Receptor Ligands

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Molecular Alliance—From Orthosteric and Allosteric Ligands to Dualsteric/Bitopic Agonists at G Protein Coupled Receptors

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agonists · allosterism · antagonists · dualsterism · receptors

Cell-membrane-spanning G protein coupled receptors (GPCRs) belong to the most important therapeutic target structures. Endogenous transmitters bind from the outer side of the membrane to the "orthosteric" binding site either deep in the binding pocket or at the extracellular N-terminal end of the receptor protein. Exogenous modulators that utilize a different, "allosteric", binding site unveil a pathway to receptor subtype-selectivity. However, receptor activation through the orthosteric area is often more powerful. Recently there has been evidence that orthosteric/allosteric, in other words "dualsteric", hybrid compounds unite subtype selectivity and receptor activation. These "bitopic" modulators channelreceptor activation and subsequent intracellular signaling into a subset of possible routes. This concept offers access to GPCR modulators with an unprecedented receptor-subtype and signaling selectivity profile and, as a consequence, to drugs with fewer side effects.

1. Introduction

> "*The structure of the M_2 receptor provides insights into both orthosteric and allosteric regulation of muscarinic receptors. The development of more selective drugs for muscarinic receptors will probably require exploitation of the more diverse allosteric surface, either as exclusively allosteric ligands or as ligands that occupy both orthosteric and allosteric sites.*"
> Haga et al.[1]

Paul Ehrlich (1854–1915) und John N. Langley (1854–1936) established the concept of receptors and active compounds that act through these receptors (chemotherapeutics, "magic bullet"). In the following decades terms were coined such as "agonists", which fully activate the receptor, and "partial agonists", which only generate submaximum activation. "Antagonists" bind to the receptor, have no intrinsic effect, and prevent activation by agonists. "Inverse agonists" abolish spontaneous, constitutive activity of a receptor. Although one can characterize ligand–receptor interactions pharmacologically by measuring radioligand binding or by a variety of other approaches, the complexity of structure–function relationships is far from being understood. In any case, there is ample evidence that the concept of drug actions being mediated solely through the orthosteric transmitter binding site is not sufficient.

In the last 100 years a great number of receptors were identified, the largest group being the so-called G protein coupled receptors (GPCRs, G: Guanylnucleotide binding), which are anchored in the cell membrane and communicate extracellular signals through an agonist-induced conformational change into the interior of the cell. For this, GPCRs recruit heterotrimeric G proteins which, utilizing guanosine triphosphate, control effector proteins such as adenylate cyclase and phospholipase C. Additional routes are the arrestin signaling pathway and the direct activation of kinases.[2]

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The fact that 30–50% of all marketed drugs bind to GPCRs and modulate signal transduction shows that GPCRs are of outstanding significance for the treatment of diseases. Currently, about 1000 different GPCRs are known, which can be subdivided into distinct families. This Minireview focuses on the family A, which consists of 700 receptors and thus makes up the largest group of GPCRs, including the highly important aminergic and cholinergic receptors.

2. GPCR Structure

GPCRs constitute a large protein family characterized by seven transmembrane helices, which are connected by three extracellular (EL) and intracellular (IL) loops. The binding site of the endogenous transmitter and of exogenous orthosteric agonists and antagonists is buried inside the helical domain of class A GPCRs.[3] However, the meanwhile numerous crystal structures reported for this GPCR family (β_1- and β_2-adrenergic receptors,[4] A_{2A} adenosine,[5] dopamine D_3,[6] histamine H_1,[7] muscarinic M_2 and M_3,[8] and opioid receptors)[9] show that despite great similarities in the overall structures and in particular of the orthosteric binding pocket, agonists, inverse agonists, and antagonists adopt diverse orientations in the binding pocket and induce different conformational changes of the receptor proteins.[10,11]

Interestingly, this family possesses a long EL2 of large structural diversity that may serve as an additional binding site. In some receptors (e.g. β_2, β_1, rhodopsin, A_{2A}, and M_2 receptors)[12] the ELs form a vestibule through which orthosteric ligands must pass and thereby is occupied by these ligands for a very brief moment.[13] This "anteroom to the orthosteric binding site" can serve as a binding site for so-called allosteric ligands. Allosteric ligands are compounds whose binding site is located in the receptor protein aside from the orthosteric binding site. These ligands can be non-self or the body's own, that is, endogenous ligands including a variety of ions like sodium, calcium, magnesium, and zinc ions.[14] The structural diversity among the extracellular vestibules provides the opportunity for a favorable, "selective" chemical addressing of one receptor or receptor subtype.[12,15] The GluAspGlyGlu(EDGE) sequence in the EL2 of the M_2 acetylcholine receptor, for instance, plays a role for, for example, the M_2/M_5 subtype selectivity of gallamine[13,16] as well as for naphmethonium,[17] while LY2033298 (Scheme 1) occupies the respective region of the M_4 acetylcholine receptor.[18] It should be kept in mind that in principle there is not a spatially defined allosteric binding site at any receptor, because the term allosteric only designates a binding site distinct from the orthosteric binding area. Accordingly, a receptor protein can exhibit several and possibly overlapping allosteric binding areas. Beyond that, the binding site of intracellular adaptor proteins, including G

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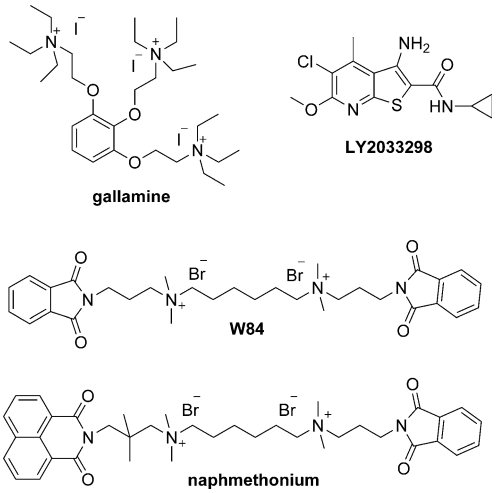


Scheme 1. Muscarinic allosteric ligands. Their binding site, particularly at the extracellular loop 2 (EL2), explains M_2 and M_4 subtype selectivity.

proteins, should also be considered as an allosteric binding site.

3. Ligand Categories

Receptor ligands can be categorized according to their influence on the receptor function or the location of their binding site, that is, orthosteric or allosteric. The function of a receptor protein can principally be modulated by means of both of these binding areas. As mentioned above, endogenous ligands as well as conventional agonists, antagonists, and inverse agonists typically occupy the orthosteric binding site. This binding site is highly conserved within the class A GPCR family (the sequence identity is assumed to be 20–50%)[19] and is even more conserved between single subtypes of one receptor. This severely hampers the development of selective orthosteric agonists and antagonists.

Proteins, like receptors, ion channels, and enzymes, however, can—as mentioned in Section 2—possess an additional allosteric binding site,[20,21] the physiological function of which is often unresolved.[22] In case of the vestibule of class A GPCRs, the removal of water molecules from the transmitter before it enters the orthosteric binding cavity is postulated[13] as well as the control of conformational rearrangements that result in receptor activation and G protein interactions.[23]

Benzodiazepines, which were discovered in the middle of the last century and have an anxiolytic effect, are the first known allosteric receptor modulators for therapeutic use. By enhancing the binding affinity of the endogenous transmitter, that is, γ-amino butyric acid (GABA), benzodiazepines potentiate the GABA effect at the $GABA_A$ receptor,[24] a ligand-gated ion channel. The same principle is basically possible at GPCRs.[20,21]

4. Allosteric–Orthosteric Interactions

The therapeutically interesting feature of allosteric–orthosteric receptor interactions is the possibility of generating ternary complexes (Figure 1). The mutual influence between

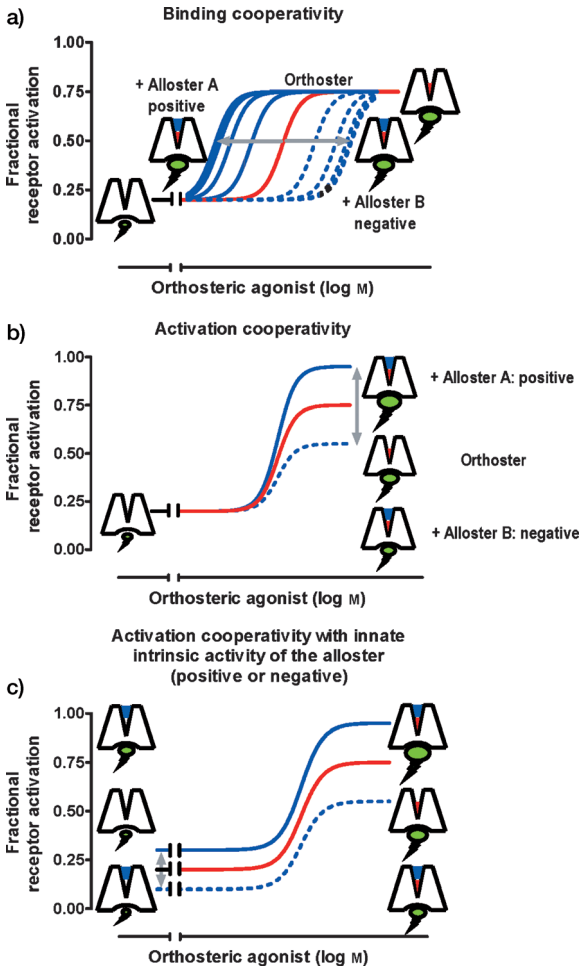


Figure 1. Influence of ternary complexes (consisting of a receptor with modest spontaneous activity, an allosteric modulator, and an orthosteric partial agonist) and of cooperativity in these complexes on a) binding affinity and b) receptor activation by the orthosteric agonist. c) Allosteric ligands with intrinsic (i.e. in absence of an orthosteric agonist) agonistic activity or inverse agonistic activity.

the partners within an allosteric/orthosteric ligand pair at a given receptor protein is bilateral and in the same direction: this phenomenon is referred to as cooperativity. Importantly, cooperative interactions are saturable, which means that the mutual influence exhibits a maximum that depends on the given receptor and the involved ligands (Figure 1a).

Thus, the allosteric and the orthosteric ligands may increase, reduce, or be neutral with respect to each other's binding—corresponding to positive, negative, or neutral binding cooperativity (Figure 1a). Moreover, cooperativity extends to the receptor's activity: positive, negative, or neutral cooperativity of receptor activation (Figure 1b).

Remarkably, the direction and magnitude of cooperativity at a given GPCR can be individually different for each pair of

orthosteric and allosteric ligands. Consequently, an allosteric inverse agonist (per se no receptor activation) may enhance the binding of an orthosteric inverse agonist (per se no receptor activation), whereas the binding of an orthosteric agonist (per se receptor activation) may be reduced or its intrinsic efficacy for receptor activation switched off. This was for example demonstrated for the pairs consisting of naphmethonium (allosteric) and acetylcholine or pilocarpine (both orthosteric) at the M_2 receptor.[17] These observations, which were also made at other receptors, are not surprising, as a receptor that is bound by an allosteric or an orthosteric ligand can be considered as a new entity that has changed in structure and, as a consequence, in its signaling behavior.

One has to be aware of this "partner dependency", when allosteric modulators are classified as negative allosteric modulators (NAM) or positive allosteric modulators (PAM) or silent allosteric modulators (SAM). In all of these cases attention has to be paid to the orthosteric partner involved.

In the present context it is important and shall be emphasized again that allosteric ligands may bind to a receptor subtype of interest with far more selectivity than their orthosteric counterparts, because the orthosteric binding area is—as outlined in Section 2— often located in close proximity to or directly at the extracellular loops which are poorly conserved among subtypes.[25] This will be illustrated in detail using muscarinic agonists, modulators, and bitopic agonists as an example (Chapter 5.1).

4.1. Negative Allosteric Modulators

Ligands that bind to the allosteric binding site and inhibit receptor function induce a rightward shift of the concentration–effect curve of, for example, the endogenous agonist (negative binding cooperativity; Figure 1 a) and may also reduce the efficacy of the agonist (negative activation cooperativity; Figure 1 b). Both effects are limited (saturable) and reach a maximum when the negative allosteric modulator (NAM) has completely occupied its binding site. This distinguishes these ligands from classical competitive antagonists which can induce a theoretically unlimited, substantially larger rightward shift of the agonist dose–response curve, because their antagonistic effect is not saturable.

It is assumed that NAMs either increase the energy barrier required for receptor activation or stabilize the inactive conformation of the receptor protein leading to a decreased affinity of the orthosteric ligand to the receptor.[26]

4.2. Positive Allosteric Modulators

Ligands that bind to the allosteric site and increase the equilibrium binding of the simultaneously bound endogenous or exogenous orthosteric ligand (Figure 1 A) enhance either the affinity of an orthosteric agonist or lower the activation barrier for the transition from the inactive to the active conformation of the receptor.[26] Again, the extent of allosteric enhancement is limited and is maximal when all allosteric binding sites are occupied. As is shown in Figure 2, this could restore insufficient receptor activation back to the standard measure, i.e. maximum efficacy. Positive allosteric modulators (PAMs) can only develop their effect in the presence of the endogenous transmitter (or another orthosteric ligand) and thus are not likely to generate off-target effects. However, a disadvantage is their lack of effect in the absence of neurotransmitter (e.g. in advanced stages of neurodegenerative diseases such as Alzheimer's disease).

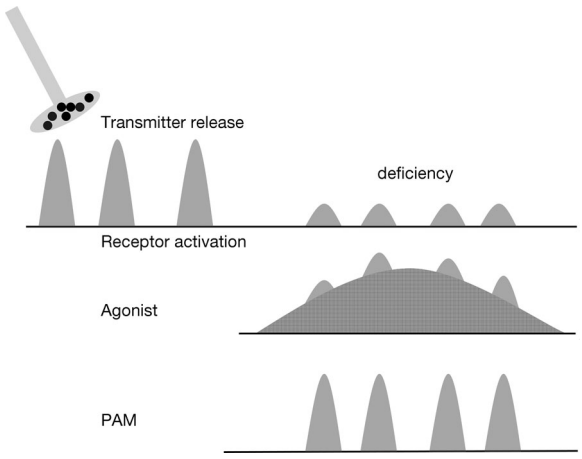


Figure 2. Approaches to compensate for a diminished release of transmitters caused by, for example, a neurodegenerative disease. Top: Pulsatile release of transmitter under physiological conditions (left) and pathological diminished release (right). Middle: Therapeutic application of an orthosteric agonist. Bottom: Therapeutic application of an allosteric enhancer; preservation of the physiological excitation pattern.

4.3. Silent Allosteric Modulators—Allosteric Antagonists

So-called silent allosteric modulators (SAMs) bind to the allosteric site but do not affect the action of an orthosteric agonist (neutral cooperativity of binding and activation). Occupying the same site as PAMs, NAMs, and allosteric agonists (see Section 4.4), SAM behave as competitors (allosteric antagonists) at this binding site inducing a rightward shift of the concentration–effect curve of allosteric modulators. Therefore, SAMs could also be classified as allosteric antagonists. In any case, these compounds are interesting pharmacological tools.[26]

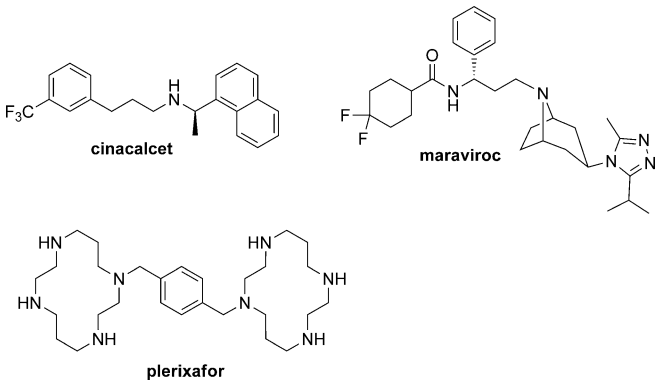
4.4. Allosteric Agonists

A number of allosteric ligands, which exclusively bind at the allosteric binding site, mediate agonistic activity in the absence of the endogenous ligand or other orthosteric agonists (Figure 1 c). These compounds are designated as allosteric agonists (or "ago-allosteric modulators").[27] A number of PAMs were reported to be allosteric agonists as well, although their agonistic activity occurs at higher

concentrations than the PAM activity. It is likely that the percentage of PAMs having additional allosteric–agonistic features is higher than has been recognized so far, because, due to missing relevance, they were not studied in the required concentrations.

4.5. First Allosteric Modulators in Clinical Use

Maraviroc (Celsentry, Scheme 2) is a high-affinity negative allosteric modulator towards the HIV-gp120 protein at the CCR5 receptor (CC-motif chemokine receptor 5). The



Scheme 2. Structural representation of the three marketed allosteric modulators already in clinical use.

drug hinders the docking of the HI virus and thus its uptake into human host cells. The antiviral effect is restricted to HI viruses. The interaction of the endogenous ligands MIP-1β and RANTES (regulated and normal T cell expressed and secreted; also known as CCL5 (chemokine ligand 5)) with CCR5 is also prevented. The recently approved drug plerixafor (Mozobil, Scheme 2) is an allosteric antagonist at the chemokine receptor CXCR4. It is applied to release stem cells into the bloodstream for autologous stem cell transplantation.[28]

Cinacalcet (Mimpara, Scheme 2) is a PAM towards Ca^{2+} ions at the calcium-sensing receptor (CaSR). Upon binding to the allosteric binding site, cinacalcet induces a conformational change which is accompanied by an enhanced sensitivity of the receptor for extracellular calcium ions and thus a reduced release of parathormone, an important hormone for bone metabolism. Cinacalcet is applied for the treatment of secondary hyperparathyroidism, a parathyroid disease.[29]

In the light of this clinical success it is not surprising that the concept of allosteric modulation has become increasingly attractive for academic and industrial institutions, for example in the quest for improved drugs for the treatment of Alzheimer's disease and schizophrenia.[30]

5. Bitopic/Dualsteric Ligands

A few years ago the repertoire of bioactive compounds was extended by GPCR modulators that are designed to bind simultaneously to the orthosteric and the allosteric GPCR binding areas. This concept is derived from the "message–address" concept of Schwyzer, which was already published in the 1970s.[31] In principle, these molecules consist of two parts: the message part that is responsible for the activation of a receptor, and the address part that guides the ligand to the receptor or receptor subtype of interest. The two parts are connected by a linker. If the message occupies the highly conserved orthosteric area and the address binds to the less conserved allosteric binding pocket, one may well refer to these constructs as orthosteric/allosteric compounds or, in short, as dualsteric ligands. As we will see later (Section 5.2) the message/address dichotomy is not strict; interestingly, orthosteric receptor activation is modulated by the allosteric address moiety, yielding a novel quality of signaling.

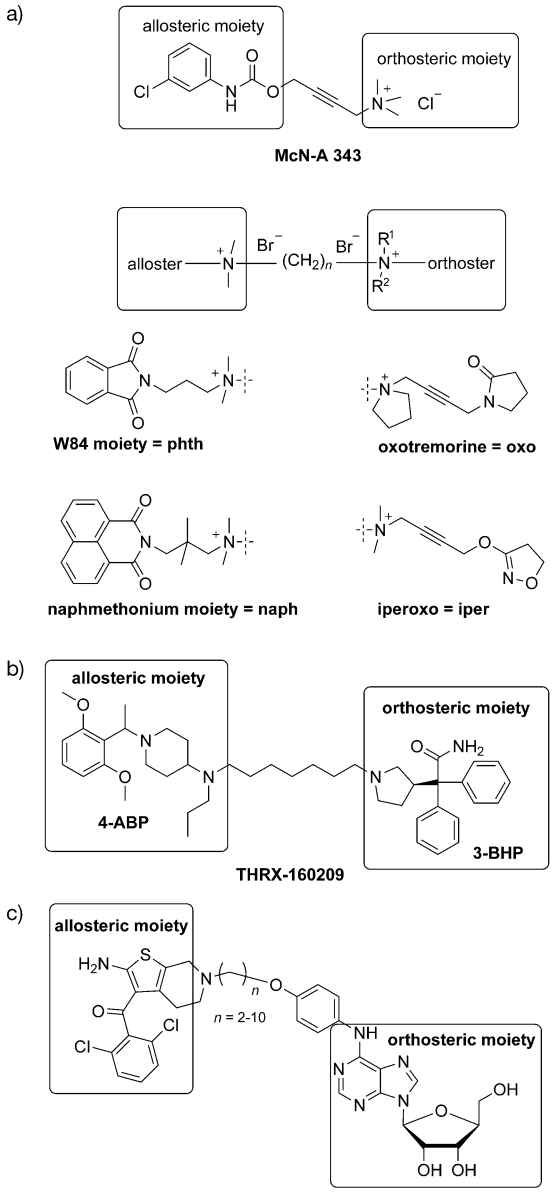
Dualsteric ligands constitute a subclass of bitopic ligands. The latter bind per definition to two areas at the receptor, but do not necessarily utilize the allosteric and the orthosteric binding sites. This is to be distinguished from the bivalent ligands, which were first synthesized by Portoghese.[32] These structures bind simultaneously to identical or different binding sites (including receptors) and contain two pharmacophores which may be homobivalent or heterobivalent.[33] This subject is not included in this Minireview.

5.1. Bitopic and Dualsteric Agonists

Allosteric/orthosteric GPCR agonism was first realized at the muscarinic M_2 acetylcholine receptor, on the one hand by molecular–mechanistic investigations of a known agonist,[34] and on the other hand by the specific de novo synthesis of a ligand intended to simultaneously occupy the allosteric and orthosteric binding sites.[33,35]

The research group led by Christopoulos was able to characterize the long-known partial agonist McN-A-343, which structurally belongs into the group of oxotremorines (Scheme 3a), as a bitopic ligand.[34a] The fragment of the endogenous ligand acetylcholine, a tetramethylammonium cation, represents the binding partner for the orthosteric binding site, whereas the 3-chlorphenylcarbamate is the partner for the allosteric EL2 vestibule—and the two parts are linked by a rigid butynyl chain. However, it seems that this molecule is too small to occupy greater parts of the allosteric area and thereby gain subtype selectively for the M_2 receptor, as it displays similar affinity to the M_4 receptor as well.[36]

Iper-6-phth and iper-6-naph were designed on the drawing board. Building blocks derived from the allosteric hexamethonium compounds W84 and naphmethonium (Scheme 1), which are NAMs regarding orthosteric agonists[17] and have preference for the M_2 acetylcholine receptor,[37] were linked to building blocks of different orthosteric agonists, which were derived from oxotremorine.[38] These dualsteric compounds display relatively good subtype selectivity for the M_2 receptor, although their potency/affinity is less than that of the orthosteric building blocks. This could be improved by linker optimization.[23] Hybrid compounds that contained iperoxo as the orthosteric part proved to be agonists; the other hybrid compounds were almost devoid of efficacy for



Scheme 3. Bitopic/dualsteric ligands: a) M_2 agonists McN-A-343 , iper-6-naph, and iper-6-phth (orthosteric building block–linker length–allosteric building block); b) M_2 antagonist THRX-160209; c) adenosine A_1 agonists.

receptor activation. Beyond that, and unexpectedly, a signaling pathway selectivity emerged in the case of the iperoxo-derived hybrids. The M_2 subtype of the muscarinic acetylcholine receptor usually activates two G protein dependent signaling pathways, which are working in opposite directions. Preferentially, the G_i pathway (inhibition of the intracellular cAMP production) is activated, but in addition, at higher agonist concentrations, the G_s pathway (stimulation of cyclic adenosine monophosphate (cAMP) production) becomes operative as well. Iper-6-phth and iper-6-naph, in contrast to the orthosteric parent compound, merely activate the G_i pathway. Hence, beyond subtype selectivity, an additional selectivity mechanism becomes available which is currently a hot topic in the GPCR field: signaling pathway selectivity, also known as functional selectivity or biased signaling.

Steinfeld et al. generated the compound THR-160209 (Scheme 3b) by connecting an orthosteric 3-benzylhydrylpyrrolinyl building block by means of a heptane chain to an allosteric 4-aminobenzylpiperidine motif. In this way, they gained an antagonist that displays a considerably higher receptor affinity than the individual components (pK_i-(total) = 9.5 vs. pK_i = 5.5 for the single compounds) and exhibits a certain preference for the M_2 acetylcholine receptor.[39]

Bitopic adenosine A1 receptor agonists were designed following a similar systematic concept as that applied to generate the M_2 hybrids iper-6-phth and iper-6-naph. An orthosteric adenosine agonist was connected through an alkyl chain linker of varying length to a PAM of the PD81,723 type (Scheme 3c).[40] The resulting LUF6258, which contains a linker length of nine carbon atoms, displayed unchanged binding affinity and functional potency in the presence and absence of the PAM PD81,723 in a concentration that saturates all of the allosteric binding sites (10 μM), suggesting a dualsteric receptor interaction of LUF6258. Nevertheless, the affinity or potency of LUF6258 was not higher than that of monovalent orthosteric agonists.

5.2. Strategies for the Design of Bitopic or Dualsteric Agonists

The development of dualsteric or bitopic compounds is a multidimensional problem, because several parts of the molecule—the orthosteric and the allosteric parts as well as the linker—must be optimized and linked in an appropriate way. The strategy resembles computational fragment-based design.

Regarding the capability for receptor activation, the following combinations of orthosteric–allosteric building blocks are possible: active–active, active–inactive, inactive–active, inactive–inactive. The dualsteric M_2 receptor agonists, which were mentioned previously, represent the constellation of "orthosteric active/allosteric inactive". To generate a highly potent dualsteric ligand, the orthosteric agonist should possess very high affinity for the receptor protein (preferably in a sub-nanomolar range) so that the bitopic construct does not prefer a purely allosteric binding mode over the dualsteric orientation. The orthosteric agonist should additionally have high efficacy for receptor activation to ensure that the dualsteric construct maintains sufficient receptor activation, since the constellation "orthosteric active/allosteric inactive" means functionally opposing receptor conformations. The allosteric building block should provide high affinity and subtype selectivity for the addressed receptor subtype.

The two parts can be optimized separately. However, it should be kept in mind that the binding of one of the building blocks can induce structural alterations in the binding site of the other building block; for instance, upon binding of the dualsteric construct the receptor may undergo conformational changes causing negative cooperativity. In other words, the affinity and/or efficacy of the other binding partner at the receptor protein would be diminished.[41] Maybe it is this

"balance of power" in the constellation "orthosteric active/allosteric inactive" that is the key to fine-tune the signaling pathway selectivity.[23]

The linker between the two building blocks plays a crucial role (Figure 3). The linker must be fixed to the building blocks in positions that allow each to bind as if they were individual components. Additionally, the linker must be tailor-made with respect length, flexibility, and chemical features. On the one hand, the linker chain must be long enough that both building blocks reach their respective binding areas and can dock there as intended; on the other hand, the linker must not be too long in order to avoid steric problems. Linker chains can consist of alkane and polyethyleneglycol chains or of polyglycins.[42] These are either chemically inert or can interact with the receptor protein.[43]

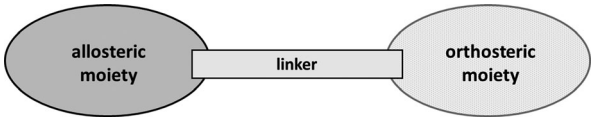


Figure 3. Basic structure of dualsteric ligands.

When all parts of the dualsteric ligand are put together, the affinity for the receptor protein might not be increased, the agonistic efficacy might be weaker than that of the applied orthosteric agonist,[43,44] and the receptor subtype selectivity might be less than that of the allosteric ligand. These effects might be improved by fine-tuning but cannot always be eliminated completely. On the other hand, the combination of high efficacy and selectivity might also considerably exceed the combined features of the individual building blocks.

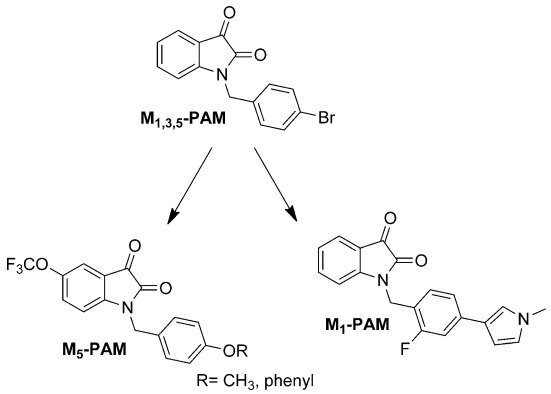
Bitopic or dualsteric ligands with affinities exceeding those of the respective building blocks can be achieved if both building blocks bind into their respective binding pockets in an ideal manner and if their binding does not induce an unfavorable conformational rearrangement of the receptor protein. This can be explained by the lower total entropy cost of the ligand–receptor complex. The mostly favorable spatial proximity between the orthosteric and the allosteric binding areas contributes to a decrease in entropy, if this is not counteracted by a too flexible linker.[32,33,45] In the end, this can differ case by case, because during the sequential binding of bitopic ligands to the orthosteric and the dualsteric binding areas, the driving force can be entropy as well as enthalpy and conformational changes in the two binding events. The general rule that an increase in entropy is the driving force for the docking of a polar agonist and a decrease in enthalpy in the driving force for the docking of a mostly hydrophobic antagonist[46] is hard to apply here, because both can be combined in one molecule.

Care has to be taken regarding the size of the molecule when the three parts are assembled. Active compounds that are to be ingested orally should, according to the "Lipinski Rule Five",[47] not exceed a molecular mass of 500 Dalton. Molecules suffering from "molecular obesity", that are too big and too lipophilic, often "perish" in clinical studies.[48] This means that allosteric and orthosteric building blocks should be as small as possible.

Nowadays, valid computational modeling can accompany optimization, since crystal structures are available for a variety of GPCRs. Nevertheless, attention should be paid to the state in which the receptor protein has been crystallized—active, inactive, or transitional states—and which function (agonist or antagonist) the ligand should have.

5.3. Molecular Switches

One may well refer to "molecular switches", when small structural changes in the molecule lead, for instance, to a switch from a PAM to a NAM. Molecular switches are also known in the context of receptor subtype selectivity. Recently it was discovered in the group of Lindsley that variations in the substitution of the *N*-benzylisatines lead to a shift from a PAM at the acetylcholine receptor subtypes M_1, M_3, and M_5 to a selective M_5 PAM (methoxy or phenoxy substitution at the benzyl residue) or a selective M_1 PAM (fluoro and pyrazolyl residues; Scheme 4).[49]



Scheme 4. *N*-benzylisatine derivatives with different subtype selectivity at muscarinic receptors.

A PAM–NAM switch was also reported by Wood et al. for modulators at the metabotropic glutamate receptors (mGLU)[50] and by Muth et al. for the antagonist binding of *N*-methylscopolamine at the muscarinic acetylcholine receptors.[51] In the latter case, mere introduction of methyl substituents into the lateral linker regions is sufficient to switch a NAM into a PAM with respect to the binding of an orthosteric radio-antagonist. This effect is particularly pronounced, if the methyl groups are attached to the naphthalimidopropyl-chain (**1b**) (Figure 4).

The group of dualsteric agonists at the muscarinic M_2 acetylcholine receptor also contains molecular switches. The dualsteric agonists described earlier can become antagonists solely by the introduction of a double bond into the isoxazoline ring (Scheme 5).[35]

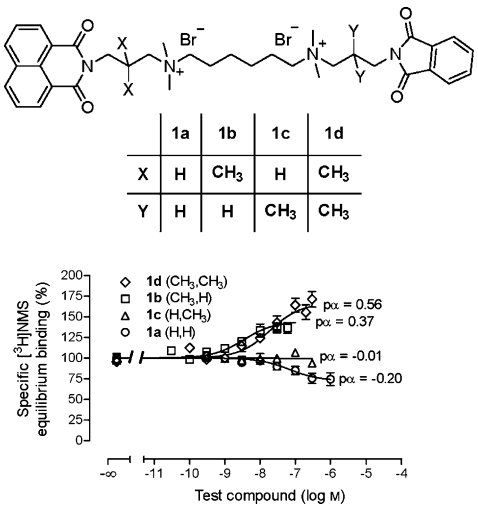
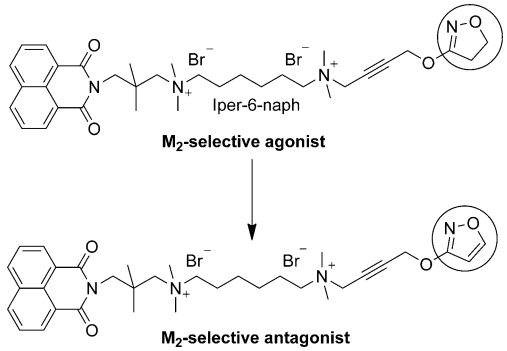


Figure 4. Molecular switching of a NAM- to a PAM-type allosteric modulator at the muscarinic M_2 receptor in the presence of the orthosteric antagonist *N*-methylscopolamine. The cooperativity factor α is a measure to quantify the quality (direction) and the extent (strength) of the allosteric interaction in the ternary complex, which consists of the allosteric modulator, orthosteric *N*-methylscopolamine, and the M_2 receptor. The negative decimal logarithm of this measure $-\log\alpha = p\alpha$ allows, solely by means of the algebraic sign, a simple distinction of positive ($p\alpha > 0$), negative ($p\alpha < 0$), or neutral cooperativity ($p\alpha = 0$).



Scheme 5. By the insertion of only one double bond, the M_2 agonist iper-6-naph becomes an antagonist.

6. Future Prospects

The design of dualsteric agents is a novel approach expanding the repertoire of medicinal chemistry and chemical biology. Dualsteric ligands not only open the door to selective drug effects, these compounds also complement classical FRET measurements,[52] molecular dynamics simulations,[13] and molecular modeling[10] as valuable tools to better understand the process of receptor activation.[23]

Some allosteric modulators have already made their way into clinical applications, others are in the pipeline.[28] The dualsteric ligands presented here provide a novel promising concept for the rational design of signaling-selective GPCR activators. The future will show whether researchers will find the first dualsteric/bitopic ligands as marketed drugs and whether these compounds, once applied to a large collective of patients, fulfill the current hopes.[53]

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[1] K. Haga, A. C. Kruse, H. Asada, T. Yurugi-Kobayashi, M. Shiroishi, C. Zhanf, W. I. Weis, T. Okada, B. K. Koblika, T. Haga, T. Kobayashi, *Nature* **2012**, *482*, 547 – 552.
[2] G. Vauquelin, B. von Mentzer, *G Protein-coupled Receptors—Molecular Pharmacology*, Wiley, Chichester, **2007**.
[3] a) D. M. Rosenbaum, S. G. F. Rasmussen, B. Kobilka, *Nature* **2009**, *459*, 356 – 363; b) P. Kolb, G. Klebe, *Angew. Chem.* **2011**, *123*, 11778 – 11780; *Angew. Chem. Int. Ed.* **2011**, *50*, 11573 – 11575.
[4] a) S. G. F. Rasmussen, H.-J. Choi, D. M. Rosenbaum, T. S. Kobilka, F. S. Thian, P. C. Edwards, M. Burghammer, V. R. P. Ratnala, R. Sanishvili, R. F. Fischetti, G. F. X. Schertler, W. I. Weis, B. K. Kobilka, *Nature* **2007**, *450*, 383 – 387; b) S. G. Rasmussen, H. J. Choi, J. J. Fung, E. Pardon, P. Casarosa, P. S. Chae, B. T. Devree, D. M. Rosenbaum, T. S. Kobilka, A. Schnapp, I. Konetzki, R. K. Sunahara, S. H. Gellman, A. Pautsch, J. Steyaert, W. I. Weis, B. K. Kobilka, *Nature* **2011**, *469*, 175 – 180; c) D. M. Rosenbaum, C. Zhang, J. A. Lyons, R. Holl, D. Aragao. D. H. Arlow, S. G. Rasmussen, H. J. Choi, B. T. Devree, R. K. Sunaharam, P. S. Chae, S. H. Gellman, R. O. Dror, D. E. Shaw, M. Caffrey, P. Gmeiner, B. K. Kobilka, *Nature* **2011**, *469*, 236 – 240.
[5] V. P. Jaakola, M. T. Griffith, M. A. Hanson, V. Cherezov, E. Y. Chien, J. R. Lane, A. P. IJzerman, R. C. Stevens, *Science* **2008**, *322*, 1211 – 1217.
[6] E. Y. Chien, W. Liu, Q. Zhao, V. Katritch, G. W. Han, M. A. Hanson, L. Shi, A. H. Newman, J. A. Javitch, V. Cherezov, R. C. Stevens, *Science* **2010**, *330*, 1091 – 1095.
[7] T. Shimamura, M. Shiroishi, S. Weyand, H. Tsujimoto, G. Winter, V. Katritch, R. Abagyan, V. Cherezov, W. Liu, G. W. Han, T. Kobayashi, R. C. Stevens, S. Iwata, *Nature* **2011**, *475*, 65 – 70.
[8] A. C. Kruse, J. Hu, A. C. Pan, D. H. Arlow, D. M. Rosenbaum, E. Rosemond, H. F. Green, T. Liu, P. S. Chae, R. O. Dror, D. E. Shaw, W. I. Weis, J. B. K. Kobilka, *Nature* **2012**, *482*, 552 – 556.
[9] a) S. Granier, A. Manglik, A. C. Kruse, T. S. Kobilka, F. S. Thian, W. I. Weis, B. K. Kobilka, *Nature* **2012**, *485*, 400 – 404; b) A. Manglik, A. C. Kruse, T. S. Kobilka, F. S. Thian, J. M. Mathiesen, R. K. Sunahara, L. Pardo, W. I. Weis, B. K. Kobilka, S. Granier, *Nature* **2012**, *485*, 321 – 326; c) H. Wu, D. Wacker, M. Mileni, V. Katritch, G. W. Han, E. Vardy, A. A. Thompson, X.-P. Huang, F. I. Carroll, S. W. Mascarella, R. B. Westkaemper, P. D. Mosier, B. L. Roth, V. Cherezov, R. C. Stevens, *Nature* **2012**, *485*, 327 – 332; d) A. A. Thompson, W. Liu, E. Chun, V. Katritch, H. Wu, E. Vardy, X.-P. Huang, C. Trapella, R. Guerrini, G. Calo, B. L. Roth, V. Cherezov, R. C. Stevens, *Nature* **2012**, *485*, 395 – 399.
[10] V. Katritch, R. Abagyan, *Trends Pharmacol. Sci.* **2011**, *32*, 637 – 643.
[11] H. Unal, S. S. Karnik, *Trends Pharmacol. Sci.* **2012**, *33*, 79 – 88.
[12] M. C. Peeters, G. J. P. van Westen, Q. Li, A. P. IJzerman, *Trends Pharmacol. Sci.* **2011**, *32*, 35 – 42.
[13] R. O. Dror, A. C. Pan, D. H. Arlow, D. W. Borhani, P. Maragakis, Y. Shan, H. Xu, D. E. Shaw, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 13118 – 13123.
[14] A. Christopoulos, *Nat. Rev. Drug Discovery* **2002**, *1*, 198 – 210.

[15] V. Katritch, V. Cherezov, R. C. Stevens, *Trends Pharmacol. Sci.* **2012**, *33*, 17 – 27.
[16] a) J. M. Stockton, N. J. M. Birdsall, A. S. V. Burgen, E. C. Hulme, *Mol. Pharmacol.* **1983**, *23*, 551 – 557; b) X.-P. Huang, S. Prilla, K. Mohr, J. Ellis, *Mol. Pharmacol.* **2005**, *68*, 769 – 778; c) A. L. Gnagey, M. Seidenberg, J. Ellis, *Mol. Pharmacol.* **1999**, *56*, 1245 – 1253.
[17] D. Jäger, C. Schmalenbach, S. Prilla, A. Kebig, M. Sennwitz, J. Schrobang, E. Heller, C. Tränkle, U. Holzgrabe, H.-D. Höltje, K. Mohr, *J. Biol. Chem.* **2007**, *282*, 34968 – 34976.
[18] V. Nawaratne, K. Leach, C. C. Felder, P. M. Sexton, A. Christopoulos, *J. Biol. Chem.* **2010**, *285*, 19012 – 19021.
[19] B. K. Shoichet, B. K. Kobilka, *Trends Pharmacol. Sci.* **2012**, *33*, 268 – 272.
[20] P. J. Conn, A. Christopoulos, C. W. Lindsley, *Nat. Rev. Drug Discovery* **2009**, *8*, 41 – 54.
[21] M. de Amici, C. Dallanoce, U. Holzgrabe, C. Tränkle, K. Mohr, *Med. Res. Rev.* **2010**, *30*, 463 – 549.
[22] J. P. Changeux, *Annu. Rev. Pharmacol. Toxicol.* **2010**, *50*, 1 – 38.
[23] A. Bock, N. Merten, R. Schrage, C. Dallanoce, J. Bätz, J. Klöckner, J. Schmitz, C. Matera, K. Simon, A. Kebig, L. Peters, A. Müller, J. Schrobang-Ley, C. Tränkle, C. Hoffmann, M. De Amici, U. Holzgrabe, E. Kostenis, K. Mohr, *Nat. Commun.* **2012**, *3*, 1044.
[24] H. Möhler, J. N. Fritschy, U. Rudolph, *J. Pharmacol. Exp. Ther.* **2002**, *300*, 2 – 8.
[25] See Ref. [12].
[26] N. T. Burford, J. Watson, R. Bertekap, A. Alt, *Biochem. Pharmacol.* **2011**, *81*, 691 – 702.
[27] T. W. Schwartz, B. Holst, *Trends Pharmacol. Sci.* **2007**, *28*, 366 – 373.
[28] D. J. Scholten,, M. Canals, D. Maussang, L. Roumen, M. J. Smit, M. Wijtmans, C. de Graaf, H. F. Vischer, R. Leurs, *Br. J. Pharmacol.* **2012**, *165*, 1617 – 1643.
[29] A. Kebig, K. Mohr, *Dtsch. Med. Wochenschr.* **2008**, *133*, 1681 – 3.
[30] a) M. Decker, U. Holzgrabe, *MedChemComm* **2012**, *3*, 752 – 762; b) G. J. Digby, J. K. Shirey, P. J. Conn, *Mol. BioSyst.* **2010**, *6*, 1345 – 1354.
[31] R. Schwyzer, *Ann. N. Y. Acad. Sci.* **1977**, *297*, 3 – 26.
[32] P. S. Portoghese, *Trends Pharmacol. Sci.* **1989**, *10*, 230 – 235.
[33] C. Valant, J. R. Lane, P. M. Sexton, A. Christopoulos, *Annu. Rev. Pharmacol. Toxicol.* **2012**, *52*, 153 – 178.
[34] a) C. Valant, K. J. Gregory, N. E. Hall, P. J. Scammels, M. J. Lew, P. M. Sexton, A. Christopoulos, *J. Biol. Chem.* **2008**, *283*, 29312 – 29321; b) K. J. Gregory, N. E. Hall, A. B. Tobin, P. M. Sexton, A. Christopoulos, *J. Biol. Chem.* **2010**, *285*, 7459 – 7474.
[35] a) T. Disingrini, M. Muth, C. Dallanoce, E. Barocelli, S. Bertoni, K. Kellershohn, K. Mohr, M. De Amici, U. Holzgrabe, *J. Med. Chem.* **2006**, *49*, 366 – 372; b) J. Antony, K. Kellershohn, M. Mohr-Andrä, A. Kebig, S. Prilla, M. Muth, E. Heller, T. Disingrini, C. Dallanoce, S. Bertoni, J. Schrobang, C. Tränkle, E. Kostenis, A. Christopoulos, H.-D. Höltje, E. Barocelli, M. De Amici, U. Holzgrabe, K. Mohr, *FASEB J.* **2009**, *23*, 442 – 450.
[36] M. P. Caulfield, N. J. M. Birdsall, *Pharmacol. Rev.* **1998**, *50*, 279 – 290.
[37] U. Voigtländer, K. Jöhren, M. Mohr, A. Raasch, C. Tränkle, S. Buller, J. Ellis, H. D. Höltje, K. Mohr, *Mol. Pharmacol.* **2003**, *64*, 21 – 31.
[38] C. Dallanoce, P. Conti, M. De Amici, C. De Micheli, E. Barocelli, M. Chiavarini, V. Ballabeni, S. Bertoni, M. Impicciatore, *Bioorg. Med. Chem.* **1999**, *7*, 1539 – 1547.
[39] T. Steinfeld, M. Mamman, J. A. M. Smith, R. D. Wilson, J. R. Jasper, *Mol. Pharmacol.* **2007**, *72*, 291 – 302.
[40] R. Narlawar, J. R. Lane, M. Doddareddy, J. Lin, J. Brussee, A. P. IJzerman, *J. Med. Chem.* **2010**, *53*, 3028 – 3037.
[41] K. Mohr, C. Tränkle, E. Kostenis, E. Barocelli, M. De Amici, U. Holzgrabe, *Br. J. Pharmacol.* **2010**, *159*, 997 – 1008.
[42] a) D. J. Daniels, N. R. Lenard, C. L. Etienne, P.-Y. Law, S. C. Roerig, P. S. Portoghese, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 19208 – 19213; b) S. Zhang, A. Yekkirala, Y. Tang, P. S. Portoghese, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6978 – 6980.
[43] J. Shonberg, P. J. Scammells, B. Capuano, *ChemMedChem* **2011**, *6*, 963 – 974.
[44] A. Soriano, R. Ventura, A. Molero, R. Hoen, V. Casadó, A. Cortés, F. Fanelli, F. Albericio, C. Lluís, R. Franco, M. Royo, *J. Med. Chem.* **2009**, *52*, 5590 – 5602.
[45] M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908 – 2953; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754 – 2794.
[46] G. Maksay, *Prog. Biophys. Mol. Biol.* **2011**, *106*, 463 – 473.
[47] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Delivery Rev.* **1997**, *23*, 3 – 25.
[48] M. M. Hann, *MedChemComm* **2011**, *2*, 349 – 355; W. P. Walters, J. Green, J. R. Weiss, M. A. Murcko, *J. Med. Chem.* **2011**, *54*, 6405 – 6416; N. A. Meanwell, *Chem. Res. Toxicol.* **2011**, *24*, 1420 – 1456.
[49] B. J. Melancon, C. R. Hopkins, M. R. Wood, K. A. Emmitte, C. N. Niswender, A. Christopoulos, P. J. Conn, C. W. Lindsley, *J. Med. Chem.* **2012**, *55*, 1445 – 1464.
[50] M. R. Wood, C. R. Hopkins, J. T. Brogan, P. J. Conn, C. W. Lindsley, *Biochemistry* **2011**, *50*, 2403 – 2410.
[51] a) M. Muth, W. Bender, O. Scharfenstein, U. Holzgrabe, E. Balatkova, C. Tränkle, K. Mohr, *J. Med. Chem.* **2003**, *46*, 1383 – 1389; b) A. Raasch, O. Scharfenstein, C. Tränkle, U. Holzgrabe, K. Mohr, *J. Med. Chem.* **2002**, *45*, 3809 – 3812.
[52] M. J. Lohse, S. Nuber, C. Hoffmann, *Pharmacol. Rev.* **2012**, *64*, 299 – 336.
[53] T. P. Kenakin, *Br. J. Pharmacol.* **2012**, *165*, 1659 – 1669.

