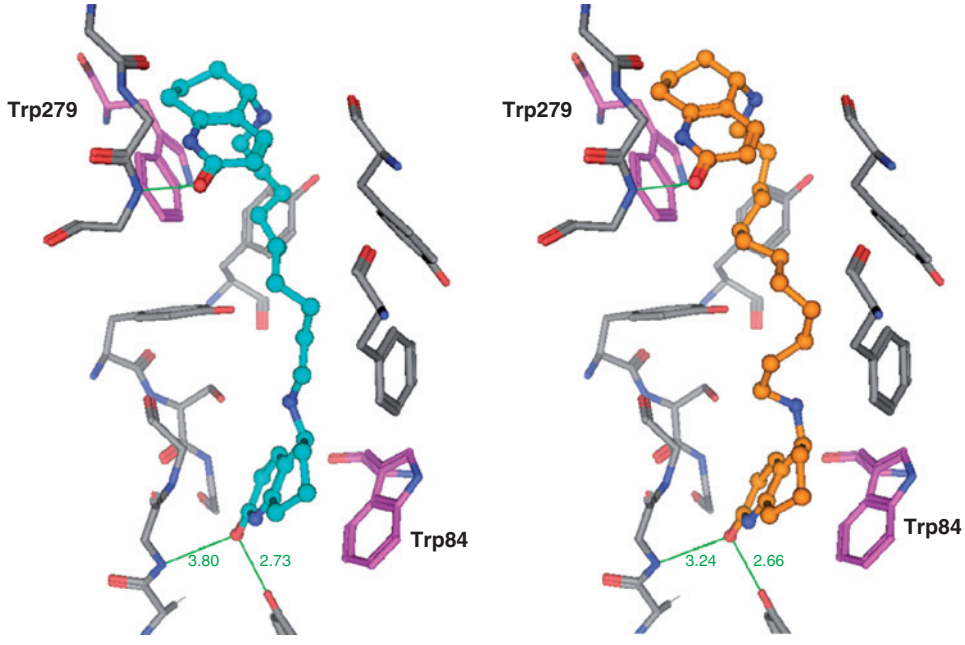


(a)



(b)

FIGURE 18.38 (a) AChE complexed with bis(7)-tacrine (left, colored orange) and bis(5)-tacrine (right, colored green). The molecular surface of the binding pocket is colored according the electrostatic potential (red = negative potential, blue = positive potential). The bis(7)-tacrine has the appropriate linker length to favorably interact with both binding sites (IC_{50} = 1.5 nM), whereas the bis(5)-tacrine can only bind in an unfavorable protein conformation (IC_{50} = 28 nM); (b) Molecular structures of the two cocrystallized AChE inhibitors.[99]



(a)

FIGURE 18.39 (a) Interaction of (−)-bis(10)-hupyridone (left side) and (−)-bis(12)-hupyridone (right side) with AChE. The two tryptophane residues of the active and peripheral binding sites are colored magenta. Hydrogen bonds between the inhibitor and the residues of the enzyme are shown as green line.

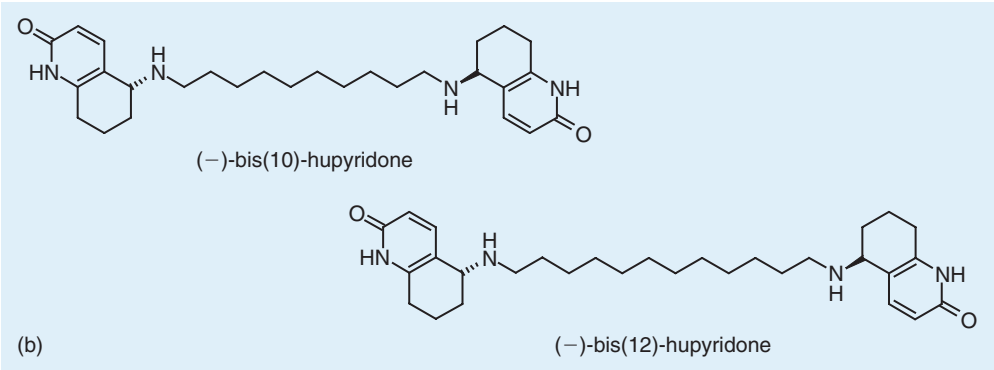


FIGURE 18.39 (Continued) (b) Molecular structures of the two cocrystallized AChE inhibitors.[104]

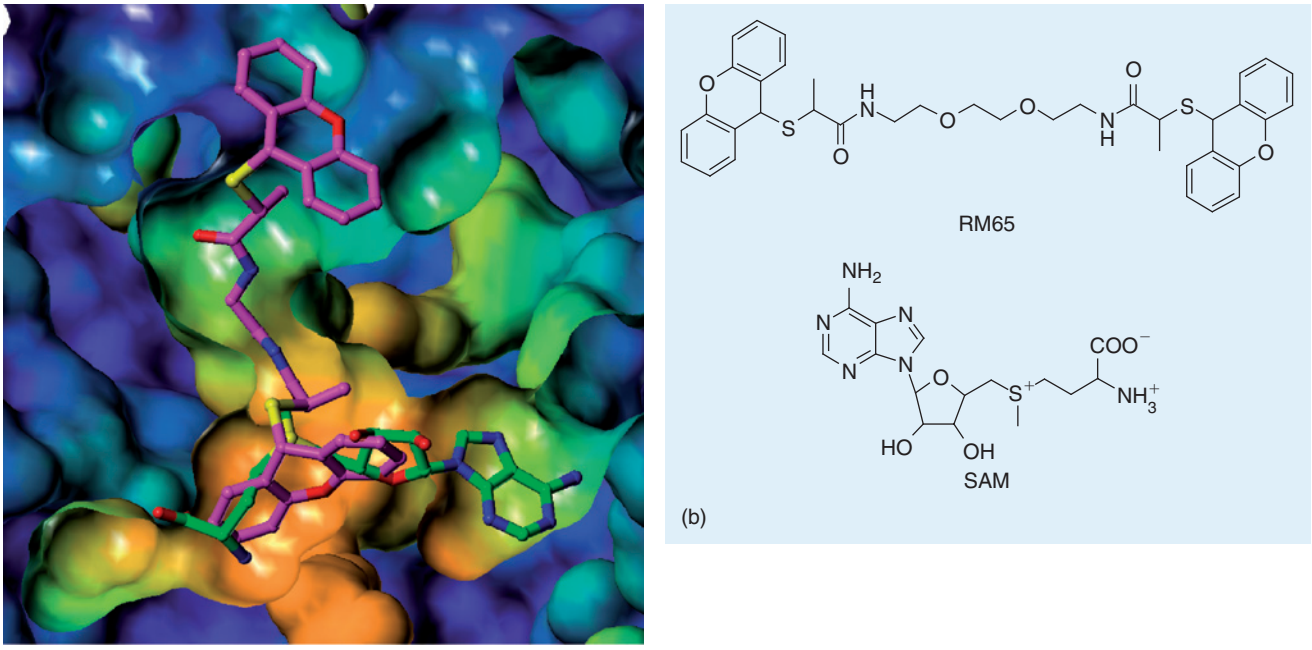


FIGURE 18.40 (a) Interaction of the bisubstrate inhibitor RM65 (magenta) and SAM (green) with the PRMT1 binding site. The symmetrical inhibitor blocks the cofactor and the substrate binding site of PRMT1; (b) Molecular structures of the bisubstrate PRMT1 inhibitor and SAM.[106]

sites of an enzyme, thus eliciting competitive inhibition. This type of ligands are called bisubstrate inhibitors. The bisubstrate concept has led to the development of compounds with powerful therapeutic properties. Mupirocin (pseudomonic acid-A) is a femtomolar inhibitor of bacterial isoleucyl-tRNA synthetase and is one of the most widely used topical antibiotic.[105] Other asymmetrical bisubstrate inhibitors have been discovered for GNC5-related *N*-acetyltransferases[106] and protein kinases.[107]

Jung *et al*. applied this approach to develop inhibitors for a histone modifying enzyme – the histone arginine methyltransferase PRMT1.[108] An identical PRMT1 inhibitor with cellular activity was obtained which blocks both the cofactor S-adenosylmethionine (SAM) and the substrate (histone) binding site simultaneously (Figure 18.40). The binding mode of RM65 is shown in Figure 18.40 in comparison with the cofactor SAM. Although the binding site is asymmetric, the symmetrical ligand shows favorable interactions with both substrate and cofactor binding site.

B. Identical twin drugs interacting with two similar binding sites located on different monomers of the same macromolecule

In this part we discuss the binding of symmetrical twin drugs to symmetrical binding sites located on different protein monomers.

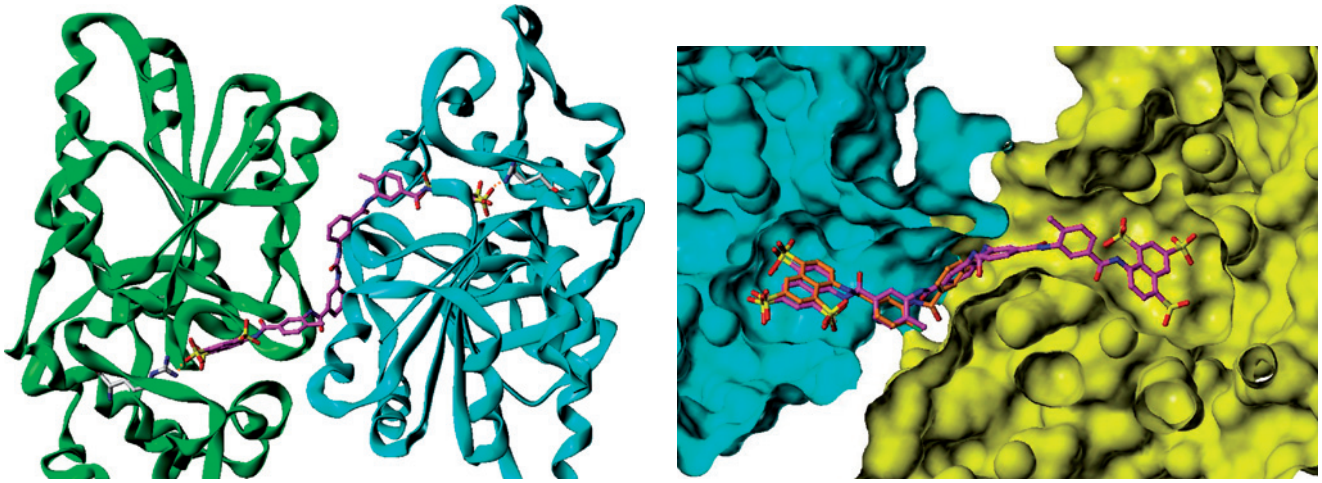
1. *Sirtuin inhibitors*

NAD^+-dependent histone deacetylases (sirtuins) are enzymes which cleave off acetyl groups from lysine residues in histones but also other proteins.[109] Reversible acetylation level is an important factor in the regulation of the activity of such proteins. Potent selective sirtuin inhibitors are interesting tools for the investigation of the biological functions of those enzymes and may be future drugs for the treatment of cancer.[110,111] The crystal structure of the sirtuin subtype Sirt5 in complex with the identical twin drug suramin revealed that two protein monomers are linked by one molecule of suramin (Figure 18.41).[112] Sirt5, which is a monomer in solution, also was found to dimerize in solution upon suramin binding, as confirmed by size-exclusion chromatography. The monomer–monomer interface is mostly non-polar and there are no direct hydrogen bonds between the two monomers, thus the dimeric structure of Sirt5 is mainly stabilized by the bound suramin molecule itself. Both in the crystal structure as well as in solution, suramin acts as a linker resulting in dimerization of Sirt5. The simultaneous binding of one molecule of suramin at the surfaces of two monomers was also observed in the crystal structure of the suramin-myotoxin II complex. For sirtuins, however, this finding might introduce a new class of inhibitors that not only bind specifically into the active site but also function as linker molecules, thus limiting enzyme mobility and accessibility. In an effort to identify more potent and selective sirtuin inhibitors the suramin structure was modified.[113] The derived derivatives were found to be potent sirtuin inhibitors, with high activity for the Sirt1 subtype. Interestingly, not only the bivalent suramin molecule was found to block Sirt1 (297 nM), but also compound NF154 (Figure 18.41a) – which resembles one half of the suramin structure – is a potent Sirt1 inhibitor (525 nM).[113] Docking studies showed that NF154 interacts in a similar way with the sirtuin binding pocket. However, cross-linking of the two sirtuin monomers is not possible (Figure 18.41b).

2. *Glutathione S-transferase inhibitors*

Glutathione *S*-transferases (GSTs)1 catalyze the conjugation of the nucleophilic tripeptide glutathione (GSH, γ-Glu-Cys-Gly) to structurally diverse hydrophobic electrophiles. Among the electrophilic substrates for GSTs are alkylating agents used in cancer chemotherapy. GSTs are known to be overexpressed in malignant tissues suggesting that they may play a role in acquired resistance to antitumor agents[114] Therefore, the coadministration of potent, selective GST inhibitors as adjuvants to chemotherapy has emerged as a possible strategy to restore the drug sensitivity of resistant cells.[115] The geometry of the GST dimer, with its two identical active sites at opposite ends of a solvent-accessible intersubunit cleft, presents an opportunity to design symmetrical twin drugs that occupy both active sites simultaneously[116] The symmetrical inhibitors developed by Atkins *et al*. were found to bind to the protein dimer possessing two equivalent active sites in close proximity (about 20 Å apart).[117]

The available crystal structure of GST in complex with an anthrachinone sulfonate derivative (Figure 18.42) was used as a basis for the development of novel potent inhibitors. The authors used a related molecule, the Uniblue A derivative shown in Figure 18.43, to design twin drug molecules and to analyze the free energy of binding by isothermal titration calorimetry (ITC) measurements.[108] Because of the proximity of the two active sites of the GST dimer and their location at opposite ends of the solvent-accessible



(a) (b)

FIGURE 18.41 (a) Cross-linking of two Sirt5 monomers (cyan and green) by suramin (magenta). Hydrogen bonds are shown as orange line; (b) Binding mode of suramin and NF154.

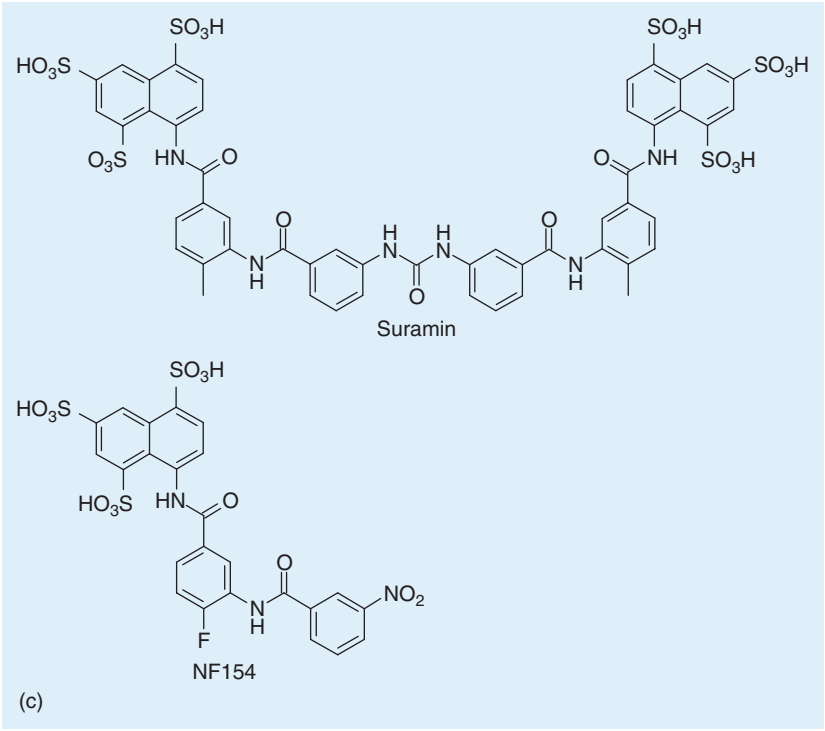


FIGURE 18.41 (Continued) (c) Molecular structures of suramin and NF154.

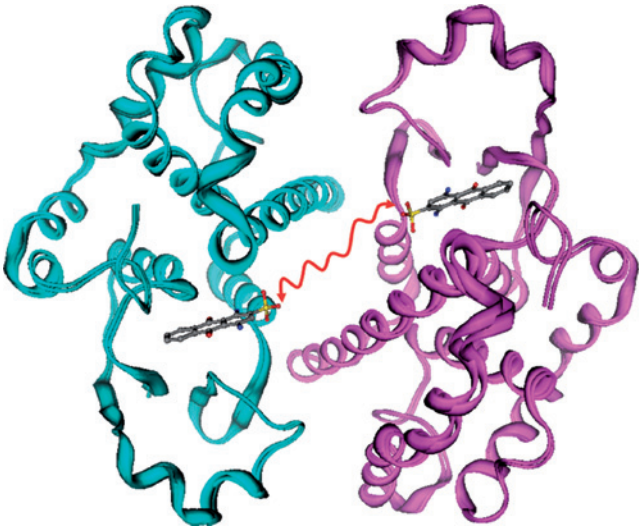


FIGURE 18.42 Ribbon diagram of the complex with the anthrachinone sulfonate moiety and the GST dimer. The anthrachinone sulfonate moieties, as observed in the X-ray structure, are shown in capped sticks. The connection of these moieties by appropriate linkers (indicated by the red arrow) resulted in potent symmetrical GST inhibitors.[108]

intersubunit cleft, the authors reasoned that bivalent inhibitors could be designed to simultaneously occupy both binding sites. The appropriate linker length to bridge both monomers was guided by docking and molecular modeling studies. Whereas the monomer Uniblue A derivative showed an IC_{50} of 5000 nM, the twin drug is more than 100-fold more active (IC_{50} 44 nM). The ITC data supported the increased affinity of the bivalent inhibitors versus the monomeric analogs as observed in the inhibition experiments. The observed difference in ΔH between the two inhibitors is very close to the expected change for a bivalent inhibitor in which the linker does not interact significantly with the protein. The ΔH value for the twin drug molecule was found to be twice as large as for the monomeric inhibitor. On the other site a decreased entropy is observed for the bivalent inhibitor. The decrease in entropy upon binding of the bivalent inhibitor was assumed to arise from immobilization of the flexible linker, which contains 10 rotatable bonds. The restriction of rotational freedom within the linker upon binding offsets any entropic gain of limiting the translational entropy of the binding element in the bivalent compound. In conclusion, the basis for the improved affinity of the identical twin drug is significantly more favorable binding enthalpy, which is partially offset by a less favorable binding entropy.

3. *G-protein-coupled receptor ligands*

G-protein-coupled receptors (GPCRs) are membrane proteins that are characterized by a common seven helix transmembrane motif. The crucial role GPCRs play in many biological processes and the availability of selective small molecule GPCR ligands explain why GPCRs are among the most important of all target families.[118] In the last decades, increasing evidence has become available that GPCRs,

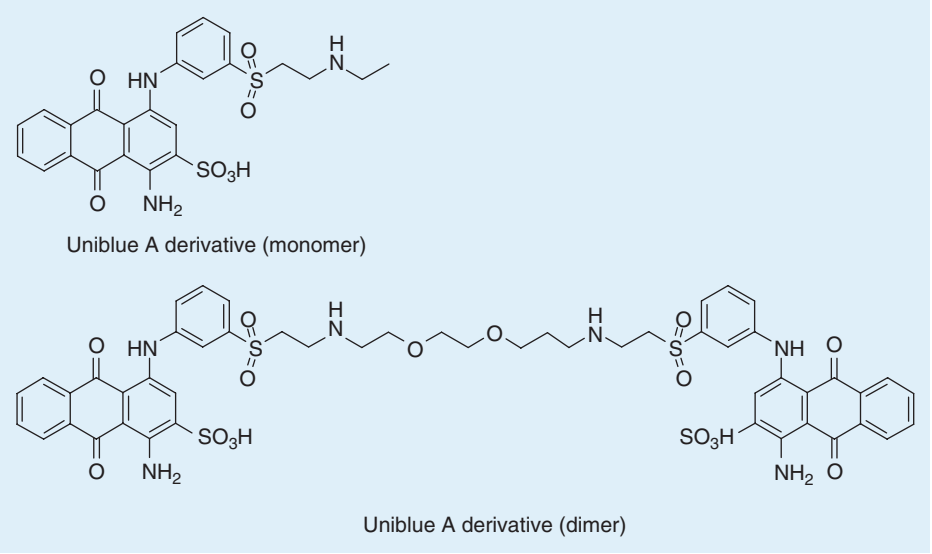


FIGURE 18.43 Molecular structures of GST inhibitors.

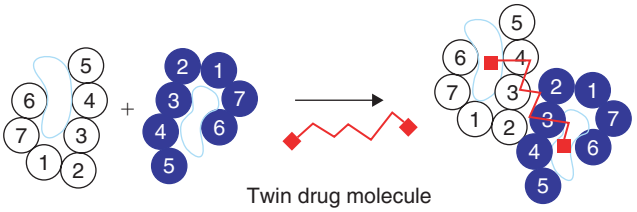


FIGURE 18.44 GPCR dimerization and binding of a twin drug molecule. The bivalent ligand bridges two independent GPCR binding sites.

upon activation, dimerize to its active form and subsequently produce its biological action. GPCRs assemble in the cell membrane as either homodimers or heterodimers.[119] Studies showing that GPCR heterodimerization can modify the receptor pharmacology have sparked an interest in the development of drugs that selectively target receptor heterodimers. One approach to target a pair of GPCRs has been to synthesize and use twin molecules targeting the two receptor binding sites on the homo or heterodimer simultaneously.[120] The rationale for employing the twin drug approach for GPCRs stems from the possibility that bivalent ligands may be capable of bridging independent receptor binding pockets on a receptor dimer resulting in a thermodynamically more favorable interactions than a monovalent binding of two ligands. This type of interaction is shown schematically in Figure 18.44.

A major breakthrough in the understanding of the GPCR family was achieved in 2000, when the crystal structure of bovine rhodopsin was resolved, and for the first time detailed structural insights were gained.[121] The structure of bovine rhodopsin was used by various groups for the generation of homology models of GPCRs. These models were used to guide the design of bivalent ligands addressing two GPCR binding sites. Portoghese *et al.* reported several identical twin molecules with varying linker length designed to investigate pharmacodynamic and organizational features of opioid receptors.[35,122,123] The twin drug approach has been shown to be generally applicable to other GPCRs. So far bivalent ligands for adenosine,[124] dopamine,[125] gonadotropin releasing hormone,[126] melatonine,[39] muscarine,[127] opioid, serotonin[128] and vasopressin receptors[129] have been reported.

Recently carried out molecular modeling studies on GPCR dimer models provided first information about the distance between the two individual binding pockets.[130] The evaluation of the minimal length between the two spacer attachment points revealed a minimal distance of approximately 22 Å.[131] Modeling with shorter spacers of (14 atoms) gave unfavorable highly extended conformation of the flexible linker, whereas a more favorable and extended conformation was obtained for longer spacers. The observed optimal distance is in good agreement with the data from the known bivalent ligands (examples are given in Figure 18.45).

C. Identical and non-identical twin drugs interacting with two different binding sites located on different macromolecules

1. GPCR (*heterodimer*) *ligands*

In the previous part we have discussed the binding of identical twin drugs to GPCR homodimers. However, it was recently also shown that many monomers from different GPCRs are able to interact with each other resulting in the formation of heterodimers.[118] An increasing number of studies point toward a role for GPCR heterodimerization

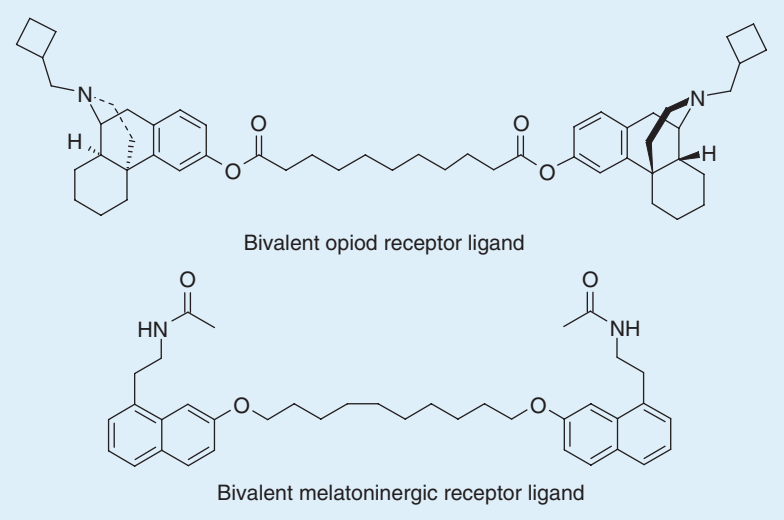


FIGURE 18.45 Molecular structure of identical twin drugs for GPCR homodimers.

in modulating receptor pharmacology and suggest that heterodimers could represent a functional unit. Thus, the design of selective bivalent ligands might be a promising strategy. A few examples have been published so far, where heterodimer selective ligands were found to have lesser side effects because of their greater selectivity. Portoghese *et al.* synthesized a series of bivalent ligands for opioid receptor dimers, consisting of a μ receptor agonist pharmacophore and a δ receptor antagonist pharmacophore separated by spacers of variable lengths.[35,120,121] The antinociceptive activity of the bivalent ligands was superior to that achieved by the coadministration of individual opioid receptor ligands.

The future analysis of the binding of such ligands to heterodimers and homodimers, guided by structure-based modeling on GPCR dimers, will provide insights into the molecular determinants required for selective occupancy and/or activation of heterodimers.[132]

V. CONCLUSION

Drugs combining two pharmacophores in a single molecule have been described in numerous domains of medicinal chemistry. Historically, they resulted from empirical structural modifications, but today rational design of homo and hetero ligands may involve the knowledge of the structure of the protein which contains these binding sites. Already, the literature contains several rational approaches to the discovery of identical and non-identical twin drugs. One problem which has to be further addressed is the physicochemical property of twin drug molecules. More sophisticated design strategies and computational tools will certainly be needed for that. Greater application of structure-based approaches and pharmacophore modeling will facilitate the design of molecules showing desired activities and optimal pharmacological profiles. Computer-based methods that can rapidly search for similar binding sites will help to predict off-site effects. Protein X-ray crystallography has revealed in several cases a high degree of symmetry resulting from the existence of dimeric (C-2), trimeric (C-3) or tetrameric protein assemblies. Simultaneous interaction of identical twin drugs (symmetrical binding sites) has to be associated with the increasing potency observed in many cases. The dimer could also show an improved selectivity profile (e.g. selectivity between different isoforms of an enzyme) compared to the initial monomer. However, combining in a same molecule two non-identical pharmacophores leads to a new compound, which may not bind simultaneously to each of the considered binding site. Recent findings in molecular pharmacology, molecular biology, enzymology and physiology will help to select pertinent pairs of targets involved in different pathologies. The search for a global synergic effect would be the goal in this approach.

For therapeutic purposes the earlier search of selective drugs is replaced by the design of non-selective dual drugs with tuning of their selectivity profile. The design of dual acting drugs is more complex but more challenging than the conventional design of a compound with a single activity. Today, the concept of "one target–one ligand" is still the major approach for drug development in pharmaceutical industry but the design of ligands, able to act on different targets simultaneously, represents probably an efficient alternative in the treatment of complex diseases or disorders. A recent review has reported and analyzed literature examples of multiple ligands combinations.[2] Even if successful examples have been reported in this chapter, some disadvantages may exist in the using of the twin drug approach: (i) Combining two pharmacophore components in a single molecule may lead to an inactive compound. A good knowledge of SAR data within each pharmacophore (types of interaction, steric hindrance-sensitive

regions, local hydrophilic and hydrophobic areas), and the choice of the linker (nature, position of the linkage) are critical for the success of the approach. (ii) The hybrid showed the awaited pharmacological profile, but the attempt failed because of non-predicted pharmacodynamic and toxicological problems. (iii) The balanced potency of the dual acting drugs has to be carefully evaluated. Design of agonist/antagonist hybrids has to take in consideration that drugs with antagonistic activity on receptors usually have to be given in concentrations significantly lesser than those needed for agonists (affinities in the nM and μM range respectively). In a similar manner, design of hybrids combining a receptor ligand and an enzyme inhibitor should take into account both efficacies, and particularly kinetic properties of the considered enzyme. However, the approach is workable and successful attempts have been obtained in cardiovascular domain, particularly for the treatment of hypertension, and in central nervous system research. This approach should be applied for the treatment of diseases that need restoration of the dopaminergic or cholinergic balance. In spite of this, numerous recent works dealing with the design of twin drugs acting in various systems have been reported in this chapter, and account for the increasing interest of this approach in drug design.

In a general manner, medicinal chemists should take into account the use of the twin drug approach as soon as they get a lead compound that need to be optimized. Dimerization of a lead or association of two different pharmacophores must be considered during the primary exploration of SARs as well as isosteric replacement, homology and conformational restriction are used during this process.

REFERENCES

1. Contreras, J. M., Bourguignon, J. J. Identical and non-identical twin drugs. In *The Practice of Medicinal Chemistry* (Wermuth, C. G., Ed.). Academic Press: London, 2003, pp. 251–273.
2. Morphy, R., Kay, C., Rankovic, Z. From magic bullets to designed multiple ligands. *Drug Discov. Today* 2004, *9*, 641–651.
3. Morphy, R., Rankovic, Z. Designed multiple ligands. An emerging drug discovery paradigm. *J. Med. Chem.* 2005, *48*, 6523–6543.
4. Csermely, P., Agoston, V., Pongor, S. The efficiency of multi-target drugs: the network approach might help drug design. *Trends Pharmacol. Sci.* 2005, *26*, 178–182.
5. Mencher, S. K., Wang, L. G. Promiscuous drugs compared to selective drugs (promiscuity can be a virtue). *BMC Clin. Pharmacol.* 2005, *5*, 3.
6. Sams-Dodd, F. Target-based drug discovery: is something wrong?. *Drug Discov. Today,* 2005, *10*, 139–147.
7. Frantz, S. Drug discovery: playing dirty. *Nature,* 2005, *437*, 942–943.
8. Morphy, R., Rankovic, Z. The physicochemical challenges of designing multiple ligands. *J. Med. Chem.* 2006, *49*, 4961–4970.
9. Espinoza-Fonseca, L. M. The benefits of the multi-target approach in drug design and discovery. *Bioorg. Med. Chem.* 2006, *14*, 896–897.
10. Morphy, R., Rankovic, Z. Fragments, network biology and designing multiple ligands. *Drug Discov. Today,* 2007, *12*, 156–160.
11. Pang, Y.-P., Quiram, P., Jelacic, T., Hong, F., Brimijoin, S. Highly potent, selective, and low cost *bis*-tetrahydroaminacrine inhibitors of acetylcholinesterase. *J. Biol. Chem.* 1996, *271*, 23646–23649.
12. Hu, M. K., Wu, L. J., Hsiao, G., Yen, M. H. Homodimeric tacrine congeners as acetylcholinesterase inhibitors. *J. Med. Chem.* 2002, *45*, 2277–2282.
13. Baldwin, J. J., Lumma, W. C., Lundell, G. F., Ponticello, G. S., Raab, A. W., Engelhardt, E. L., Hirschmann, R., Sweet, C. S., Scriabine, A. Symbiotic approach to drug design: antihypertensive β-adrenergic blocking agents. *J. Med. Chem.* 1979, *22*, 1284–1290.
14. Cecchetti, V., Fravolini, A., Schiaffella, F., Tabarrini, O., Bruni, G., Segre, G. *o*-Chlorobenzenesulfonamidic derivatives of (aryloxy)propanolamines as β-blocking/diuretic agents. *J. Med. Chem.* 1993, *36*, 157–161.
15. Murugesan, N., Gu, Z., Fadnis, L., Tellew, J. E., Baska, R. A., Yang, Y., Beyer, S. M., Monshizadegan, H., Dickinson, K. E., Valentine, M. T., Humphreys, W. G., Lan, S. J., Ewing, W. R., Carlson, K. E., Kowala, M. C., Zahler, R., Macor, J. E. Dual angiotensin II and endothelin A receptor antagonists: synthesis of 2′-substituted *N*-3-isoxazolyl biphenylsulfonamides with improved potency and pharmacokinetics. *J. Med. Chem.* 2005, *48*, 171–179.
16. Piwinski, J. J., Wong, J. K., Green, M. J., Ganguly, A. K., Billah, M. M., West, R. E., Jr, Kreutner, W. Dual antagonists of platelet activating factor and histamine. Identification of structural requirements for dual activity of *N*-acyl-4-(5,6-dihydro-11*H*-benzo[5,6]cyclohepta-[1,2-*b*]pyridin-11-ylene) piperidines. *J. Med. Chem.* 1991, *34*, 457–461.
17. Stephenson, V. C., Heyding, R. A., Weaver, D. F. The "promiscuous drug concept" with applications to Alzheimer's disease. *FEBS Lett.* 2005, *579*, 1338–1342.
18. Changeux, J. P. Remarks on the symmetry and cooperative properties of biological membranes. In *Symmetry and Function of Biological Systems at the Macromolecular Level* (Engström, A., Strandberg, B., Eds), Almqvist and Wiksell: Stockholm, 1969, pp. 235–256.
19. Blundell, T., Sewell, T., Turnell, B. Symmetry in the structure and organization of proteins. In *Structural Studies on Molecules of Biological Interest* (Dodson, G., Glusker, J. P., Sayre, D., Eds), Oxford University Press: London, 1981, pp. 390–403.
20. Sidddle, K. The insulin receptor. In *Receptor Subunits and Complexes* (Burgen, A., Barnard, E. A., Eds), Cambridge University Press: Cambridge, 1992, pp. 261–351.
21. Ariëns, E. J. Drug design: a general introduction. In *Drug Design* (Ariëns, E. J., Ed.), Vol. 1. Academic Press: New York, 1971, pp. 1–270.
22. Bell, M. R., Batzold, F. H., Winneker, R. C. Chemical control of fertility. In *Annual Reports in Medicinal Chemistry* (Bailey, D. M., Ed.), Vol. 21. Academic Press: San Diego, 1986, pp. 169–177.
23. Neupert-Laves, K., Dobber, M. The crystal structure of a K+ complex of valinomycin. *Helv. Chim. Acta.* 1975, *58*, 432–442.
24. Tamiz, A. P., Bandyopadhyay, B. C., Zhang, J., Flippen-Anderson, J. L., Zhang, M., Wang, C. Z., Johnson, K. M., Tella, S., Kozikowski, A. P. Pharmacological and behavioral analysis of the effects of some bivalent ligand-based monoamine reuptake inhibitors. *J. Med. Chem.* 2001, *44*, 1615–1622.
25. Kierstead, R. W., Faraone, A., Mennona, F., Mullin, J., Guthrie, R. W., Crowley, H. Beta-1-selective adrenoceptor antagonists. Blocking activity of a series of binary (aryloxy) propanolamines. *J. Med. Chem.* 1983, *26*, 1561–1569.
26. Melchiorre, C., Bolognesi, M. L., Chiarini, A., Minarini, A., Spampinato, S. Synthesis and biological activity of some methoctramine-related tetraamines bearing a 11-acetyl-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]-benzodiazepin-6-one moiety as antimuscarinics: a second generation of highly selective M_2 muscarinic receptor antagonists. *J. Med. Chem.* 1993, *36*, 3734–3737.
27. Rosini, M., Budriesi, R., Bixel, M. G., Bolognesi, M. L., Chiarini, A., Hucho, F., Krogsgaard-Larsen, P., Mellor, I. R., Minarini, A., Tumiatti, V., Usherwood, P. N. R., Melchiorre, C. Design, synthesis, and biological evaluation of symmetrically and unsymmetrically substituted methoctramine-related polyamines as muscular nicotinic

receptor noncompetitive antagonists. *J. Med. Chem.* 1999, *42*, 5212–5223.
28. Rosini, M., Bixel, M. G., Marucci, G., Budriesi, R., Krauss, M., Bolognesi, M. L., Minarini, A., Tumiatti, V., Hucho, F., Melchiorre, C. Structure-activity relationships of methoctramine-related polyamines as muscular nicotinic receptor noncompetitive antagonists. 2. Role of polymethylene chain lengths separating amine functions and of substituents on the terminal nitrogen atoms. *J. Med. Chem.* 2002, *45*, 1860–1878.
29. Wei, Z. L., Xiao, Y., Kellar, K. J., Kozikowski, A. P. Synthesis and pharmacological characterization of bivalent ligands of epibatidine at neuronal nicotinic acetylcholine receptors. *Bioorg. Med. Chem. Lett.* 2004, *14*, 1855–1858.
30. Roth, R. A., Cassell, D. J., Morgan, D. O., Tatnell, M. A., Jones, R. H., Schüttler, A., Brandenburg, D. Effects of covalently linked insulin dimers on receptor kinase activity and receptor down regulation. *FEBS Lett.* 1984, *170*, 360–364.
31. Fauchère, J. C., Rossier, M., Capponi, A., Vallotton, M. B. Potentiation of the antagonistic effect of $ACTH_{11–24}$ on steroidogenesis by synthesis of covalent dimeric conjugates. *FEBS Lett.* 1985, *183*, 283–286.
32. Chino, N., Yoshizawa-Kumagaye, K., Noda, Y., Watanabe, T. X., Kimura, T., Sakakibara, S. Synthesis and biological properties of antiparrallel and parallel dimers of human α-human atrial natriuretic peptide. *Biochem. Biophys. Res. Comm.* 1986, *141*, 665–672.
33. Salvino, J. M., Seoane, P. R., Douty, B. D., Awad, M. A., Dolle, R. E., Houck, W. T., Faunce, D. M., Sawutz, D. G. Design of potent nonpeptide competitive antagonists of the human bradykinin B_2 receptor. *J. Med. Chem.* 1993, *36*, 2583–2584.
34. Costa, T., Wüster, M., Herz, A., Shimohigashi, Y., Chen, H.-C., Rodbard, D. Receptor binding and biological activity of bivalent enkephalins. *Biochem. Pharmacol.* 1985, *34*, 25–30.
35. Portoghese, P. S., Larson, D. L., Sayre, L. M., Yim, C. B., Ronsisvalle, G., Tam, S. W., Takemori, A. E. Opioid agonist and antagonist bivalent ligands. The relationship between spacer length and selectivity at multiple opioid receptors. *J. Med. Chem.* 1986, *29*, 1855–1861.
36. Lin, C.-E., Takemori, A. E., Portoghese, P. S. Synthesis and κ-opioid antagonist selectivity of a norbinaltorphimine congener. Identification of the address moiety required for κ-antagonist activity. *J. Med. Chem.* 1993, *36*, 2412–2415.
37. Portoghese, P. S. Bivalent ligands and the message-address concept in the design of selective opioid receptor antagonists. *Trends Pharmacol. Sci.* 1989, *10*, 230–235.
38. Joslyn, A. F., Luchowski, E., Triggle, D. J. Dimeric 1,4-dihydropyridines as calcium channel antagonists. *J. Med. Chem.* 1988, *31*, 1489–1492.
39. Descamps-Francois, C., Yous, S., Chavatte, P., Audinot, V., Bonnaud, A., Boutin, J. A., Delagrange, P., Bennejean, C., Renard, P., Lesieur, D. Design and synthesis of naphthalenic dimers as selective MT1 melatoninergic ligands. *J. Med. Chem.* 2003, *46*, 1127–1129.
40. Sauerberg, P., Bury, P. S., Mogensen, J. P., Deussen, H. J., Pettersson, I., Fleckner, J., Nehlin, J., Frederiksen, K. S., Albrektsen, T., Din, N., Svensson, L. A., Ynddal, L., Wulff, E. M., Jeppesen, L. Large dimeric ligands with favorable pharmacokinetic properties and peroxisome proliferator-activated receptor agonist activity *in vitro* and *in vivo*. *J. Med. Chem.* 2003, *46*, 4883–4894.
41. De Clerq, E. Toward improved anti-HIV chemotherapy: therapeutic strategies for intervention with HIV infections. *J. Med. Chem.* 1995, *38*, 2491–2517.
42. Appelt, K. Crystal structures of HIV-1 protease-inhibitor complexes. *Persp. Drug Discov. Des.* 1993, *1*, 23–48.
43. Bone, R., Vacca, J. P., Anderson, P. S., Holloway, M. K. X-ray crystal structure of the HIV protease complex with L-700,417, an inhibitor with pseudo C_2 symmetry. *J. Am. Chem. Soc.* 1991, *113*, 9382–9384.
44. Tan, G. T., Wickramasinghe, A., Verma, S., Singh, R., Hughes, S. H., Pezzuto, J. M., Baba, M., Mohan, P. Potential anti-AIDS naphtalenesulfonic acid derivatives. Synthesis and inhibition of HIV-1 induced cytopathogenesis and HIV-1 and HIV-2 reverse transcriptase activity. *J. Med. Chem.* 1992, *35*, 4846–4853.
45. Erickson, J. W., Neidhardt, D. J., VanDrie, J., Kempf, D. J., Wang, X. C., Norbeck, D. W., Plattner, J. J., Rittenhouse, J. W., Turon, M., Wideburg, N., Kohlbrenner, W. E., Simmer, R., Helfrich, R., Paul, D. A., Knigge, M. Design, activity, and 2.8 angström crystal structure of a C_2 symmetric inhibitor complexed to HIV-1 protease. *Science* 1990, *249*, 527–533.
46. Hulten, J., Bonham, N. M., Nillroth, U., Hansson, T., Zuccarello, G., Bouzide, A., Aqvist, J., Classon, B., Danielson, U. H., Karlen, A., Kvarnstrom, I., Samuelsson, B., Hallberg, A. Cyclic HIV-1 protease inhibitors derived from mannitol: synthesis, inhibitory potencies, and computational predictions of binding affinities. *J. Med. Chem.* 1997, *40*, 885–897.
47. Alterman, M., Bjorsne, M., Muhlman, A., Classon, B., Kvarnstrom, I., Danielsson, H., Markgren, P.-O., Nillroth, U., Unge, T., Hallberg, A., Samuelsson, B. Design and synthesis of new potent C_2-symmetric HIV-1 protease inhibitors. Use of the l-mannaric acid as a peptidomimetic scaffold. *J. Med. Chem.* 1998, *41*, 3782–3792.
48. Sridhar, J., Wei, Z. L., Nowak, I., Lewin, N. E., Ayres, J. A., Pearce, L. V., Blumberg, P. M., Kozikowski, A. P. New bivalent PKC ligands linked by a carbon spacer: enhancement in binding affinity. *J. Med. Chem.* 2003, *46*, 4196–4204.
49. Qin, D., Sullivan, R., Berkowitz, W. F., Bittman, R., Rotenberg, S. A. Inhibition of protein kinase Cα by dequalinium analogues: dependence on the linker length and geometry. *J. Med. Chem.* 2003, *43*, 1413–1417.
50. Rossello, A., Nuti, E., Catalani, M. P., Carelli, P., Orlandini, E., Rapposelli, S., Tuccinardi, T., Atkinson, S. J., Murphy, G., Balsamo, A. A new development of matrix metalloproteinase inhibitors: twin hydroxamic acids as potent inhibitors of MMPs. *Bioorg. Med. Chem. Lett.* 2005, *15*, 2311–2314.
51. Shaw, K. J., Guilford, W. J., Dallas, J. L., Koovakkaat, S. K., McCarrick, M. A., Liang, A., Light, D. R., Morrissey, M. M. (Z,Z)-2,7-Bis(4-amidinobenzylidene)cycloheptan-1-one: identification of a highly active inhibitor of blood coagulation factor Xa. *J. Med. Chem.* 1998, *41*, 3551–3556.
52. Phillips, G., Davey, D. D., Eagen, K. A., Koovakkat, S. K., Liang, A., Ng, H. P., Pinkerton, M., Trinh, L., Whitlow, M., Beatty, A. M., Morrissey, M. M. Design, synthesis, and activity of 2,6-diphenoxypyridine-derived factor Xa inhibitors. *J. Med. Chem.* 1999, *42*, 1749–1756.
53. Stanek, J., Caravatti, G., Capraro, H.-G., Furet, P., Mett, H., Schneider, P., Regenass, U. *S*-Adenosylmethionine decarboxylase inhibitors: new aryl and heteroaryl analogues of methylglyoxal bis(guanylhydrazone). *J. Med. Chem.* 1993, *36*, 46–54.
54. Taylor, P., Lappi, S. Interaction of fluorescence probes with acetylcholinesterase. The site and specificity of propidium binding. *Biochemistry* 1975, *14*, 1989–1997.
55. Fairley, T. A., Tidwell, R. R., Donkor, I., Naiman, N. A., Ohemeng, K. A., Lombardy, R. J., Bentley, J. A., Cory, M. Structure, DNA minor groove binding, and base pair specificity of alkyl- and aryl-linked bis(amidinobenzimidazoles) and bis(amidinoindoles). *J. Med. Chem.* 1993, *36*, 1746–1753.
56. Gamage, S. A., Spicer, J. A., Atwell, G. J., Finlay, G. J., Baguley, B. C., Denny, W. A. Structure-activity relationships for substituted bis(acridine-4-carboxamides): a new class of anticancer agents. *J. Med. Chem.* 1999, *42*, 2383–2393.
57. Vennerström, J. L., Ellis, W. Y., Ager, A. L., Jr, Anderson, S. L., Gerena, L., Milhous, W. K. Bisquinolines. 1. *N*,*N*-bis(7-Chloroquinolin-4-yl) alkanediamines with potential against chloroquine-resistant malaria. *J. Med. Chem.* 1992, *35*, 2129–2134.

58. Girault, S., Grellier, P., Berecibar, A., Maes, L., Mouray, E., Lemière, P., Debreu, M.-A., Davioud-Charvet, E., Sergheraert, C. Antimalarial, antitrypanosomal, and antileishmanial activities and cytotoxiciy of bis(9-amino-6-chloro-2-methoxyacridines): influence of the linker. *J. Med. Chem.* 2000, *43*, 2646–2654.
59. Mewshaw, R. E., Silverman, L. S., Mathew, R. M., Kaiser, C., Sherrill, R. G., Cheng, M., Tiffany, C. W., Karbon, E. W., Bailey, M. A., Borosky, S. A., Ferkany, J. W., Abreu, M. E. Bridged γ-carbolines and derivatives possessing selective and combined affinity for 5-HT_2 and D_2 receptors. *J. Med. Chem.* 1993, *36*, 1488–1495.
60. LoweIII, J. A., Seeger, T. F., Nagel, A. A., Howard, H. R., Seymour, P. A., Heym, J. H., Ewing, F. E., Newman, M. E., Schmidt, A. W., Furman, J. S. *et al* 1-Naphthylpiperazine derivatives as potential atypical antipsychotic agents. *J. Med. Chem.* 1991, *34*, 1860–1866.
61. Howard, H. R., LoweIII, J. A., Seeger, T. F., Seymour, P. A., Zorn, S. H., Maloney, P. R., Ewing, F. E., Newman, M. E., Schmidt, A. W., Furman, J. S., Robinson, G. L., Jackson, E., Johnson, C., Morrone, J. 3-Benzisothiazolylpiperazine derivatives as potential atypical antipsychotic agents. *J. Med. Chem.* 1996, *39*, 143–148.
62. Buschauer, A. Synthesis and *in vitro* pharmacology of arpromidine and related phenyl(pyridylalkyl)guanidines, a potential new class of positive inotropic drugs. *J. Med. Chem.* 1989, *32*, 1963–1970.
63. Ohshima, E., Takami, H., Harakawa, H., Sato, H., Obase, H., Miki, I., Ishii, A., Ishii, H., Sasaki, Y., Ohmori, K., Karasawa, A., Kubo, K. Dibenz[*b,e*]oxepin derivatives: novel antiallergic agents possessing thromboxane A_2 and histamine H_1 dual antagonizing activity 1. *J. Med. Chem.* 1993, *36*, 417–420.
64. Zhang, J. C., Entzeroth, M., Wienen, W. Characterization of BIBS 39 and BIBS 222: two new non peptide angiotensine II receptor antagonists. *Eur. J. Pharmacol.* 1992, *218*, 35–41.
65. de Laszlo, S. E., Quagliato, C. S., Greenlee, W. J., Patchett, A. A., Chang, R. S., Lotti, V. J., Chen, T. B., Scheck, S. A., Faust, K. A., Kivlighn, S. S. *et al* A potent, orally active balanced affinity angiotensin II AT1 antagonist and AT2 binding inhibitor. *J. Med. Chem.* 1993, *36*, 3207–3210.
66. Ikeda, T., Ohta, H., Okada, M., Kawai, N., Nakao, R., Siegl, P. K., Kobayashi, T., Miyauchi, T., Nishikibe, M. Antihypertensive effects of a mixed endothelin-A- and -B-receptor antagonist, J-104132, were augmented in the presence of an AT1-receptor antagonist, MK-954. *J. Cardiovasc. Pharmacol.* 2000, *36*, S337–S341.
67. Bohlender, J., Gerbaulet, S., Kramer, J., Gross, M., Kirchengast, M., Dietz, R. Synergistic effects of AT(1) and ET(A) receptor blockade in a transgenic, angiotensin II-dependent, rat model. *Hypertension*, 2000, *35*, 992–997.
68. Murugesan, N., Tellew, J. E., Gu, Z., Kunst, B. L., Fadnis, L., Cornelius, L. A., Baska, R. A., Yang, Y., Beyer, S. M., Monshizadegan, H., Dickinson, K. E., Panchal, B., Valentine, M. T., Chong, S., Morrison, R. A., Carlson, K. E., Powell, J. R., Moreland, S., Barrish, J. C., Kowala, M. C., Macor, J. E. Discovery of *N*-isoxazolyl biphenylsulfonamides as potent dual angiotensin II and endothelin A receptor antagonists. *J. Med. Chem.* 2002, *45*, 3829–3835.
69. Jacobson, K. A., Lipkowski, A. W., Moody, T. W., Padgett, W., Pijl, E., Kirk, K. L., Daly, J. W. Binary drugs: conjugates of purines and a peptide that bind to both adenosine and substance P. *J. Med. Chem.* 1987, *30*, 1529–1532.
70. Willson, T. M., Brown, P. J., Sternbach, D. D., Henke, B. R. The PPARs: from orphan receptors to drug discovery. *J. Med. Chem.* 2000, *43*, 527–550.
71. Brooks, D. A., Etgen, G. J., Rito, C. J., Shuker, A. J., Dominianni, S. J., Warshawsky, A. M., Ardecky, R., Paterniti, J. R., Typhonas, J., Karanewsky, D. S., Kauffman, R. F., Broderick, C. L., Oldham, B. A., Montrose-Rafizadeh, C., Winneroski, L. L., Faul, M. M., McCarthy, J. R. Design and synthesis of 2-methyl-2-(4-[2-(5-methyl-2-aryloxazol-4-yl)ethoxy]phenoxy)propionic acids: a new class of dual PPARα/γ agonists. *J. Med. Chem.* 2001, *44*, 2061–2064.
72. Kasuga, J., Yamasaki, D., Araya, Y., Nakagawa, A., Makishima, M., Doi, T., Hashimoto, Y., Miyachi, H. Design, synthesis, and evaluation of a novel series of alpha-substituted phenylpropanoic acid derivatives as human peroxisome proliferator-activated receptor (PPAR) alpha/delta dual agonists for the treatment of metabolic syndrome. *Bioorg. Med. Chem.* 2006, *14*, 8405–8414.
73. Unangst, P. C., Connor, D. T., Cetenko, W. A., Sorenson, R. J., Kostlan, C. R., Sircar, J. C., Wright, C. D., Schrier, D. J., Dyer, R. D. Synthesis and biological evaluation of 5-[[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]methylene]oxazoles, -thiazoles, and imidazoles: novel dual 5-lipoxygenase and cyclooxygenase inhibitors with antiinflammatory activity. *J. Med. Chem.* 1994, *37*, 322–328.
74. Barbey, S., Goossens, L., Taverne, T., Cornet, J., Choesmel, V., Rouaud, C., Gimeno, G., Yannic-Arnoult, S., Michaux, C., Charlier, C., Houssin, R., Henichart, J. P. Synthesis and activity of a new methoxytetrahydropyran derivative as dual cyclooxygenase-2/5-lipoxygenase inhibitor. *Bioorg. Med. Chem. Lett.* 2002, *12*, 779–782.
75. Flynn, G. A., Beight, D. W., Mehdi, S., Koehl, J. R., Giroux, E. L., French, J. F., Hake, P. W., Dage, R. C. Application of a conformationally restricted phe-leu dipeptide mimetic to the design of a combined inhibitor of angiotensin I-converting enzyme and neutral endopeptidase 24.11. *J. Med. Chem.* 1993, *36*, 2420–2423.
76. Robl, J. A., Sun, C. Q., Stevenson, J., Ryono, D. E., Simpkins, L. M., Cimarusti, M. P., Dejneka, T., Slusarchyk, W. A., Chao, S., Stratton, L., Misra, R. N., Bednarz, M. S., Asaad, M. M., Cheung, H. S., Abboa-Offei, B. E., Smith, P. L., Mathers, P. D., Fox, M., Schaeffer, T. R., Seymour, A. A., Trippodo, N. C. Dual metalloprotease inhibitors: mercaptoacetyl-based fused heterocyclic dipeptide mimetics as inhibitors of angiotensin-converting enzyme and neutral endopeptidase. *J. Med. Chem.* 1997, *40*, 1570–1577.
77. Pontikis, R., Dollé, V., Guillaumel, J., Dechaux, E., Note, R., Nguyen, C. H., Legraverend, M., Bisagni, E., Aubertin, A.-M., Grierson, D. S., Monneret, C. Synthesis and evaluation of "AZT-HEPT", "AZT-pyridinone", and "ddC-HEPT" conjugates as inhibitors of HIV reverse transcriptase. *J. Med. Chem.* 2000, *43*, 1927–1939.
78. Camps, P., El Achab, R., Gorbig, D. M., Morral, J., Munoz-Torrero, D., Badia, A., Banos, J. E., Vivas, N. M., Barril, X., Orozco, M., Luque, F. J. Synthesis, *in vitro* pharmacology, and molecular modeling of very potent tacrine-huperzine A hybrids as acetylcholinesterase inhibitors of potential interest for the treatment of Alzheimer's disease. *J. Med. Chem.* 1999, *42*, 3227–3242.
79. Mary, A., Renko, D. Z., Guillou, C., Thal, C. Potent acetylcholinesterase inhibitors: design, synthesis, and structure-activity relationships of *bis*-interacting ligands in the galanthamine series. *Bioorg. Med. Chem.* 1998, *6*, 1835–1850.
80. Van der Schyf, C. J., Geldenhuys, W. J., Youdim, M. B. Multifunctional drugs with different CNS targets for neuropsychiatric disorders. *J. Neurochem.* 2006, *99*, 1033–1048.
81. Kogen, H., Toda, N., Tago, K., Marumoto, S., Takami, K., Ori, M., Yamada, N., Koyama, K., Naruto, S., Abe, K., Yamazaki, R., Hara, T., Aoyagi, A., Abe, Y., Kaneko, T. Design and synthesis of dual inhibitors of acetylcholinesterase and serotonin transporter targeting potential agents for Alzheimer's disease. *Org. Lett.* 2002, *4*, 3359–3362.
82. Toda, N., Tago, K., Marumoto, S., Takami, K., Ori, M., Yamada, N., Koyama, K., Naruto, S., Abe, K., Yamazaki, R., Hara, T., Aoyagi, A., Abe, Y., Kaneko, T., Kogen, H. Design, synthesis and structure-activity relationships of dual inhibitors of acetylcholinesterase and serotonin transporter as potential agents for Alzheimer's disease. *Bioorg. Med. Chem.* 2003, *11*, 1935–1955.
83. Toda, N., Tago, K., Marumoto, S., Takami, K., Ori, M., Yamada, N., Koyama, K., Naruto, S., Abe, K., Yamazaki, R., Hara, T., Aoyagi, A., Abe, Y., Kaneko, T., Kogen, H. A conformational restriction approach to the development of dual inhibitors of acetylcholinesterase and serotonin transporter as potential agents for Alzheimer's disease. *Bioorg. Med. Chem.* 2003, *11*, 4389–4415.

84. Soyka, R., Heckel, A., Nickl, J., Eisert, W., Muller, T. H., Weisenberger, H. 6,6-Disubstituted hex-5-enoic acid derivatives as combined thromboxane A_2 receptor antagonists and synthetase inhibitors. *J. Med. Chem.* 1994, *37*, 26–39.
85. Soyka, R., Guth, B. D., Weisenberger, H. M., Luger, P., Muller, T. H. Guanidine derivatives as combined thromboxane A_2 receptor antagonists and synthase inhibitors. *J. Med. Chem.* 1999, *42*, 1235–1249.
86. Cozzi, P., Carganico, G., Fusar, D., Grossoni, M., Menichincheri, M., Pinciroli, V., Tonani, R., Vaghi, F., Salvati, P. Imidazol-1-yl and pyridin-3-yl derivatives of 4-phenyl-1,4-dihydropyridines combining Ca^{++} antagonism and thromboxane A_2 synthase inhibition. *J. Med. Chem.* 1993, *36*, 2964–2972.
87. Fujita, M., Seki, T., Inada, H., Shimizu, K., Takahama, A., Sano, T. Approach to dual-acting platelet activating factor (PAF) receptor antagonist/thromboxane synthase inhibitor (TxSI) based on the link of PAF antagonists and TxSIs. *Bioorg. Med. Chem. Lett.* 2002, *12*, 341–344.
88. Allan, G., Cambridge, D., Hardy, G. W., Follenfant, M. J. BW A575C a chemically novel agent with angiotensin converting enzyme inhibitor and beta-adrenoceptor-blocking properties. *Br. J. Pharmacol.* 1987, *90*, 609–615.
89. Cecchetti, V., Schiaffella, F., Tabarrini, O., Zhou, W., Fravolini, A., Goi, A., Bruni, G., Segre, G. Symbiotic approach to drug design: *N*-[(4-Chloro-3-sulfamoylbenzamido)ethyl]propanolamine derivatives as beta-adrenergic blocking agents with diuretic activity. *Eur. J. Med. Chem.* 1991, *26*, 381–386.
90. Albrecht, H. A., Beskid, G., Christenson, J. G., Deitcher, K. H., Georgopapadakou, N. H., Keith, D. D., Konzelmann, F. M., Pruess, D. L., Wei, C. C. Dual-action cephalosporins incorporating a 3′-tertiary-amine-linked quinolone. *J. Med. Chem.* 1994, *37*, 400–407.
91. Martinez-Esparza, J., Oficialdegui, A.-M., Perez-Silanes, S., Heras, B., Orus, L., Palop, J.-A., Lasheras, B., Roca, J., Mourelle, M., Bosch, A., Del Castillo, J.-C., Tordera, R., Del Rio, J., Monge, A. New 1-aryl-3-(4-arylpiperazin-1-yl)propane derivatives, with dual action at 5-HT_{1A} serotonin receptors and serotonin transporter as a new class of antidepressants. *J. Med. Chem.* 2001, *44*, 418–428.
92. Matzen, L., van Amsterdam, C., Rautenberg, W., Greiner, H. E., Harting, J., Seyfriend, C. A., Bottcher, H. 5-HT reuptake inhibitors with 5-HT1B/1D antagonistic activity: a new approach toward efficient antidepressants. *J. Med. Chem.* 2000, *43*, 1149–1157.
93. Ocampo, M. T., Chaung, W., Marenstein, D. R., Chan, M. K., Altamirano, A., Basu, A. K., Boorstein, R. J., Cunningham, R. P., Teebor, G. W. Targeted deletion of mNth1 reveals a novel DNA repair enzyme activity. *Mol. Cell. Biol.* 2002, *22*, 6111–6121.
94. Papp, B., Pal, C., Hurst, L. D. Metabolic network analysis of the causes and evolution of enzyme dispensability in yeast. *Nature,* 2004, *429*, 661–664.
95. Sussman, J. L., Harel, M., Frolow, F., Oefner, C., Goldman, A., Toker, L., Silman, I. Atomic structure of acetylcholinesterase from *Torpedo californica*: a prototypic acetylcholine-binding protein. *Science*, 1991, *253*, 872–879.
96. Silman, I., Sussman, J. L. Acetylcholinesterase: "classical" and "non-classical" functions and pharmacology. *Curr. Opin. Pharmacol.* 2005, *5*, 293–302.
97. Du, D. M., Carlier, P. R. Development of bivalent acetylcholinesterase inhibitors as potential therapeutic drugs for Alzheimer's disease. *Curr. Pharm. Des.* 2004, *10*, 3141–3156.
98. Sippl, W., Contreras, J. M., Parrot, I., Rival, Y. M., Wermuth, C. G. Structure-based 3D QSAR and design of novel acetylcholinesterase inhibitors. *J. Comput. Aided Mol. Des.* 2001, *15*, 395–410.
99. Rydberg, E. H., Brumshtein, B., Greenblatt, H. M., Wong, D. M., Shaya, D., Williams, L. D., Carlier, P. R., Pang, Y. P., Silman, I., Sussman, J. L. Complexes of alkylene-linked tacrine dimers with *Torpedo californica* acetylcholinesterase: Binding of Bis5-tacrine produces a dramatic rearrangement in the active-site gorge. *J. Med. Chem.* 2006, *49*, 5491–5500.
100. Greenblatt, H. M., Guillou, C., Guenard, D., Argaman, A., Botti, S., Badet, B., Thal, C., Silman, I., Sussman, J. L. The complex of a bivalent derivative of galanthamine with torpedo acetylcholinesterase displays drastic deformation of the active-site gorge: implications for structure-based drug design. *J. Am. Chem. Soc.* 2004, *126*, 15405–15411.
101. Barril, X., Orozco, M., Luque, F. J. Predicting relative binding free energies of tacrine-huperzine A hybrids as inhibitors of acetylcholinesterase. *J. Med. Chem.* 1999, *42*, 5110–5119.
102. De Ferrari, G. V., Canales, M. A., Shin, I., Weiner, L. M., Silman, I., Inestrosa, N. C. A structural motif of acetylcholinesterase that promotes amyloid beta-peptide fibril formation. *Biochemistry,* 2001, *40*, 10447–10457.
103. Piazzi, L., Rampa, A., Bisi, A., Gobbi, S., Belluti, F., Cavalli, A., Bartolini, M., Andrisano, V., Valenti, P., Recanatini, M. 3-(4-[[Benzyl(methyl)amino]methyl]phenyl)-6,7-dimethoxy-2H-2-chromenone (AP2238) inhibits both acetylcholinesterase and acetylcholinesterase-induced beta-amyloid aggregation: a dual function lead for Alzheimer's disease therapy. *J. Med. Chem.* 2003, *46*, 2279–2282.
104. Wong, D. M., Greenblatt, H. M., Dvir, H., Carlier, P. R., Han, Y. F., Pang, Y. P., Silman, I., Sussman, J. L. Acetylcholinesterase complexed with bivalent ligands related to huperzine a: experimental evidence for species-dependent protein-ligand complementarity. *J. Am. Chem. Soc.* 2003, *125*, 363–373.
105. Parenti, M. A., Hatfield, S. M., Leyden, J. J. Mupirocin: a topical antibiotic with a unique structure and mechanism of action. *Clin. Pharm.* 1987, *6*, 761–770.
106. Poux, A. N., Cebrat, M., Kim, C. M., Cole, P. A., Marmorstein, R. Structure of the GCN5 histone acetyltransferase bound to a bisubstrate inhibitor. *Proc. Natl. Acad. Sci. USA* 2002, *99*, 14065–14070.
107. Parang, K., Cole, P. A. Designing bisubstrate analog inhibitors for protein kinases. *Pharmacol. Ther.* 2002, *3*, 145–157.
108. Spannhoff, A., Machmur, R., Heinke, R., Trojer, P., Bauer, I., Brosch, G., Schule, R., Hanefeld, W., Sippl, W., Jung, M. A novel arginine methyltransferase inhibitor with cellular activity. *Bioorg. Med. Chem. Lett.* 2007, *17*, 4150–4153.
109. North, B. J., Verdin, E. Sirtuins: Sir2-related NAD-dependent protein deacetylases. *Genome Biol.* 2004, *5*, 224–234.
110. Schafer, S., Jung, M. Chromatin modifications as targets for new anticancer drugs. *Arch. Pharm. (Weinheim)* 2005, *338*, 347–357.
111. Heltweg, B., Gatbonton, T., Schuler, A. D., Posakony, J., Li, H., Goehle, S., Kollipara, R., Depinho, R. A., Gu, Y., Simon, J. A., Bedalov, A. Antitumor activity of a small-molecule inhibitor of human silent information regulator 2 enzymes. *Cancer Res.* 2006, *66*, 4368–4377.
112. Schuetz, A., Min, J., Antoshenko, T., Wang, C. L., Allali-Hassani, A., Dong, A., Loppnau, P., Vedadi, M., Bochkarev, A., Sternglanz, R., Plotnikov, A. N. Structural basis of inhibition of the human NAD^+-dependent deacetylase SIRT5 by suramin. *Structure,* 2007, *15*, 377–389.
113. Trapp, J., Meier, R., Hongwiset, D., Kassack, M. U., Sippl, W., Jung, M. Structure-activity studies on suramin analogues as inhibitors of NAD(+)-dependent histone deacetylases (sirtuins). *Chem. Med. Chem.* 2007, 2(10), 1419–1431.
114. Tew, K. D. Glutathione-associated enzymes in anticancer drug resistance. *Cancer Res.* 1994, *54*, 4313–4320.
115. Lyttle, M. H., Hocker, M. D., Hui, H. C., Caldwell, C. G., Aaron, D. T., Engqvist-Goldstein, A., Flatgaard, J. E., Bauer, K. E. Isozyme-specific glutathione-S-transferase inhibitors: design and synthesis. *J. Med. Chem.* 1994, *37*, 189–194.
116. Oakley, A. J., Lo Bello, M., Ricci, G., Federici, G., Parker, M. W. Evidence for an induced-fit mechanism operating in pi class glutathione transferases. *Biochemistry,* 1998, *37*, 9912–9917.
117. Lyon, R. P., Hill, J. J., Atkins, W. M. Novel class of bivalent glutathione S-transferase inhibitors. *Biochemistry* 2003, *42*, 10418–10428.

118. Drews, J. Genomic sciences and the medicine of tomorrow. *Nat. Biotechnol.* 1996, *14*, 1516–1518.
119. Milligan, G. G protein-coupled receptor dimerization: function and ligand pharmacology. *Mol. Pharmacol.* 2004, *66*, 1–7.
120. Rozenfeld, R., Decaillot, F. M., IJzerman, A. P., Devi, L. A. Heterodimers of G protein-coupled receptors as novel and distinct drug targets. *Drug Discov. Today*, 2006, *3*, 437–443.
121. Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Le Trong, I., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M., Miyano, M. Crystal structure of rhodopsin: A G protein-coupled receptor. *Science*, 2000, *289*, 739–745.
122. Daniels, D. J., Kulkarni, A., Xie, Z., Bhushan, R. G., Portoghese, P. S. A bivalent ligand (KDAN-18) containing delta-antagonist and kappa-agonist pharmacophores bridges delta2 and kappa1 opioid receptor phenotypes. *J. Med. Chem.* 2005, *48*, 1713–1716.
123. Bhushan, R. G., Sharma, S. K., Xie, Z., Daniels, D. J., Portoghese, P. S. A bivalent ligand (KDN-21) reveals spinal delta and kappa opioid receptors are organized as heterodimers that give rise to delta(1) and kappa(2) phenotypes. Selective targeting of delta-kappa heterodimers. *J. Med. Chem.* 2004, *47*, 2969–2972.
124. Jacobson, K. A., Xie, R., Young, L., Chang, L., Liang, B. T. A novel pharmacological approach to treating cardiac ischemia. Binary conjugates of A1 and A3 adenosine receptor agonists. *J. Biol. Chem.* 2000, *275*, 30272–30279.
125. Abadi, A. H., Lankow, S., Hoefgen, B., Decker, M., Kassack, M. U., Lehmann, J. Dopamine/serotonin receptor ligands, part III[1]: synthesis and biological activities of 7, 7′-alkylene-*bis*-6, 7, 8, 9, 14, 15-hexahydro-5H-benz[d]indolo[2, 3-g]azecines – application of the bivalent ligand approach to a novel type of dopamine receptor antagonist. *Arch. Pharm. (Weinheim)* 2002, *335*, 367–373.
126. Bonger, K. M., van den Berg, R. J., Heitman, L. H., AP, I. J., Oosterom, J., Timmers, C. M., Overkleeft, H. S., van der Marel, G. A. Synthesis and evaluation of homo-bivalent GnRHR ligands. *Bioorg. Med. Chem.* 2007, *15*, 4841–4856.
127. Rajeswaran, W. G., Cao, Y., Huang, X. P., Wroblewski, M. E., Colclough, T., Lee, S., Liu, F., Nagy, P. I., Ellis, J., Levine, B. A., Nocka, K. H., Messer, W. S., Jr Design, synthesis, and biological characterization of bivalent 1-methyl-1,2,5,6-tetrahydropyridyl-1,2,5-thiadiazole derivatives as selective muscarinic agonists. *J. Med. Chem.* 2001, *44*, 4563–4576.
128. Soulier, J. L., Russo, O., Giner, M., Rivail, L., Berthouze, M., Ongeri, S., Maigret, B., Fischmeister, R., Lezoualc'h, F., Sicsic, S., Berque-Bestel, I. Design and synthesis of specific probes for human 5-HT4 receptor dimerization studies. *J. Med. Chem.* 2005, *48*, 6220–6228.
129. Chini, B., Manning, M. Agonist selectivity in the oxytocin/vasopressin receptor family: new insights and challenges. *Biochem. Soc. Trans.* 2007, *35*, 737–741.
130. Fotiadis, D., Jastrzebska, B., Philippsen, A., Muller, D. J., Palczewski, K., Engel, A. Structure of the rhodopsin dimer: a working model for G-protein-coupled receptors. *Curr. Opin. Struct. Biol.* 2006, *16*, 252–259.
131. Russo, O., Berthouze, M., Giner, M., Soulier, J. L., Rivail, L., Sicsic, S., Lezoualc'h, F., Jockers, R., Berque-Bestel, I. Synthesis of specific bivalent probes that functionally interact with 5-HT_4 receptor dimers. *J. Med. Chem.* 2007, 50(18), 4482–4492.
132. Filizola, M., Wang, S. X., Weinstein, H. Dynamic models of G-protein coupled receptor dimers: indications of asymmetry in the rhodopsin dimer from molecular dynamics simulations in a POPC bilayer. *J. Comput. Aided Mol. Des.* 2006, *20*, 405–416.