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Review

Emerging Approaches to GPCR Ligand Screening for Drug Discovery

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The superfamily of G-protein-coupled receptors (GPCRs) represents the largest class of cell surface receptors and, thus, a prominent family of drug targets. Recently, there has been significant progress in determination of GPCR crystal structures. The structure-based ligand discovery of GPCRs is emerging as a powerful path to drug development. Sensor surface-immobilized GPCRs can identify direct receptor–ligand interactions of a range of chemical libraries. This type of screening shows great promise as an alternative strategy for ligand discovery. Here, we summarize the most recent developments of structure- and sensor-based GPCR ligand discovery. We also highlight certain areas where GPCRs harbor great potential for the development of novel therapeutics, emphasizing the strategic approaches that may yield significant breakthroughs.

G-Protein-Coupled Receptors as Drug Targets and High-Throughput Ligand Screening

GPCRs, also referred to as seven transmembrane receptors (7TMRs), are key players in innumerable cellular signaling cascades [1,2]. GPCRs participate directly or indirectly in most physiological functions in the human body and are often at the center stage in the symptomatic manifestation of many diseases [3]. Upon ligand binding, GPCRs couple to heterotrimeric G proteins, followed by the generation of second messengers and the initiation of downstream signaling events. Activated GPCRs are phosphorylated by GPCR kinases (GRKs), which trigger the recruitment of multifunctional protein β-arrestins, which in turn leads to the desensitization of G protein signaling through steric hindrance [4,5]. At the same time, β-arrestins also facilitate GPCR endocytosis and initiate a G protein-independent wave of downstream signaling [5,6]. GPCRs represent a fascinating example of fine-tuned molecular recognition modules because they recognize many different types of ligand with strikingly diverse physicochemical and structural properties, despite sharing an overall, highly conserved 7TM architecture. Furthermore, despite their ability to engage diverse ligands, from small nucleotides to entire polypeptides, there exists a remarkable convergence in the signaling and regulatory mechanisms of GPCRs.

Given their central role in many pathophysiological conditions, GPCRs have traditionally been, and continue to be, attractive drug targets [7,8]. There have been innumerable efforts to screen and identify novel GPCR ligands both in industry and academia and this is one of the key focus areas in GPCR biology. Not surprisingly, approximately one-third of the currently marketed drugs target GPCRs and work by turning them 'on' or 'off' [7]. It is interesting to note that approximately 100 nonolfactory GPCRs are still orphan and represent as yet unexplored targets for novel drug discovery. Furthermore, new paradigms of GPCR signaling, especially **biased agonism** (i.e., the ability of certain ligands to selectively signal through either the G protein or β-arrestin pathways; see Glossary), have opened up avenues for novel drug design even using

Trends

G-Protein-coupled receptors (GPCRs) are the largest class of cell surface receptors and, thus, represent targets for approximately one-third of currently marketed drugs.

The emergence of GPCR crystal structures has allowed the virtual screening and identification of novel antagonist and agonist ligands with reasonable affinity and selectivity.

The sensor-based screening approach is emerging as a powerful strategy to uncover novel GPCR ligands.

The sensor-based approach harbors a unique potential for the identification of allosteric ligands and the screening of GPCR–ligand signaling complexes.

Recent advances in the area of GPCR biology open up new frontiers in GPCR targeting, such as biased ligands, allosteric ligands, and intracellular modulators.

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existing targets [9,10]. Therefore, the identification of novel GPCR ligands continues to be a major area in GPCR research with great promise for novel therapeutics.

Currently, the most commonly used strategy for novel GPCR ligand identification is high-throughput screening (HTS) of small molecule compounds [11]. This approach relies on screening a large number of chemical compounds (libraries) on heterologous cells overexpressing target GPCRs with effector coupling or second messenger generation as the primary readout (Figure 1A, Key Figure). Candidate hits identified through such screens are confirmed by in-depth analysis with respect to their affinity and efficacy, followed by optimization of selected **leads** in extensive **structure–activity relationship** (SAR) studies. After satisfactory lead optimization, to determine their potential as promising drugs, compounds are tested for their *in vivo* efficacy and safety, which includes clinical trials. Although the drug discovery pipeline for any target is expensive and time consuming, even the first phase of ligand identification through HTS is cumbersome for GPCRs. HTS sometimes fails to deliver compounds that are suitable for subsequent optimization. In addition, inherently focusing on potency during HTS often results in potential hits with high molecular weight and suboptimal physicochemical characteristics that pose a significant challenge during optimization. Therefore, novel strategies for GPCR ligand discovery are desirable.

Recent technical advances have allowed the isolation of stable and functional recombinant GPCRs and, together with methodological breakthroughs in the area of membrane protein crystallography, has catalyzed rapid progress in GPCR crystallography. The availability of stable protein preparations and high-resolution GPCR crystal structures has opened up new avenues for GPCR ligand screening and two approaches in particular, one **structure-based** and the other **sensor-based**, have emerged as robust GPCR ligand discovery tools. Compared with conventional HTS, the structure-based approach offers relatively better time and cost effectiveness, high throughput, typically high hit rates, and the identification of novel scaffolds. The sensor-based approach, although less amenable to large-scale screening, can provide detailed association and dissociation parameters of candidate hits as well as be potentially used to target novel **allosteric binding sites** on the receptors. Here, we discuss in detail the successful examples of these approaches, their advantages, and limitations for novel GPCR ligand discovery. Although GPCR homology models were used for ligand discovery before the crystal structures became available, it resulted in only limited success; therefore, high-resolution crystal structures of GPCRs have provided a much-needed breakthrough to facilitate structure-based GPCR ligand discovery. Considering the rapidly expanding structural coverage of the GPCR superfamily, including activated and effector bound conformations, it is an opportune time to assess the current capabilities of virtual screening and push the boundaries to fully leverage the new structural information on GPCRs that is likely to become available in coming years.

Seeing the Pocket: Crystallography of GPCRs

Structure-based drug design (SBDD) has been a prominent approach for many non-GPCR targets (e.g., viral proteases) and has led to several clinically used drugs (e.g., aliskiren against renin and telaprevir against hepatitis C virus protease) [12,13]. Typically, crystal structure determination of the target in complex with the lead compound is an integral component of SBDD. High-resolution structural details gained from target-lead complex structures are factored into lead optimization and SAR studies through directed chemistry. However, for GPCRs, SBDD had only limited success until recently due to the paucity of GPCR crystal structures. Furthermore, it has also been difficult to incorporate the co-crystallization of target complexes during lead optimization phase because of problems associated with their crystallization. As described below, the past few years have seen remarkable progress in the structural coverage of the GPCR family, which has led to a new wave of structure-based ligand discovery on GPCRs.

Glossary

Allosteric binding site: the binding site of synthetic ligands other than orthosteric binding sites on the receptor.
Biased agonism: the phenomenon of certain ligands (referred to as biased agonists) selectively engaging one or other signaling pathways (e.g., G protein versus β-arrestin pathway).
Lead identification (hit to lead): the process of limited optimization of initial hits (e.g., positive-scoring compounds from a screen) to generate a smaller set of promising leads (e.g., modification of side chains of the hits) for extensive optimization.
Orthosteric binding site: the binding pocket on the receptor that accommodates the endogenous ligand.
Sensor-based ligand screening: the process of identifying potential ligands against a given target using SPR-based detection of direct binding between chemical compounds (ligands) and the target.
Structure-based drug design (SBDD): the structure-based optimization of a lead compound (e.g., identified through HTS). It involves cycles of co-crystallization and structure determination of the drug target with chemical derivatives of the lead compound, and subsequent structure-based design of new derivatives to improve the affinity, potency, and efficacy of that compound.
Structure–activity relationship (SAR): a process of understanding through chemical (and structural) modification how different chemical moieties of a compound are responsible for, and govern, its biological activity.
Surface plasmon resonance (SPR): a detection technique for the interaction of two molecules based on a change in refractive index on a sensor surface.

Key Figure

A Simplified Schematic Representation of Cell-based High-Throughput Screening (HTS), Structure-based and Sensor-based Ligand Discovery Approaches

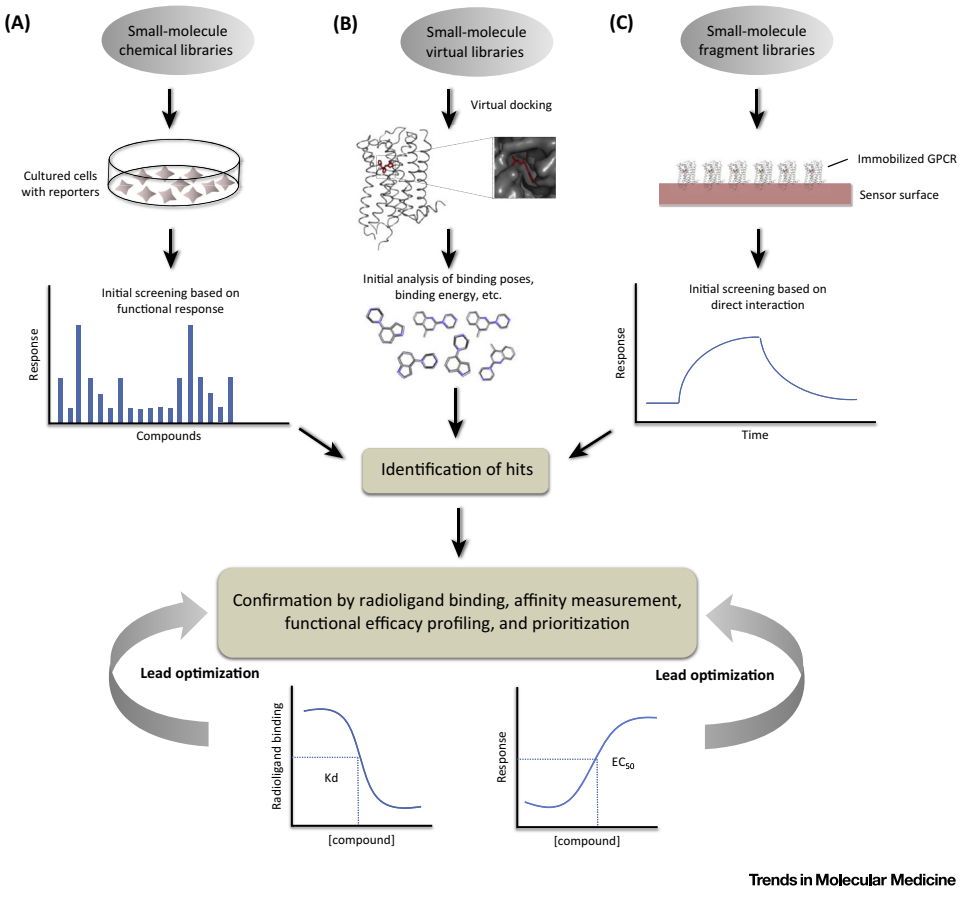

Figure 1. (A) In an HTS approach, heterologous cells overexpressing the target of interest are stimulated with individual compounds of a large chemical library and hits are identified with coupling of an effector or the generation of second messenger as readout. (B) In structure-based ligand discovery, once the target receptor and potential docking interface have been identified, a virtual screening of a large chemical library is performed *in silico*. Subsequently, top-scoring 'candidate hits' (or 'potential hits') can be analyzed manually based on their binding energy calculations and binding poses. This is followed by selection of a handful of 'candidate hits' for subsequent characterization. (C) In a sensor-based approach, a purified receptor is first immobilized on a solid sensor surface and subsequently validated using known ligands for the target receptor and for assessing the stability of the system. Subsequently, compound libraries are added to the immobilized target, one compound at a time, and 'candidate hits' are identified based on a direct interaction with the target receptor. In all of these approaches, 'candidate hits' are further characterized by radioligand binding (measuring their affinity) and cell-based functional assays (measuring their efficacies). These extensive characterizations are followed by further optimization cycles through directed chemistry and subsequent *in vivo* efficacy profiling.

The crystal structure of the visual receptor rhodopsin was determined approximately 15 years ago, and it remained the only GPCR structure for nearly a decade [14]. Homology models of other GPCRs built using rhodopsin as a template resulted in some success for new ligand identification [15–17]. This was not an optimal approach because of the low sequence homology

of rhodopsin with class A GPCRs, which was even lower with other classes of GPCR. Furthermore, *cis*-retinal is a covalently attached inverse agonist for rhodopsin that undergoes isomerization and is converted into an agonist. This differs from the diffusible inverse agonist and agonist ligand binding that occurs with other GPCRs. In addition, accurate modeling of the extracellular loops of GPCRs based on the rhodopsin template has also been challenging. A major breakthrough came with the structure determination of the human β_2 adrenergic receptor (β_2AR) approximately 7 years ago [18]. Since then, several generally applicable technological advances (reviewed in [19–21]) have facilitated the crystallization and structure determination of many GPCRs bound to different types of ligand (summarized and discussed in recent reviews [19,22–26]). The emergence of structural coverage has provided much awaited visualization of the atomic details of ligand-binding pockets to test the feasibility of structure-based ligand discovery using GPCRs. Furthermore, the availability of both inactive and active conformations of at least a few GPCRs, such as adenosine receptor subtype 2A (A_{2A}R) [27,28], β_2AR [18,29], muscarinic receptor subtype 2 (M_2R) [30,31], and μ-opioid receptor (μ-OR) [32,33] not only allows assessment of the capabilities of structure-based screening approaches to identify ligands with different functional efficacies, but also permits cross-screening and validation of different conformations to optimize this approach.

Structure-based Virtual Ligand Screening on GPCRs

A typical cycle of structure-based ligand discovery on GPCRs starts with the virtual screening of large chemical databases against the crystal structure of target receptors [34,35] (Figure 1B). Although the known ligand-binding pocket as observed in the crystal structure is used primarily as the docking interface, theoretically, other nonorthosteric binding pockets can also be targeted as potential sites for the binding of allosteric ligands. Subsequently, based on energy calculations and binding poses, top-scoring potential hits are procured. This is followed generally by the validation of potential hits through cell-based functional assays of effector coupling and second messenger generation, which, in turn, helps to prioritize hits for subsequent optimization. Potential hits can also be characterized by biophysical approaches, such as surface plasmon resonance (SPR) or radioligand binding assays, to confirm their direct binding to target receptors and to measure their binding affinities. One would envisage crystallization and structure determination of the target receptor with the lead compound and further SAR studies as a part of lead optimization. However, this remains a challenging step for GPCR targets because most of the lead compounds have relatively low affinity and are not suitable for co-crystallization.

Antagonist-bound GPCR Templates

The crystal structure of β_2AR, also the first nonrhodopsin GPCR, was first determined in complex with the high-affinity inverse agonist carazolol [18]. This provided both the possibility of direct structure-based virtual docking (unlike homology models used earlier) and an additional template to generate better homology models for class A GPCRs. Soon after structure determination, the carazolol-bound β_2AR crystal structure (Figure 2A) was used as a template for virtual docking of the ZINC library, comprising close to 1 million compounds [36]. Approximately 25 high-scoring hits were chosen for further characterization and six of these showed direct binding to β_2AR, as assessed by radioligand binding, with affinities ranging from 9 nM to 4 μM. Interestingly, some of these hits displayed significant chemical dissimilarity with the known β_2AR inverse agonists, indicating the feasibility of identifying novel chemotypes and scaffolds by structure-based screening. Subsequently, the β_2AR bound to the most potent hit from this screen was crystallized; the crystal structure revealed a binding pose that was similar to the well-characterized inverse agonist ICI118551 and carazolol [37] (Figure 2B,C). As additional GPCR crystal structures became available, a similar approach was applied to A_{2A}R [38–41], histamine subtype 1 receptor (H_1R) [42], dopamine subtype 3 receptor (D_3R) [43–45], M_2R and M_3R [46], serotonin receptors 5-HT_{1B} and 5-HT_{2B} [47], κ-opioid receptor (κ-OR) [48], and chemokine

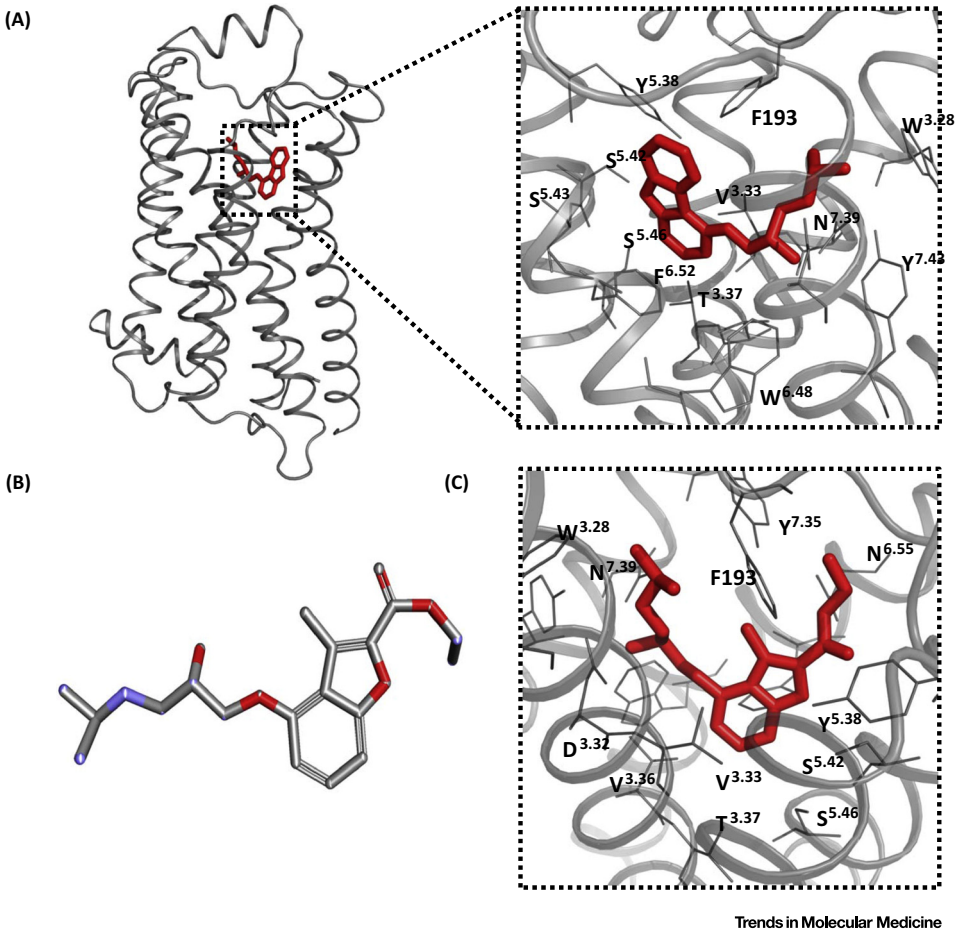

Figure 2. Structure-based Identification of a Novel β_2 Adrenergic Receptor (β_2AR) Inverse Agonist. (A) The crystal structure of the human β_2AR [Protein Data Bank (PDB) ID 2RH1] bound to the inverse agonist carazolol was used as a docking template for structure-based ligand screening. A ZINC library comprising approximately 1 million compounds was used for virtual screening. (B) Among several hits, a novel chemotype with an affinity of 9 nM (as assessed by a radioligand binding assay) was identified. (C) Subsequently, the crystal structure of β_2AR in complex with this hit was determined (PDB ID 3NY9) and revealed a binding pose similar to that of the inverse agonists carazolol and ICI118551. Residues in the orthosteric ligand-binding pocket of β_2AR that interact with the ligands are labeled and presented with their side chains.

(C-X-C motif) receptor 4 ($CXCR_4$) [49] to identify low- to moderate-affinity novel chemotype and ligand scaffolds (Table 1). Interestingly, a correlation appears to exist between the efficacy of ligand bound to the receptor template used for screening and the efficacy of potential hits that are identified. Furthermore, in most cases, the hit rate is significantly higher compared with typical hit rates in conventional HTS, underscoring the advantage of structure-based screening. These studies establish that GPCR crystal structures provide a feasible template for structure-based ligand discovery.

Active Receptor Structures as Screening Templates

When the crystal structure of fully active agonist-bound β_2AR became available, first stabilized by an active state-selective nanobody [29] and then in complex with heterotrimeric G protein [50], it provided a unique opportunity for virtual screening to probe the feasibility of agonist discovery. Indeed, screening of a larger database comprising approximately 3 million compounds on fully active β_2AR yielded several hits that turned out to be *bona fide* agonists in cell-based functional assays [51]. Four of these hits exhibited full agonist-like properties, stimulating both the G protein

Table 1. Summary of Structure-based GPCR Ligand Discovery

Receptor/Ligand/ Efficacy	Library/Size/ Tool	Candidate Hits (Confirmed Hits) (Hit Rate)[a]	Validation Assay(s)	Affinity Thresholds [% Inhibition or pK_i/IC_{50} (μM)]	Efficacy and Selectivity	Refs
β_2AR/carazolol/ inverse agonist	ZINC/1 million/ Dock 3.5.54	25 (6) (24%)	Radioligand binding and G protein coupling	>10% inhibition at 20 μM	Inverse agonists	[36]
	Proprietary and commercial/ 4.4 million/ GLIDE	300 (31) (10%)	Radioligand binding assay	>35% inhibition at 10 μM	Antagonists	[96]
	MSD Organon/ 50 000	900 (6) (0.7%)	Radioligand binding assay	pK_i >4.0	Antagonists	[97]
β_2AR/BI-167017/ agonist	ZINC/3million/ Dock 3.6	22 (6) (27%)	Radioligand binding, G protein and β-arrestin coupling	pEC_{50} >/= 4.5	4 Full agonists, 2 partial agonists	[51]
A_{2A}AR/ ZM241385/ antagonist	Multiple libraries/4 million/ICM-VLS	56 (23) (42%)	Radioligand binding assay	pK_i >5.0	Antagonists	[39]
	ZINC/1 million/ Dock 3.5.54	20 (7) (35%)	Radioligand binding and G protein coupling assay	>40% inhibition at 20 μM	Antagonists	[98]
	MSD Organon/ 50 000	900 (18) (2%)	Radioligand binding assay	pK_i >5.0	Antagonists	[97]
	Zinc/791 162	54 (6) (11%)	Radioligand binding assay	>50% inhibition at 10 μM	Antagonists	[99]
A_{2A}AR/UK-432097/agonist	ChemBridge/ 2000/ICM-VLS	16 (15) (94%)	Radioligand binding and G protein coupling assay	pK_i >6.0	Agonists	[53]
A_{2A}AR/UK-432097, NECA, adenosine/agonist	ZINC/6 million/ Dock 3.6	20 (9) (45%)	Radioligand binding and G protein coupling assay	>50% inhibition at 10 μM	Antagonists (selective for A_{2A}R over A_{1A}R)	[38]
D_3R/eticlopride/ inverse agonist	Fragment library/13 000/ Glide 5.7	92 (25) (27%)	Radioligand binding assay	>20% inhibition at 10 μM	Agonists	[43]
	ZINC/3.6 million/Dock 3.6	25 (5) (20%)	Radioligand binding and β-arrestin coupling assay	pK_i >5.0	Antagonists (one weak partial agonist)	[45]
D_3R/Apo and D_3R/ dopamine/agonist	Commercial/ 4.1 million/ICM	25 (14) (56%)^{Apo} and 25 (8) (32%)^{Dopa}	Radioligand binding, ERK activation and β-arrestin coupling assay	pK_i >5.0	Antagonists and allosteric modulators	[44]
M_2R/3-QNB/ inverse agonist	ZINC/3 million/ Dock 3.6	18 (11) (61%)	Radioligand binding and G Protein coupling assays	pK_i >4.0	Antagonists	[46]

Table 1. (continued)

Receptor/Ligand/ Efficacy	Library/Size/ Tool	Candidate Hits (Confirmed Hits) (Hit Rate)[a]	Validation Assay(s)	Affinity Thresholds [% Inhibition or pK_i/IC_{50} (μM)]	Efficacy and Selectivity	Refs
M_3R/tiotropium/ inverse agonist	ZINC/3 million/ Dock 3.6	16 (8) (50%)	Radioligand binding and G Protein coupling assays	pK_i >4.0	Antagonists expect 1 that is M_3R selective partial agonist	[46]
H_1R/doxepin/ inverse agonist	ZINC/100 000/	26 (19) (73%)	Radioligand binding and G Protein coupling assays	pK_i >5.0	9 Inverse agonists and 1 partial agonist	[42]
5-HT_{1B} and 5-HT_{2B}/ergotamine/ agonist	ZINC/1.3 million/Dock 3.6	22 (13) (60%)	Radioligand binding and G protein coupling assay	pK_i >5.0	Agonists, one shows selectivity for 5-HT_{1B} over 5-HT_{2B}	[47]
κ-OR/JDTic/ inverse agonist	ZINC/4.5 million/Dock 3.6	22 (4) (18%)	Radioligand binding, G protein and β-arrestin coupling	pK_i >4.0	Antagonists, one shows weak but selective agonism at κ-OR	[48]
CXCR4/IT1t/ antagonist	ZINC/4.2 million/Dock 3.6	23 (4) (17%)	Radioligand binding and G protein coupling assay	IC_{50} >100 μM	Competitive antagonists	[49]

[a]The hit rate presented here represents the % of hits that were confirmed based on radioligand binding and/or functional assays out of the compounds that were chosen for such assays. The affinity threshold cut-off values represent a close approximation whenever not clearly calculated in the primary papers.

and β-arrestin coupling to β_2AR, while two appeared to be partial agonists for G protein coupling with no measurable β-arrestin recruitment (Table 1). The **orthosteric binding** pocket of the β_2AR between the inactive and active state structures shows relatively modest but well-defined conformational differences and these were also predicted computationally [52]. These findings support the notion that a snapshot of the ligand-binding pocket in complex with the relevant ligand might have a key role in the efficacy of hits that are identified. A similar trend has been observed for agonist-bound A_{2A}R [53], M_3R [46], and 5-HT_{1B} [47] that have been subjected to structure-based virtual screening and subsequent functional assessment of the hits (Table 1). However, relatively fewer GPCR structures bound to agonist are currently available compared with antagonist-bound structures and this represents a potential limiting step in the structure-based agonist discovery of GPCR agonists.

The Issue of Subtype Selectivity

Subtype selectivity of GPCR targeting drugs is crucial for minimizing their adverse effects. In the drug discovery pipeline, receptor subtype selectivity is typically tested and tweaked during the lead optimization phase. As crystal structures of different subtypes of some GPCRs have become available (e.g., β_2AR and β_1AR, M_2R and M_3R, and 5-HT_{1B} and 5-HT_{2B}), it has enabled researchers to test whether a structure-guided approach can be leveraged to yield subtype-selective ligands. The crystal structures of M_2R and M_3R were described in close succession and, therefore, presented an ideal system for testing the potential of a structure-based approach with respect to subtype-specific ligand identification [31,54]. Although the orthosteric binding pockets of the two subtypes show modest differences (Figure 3A), compounds with some

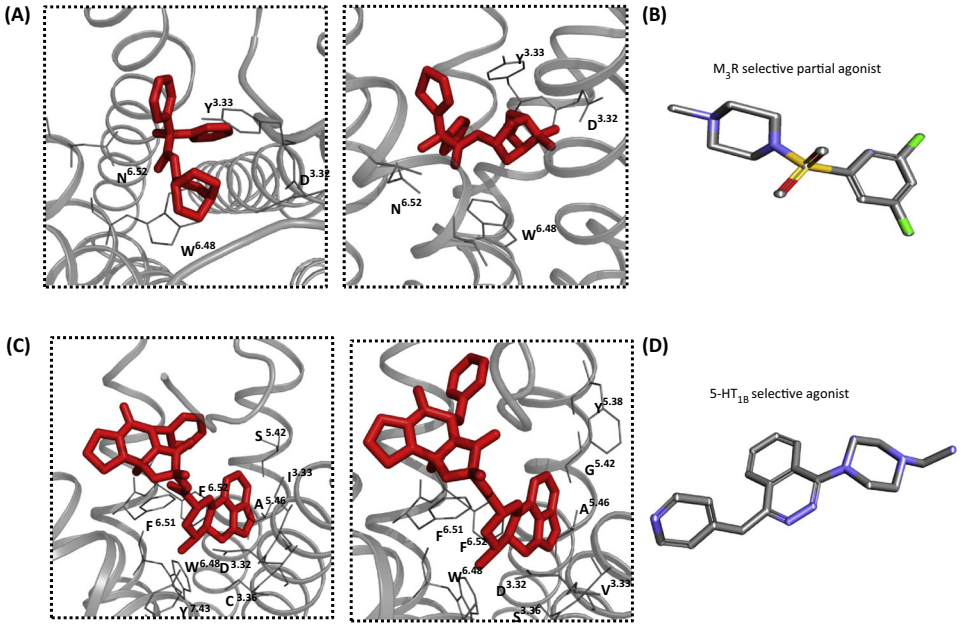

Figure 3. Structure-based Ligand Screening Enables the Discovery of Receptor Subtype-Selective Ligands. (A) Crystal structures of the two subtypes of muscarinic receptor [M_2R, Protein Data Bank (PDB) ID 3UON and M_3R, PDB ID 4DAJ] were determined in quick succession and revealed remarkably similar orthosteric ligand-binding pockets. (B) However, structure-based screening was able to identify at least one compound that exhibited significant subtype selectivity for M_3R over M_2R in a cell-based functional assay of cAMP generation. (C) The crystal structures of ergotamine bound serotonin 5-HT_{1B} (PDB ID 4IAR) and 5-HT_{2B} (PDB ID 4IB4) were determined simultaneously and subsequently used as a template for the virtual screening of approximately 3 million compounds. (D) One of the hits exhibited approximately 300-fold binding selectivity to 5-HT_{1B} over 5-HT_{2B}, as determined by radioligand binding and functional assays.

selectivity of M_3R were identified, with at least one compound that serves as a M_3 partial agonist with lack of any measurable activity at M_2R [46] (Figure 3B). The other example of the discovery of receptor subtype-selective hits resulted from virtual screening of 5-HT_{1A} and 5-HT_{2B} crystal structures [55,56]. Although both crystal structures contained the same ligand, ergotamine, in their orthosteric binding pocket, virtual screening resulted in at least one hit with an approximately 300-fold preference for 5-HT_{1A} over 5-HT_{2B} [47]. Similarly, structure-based virtual screening of A_2AR also yielded at least two hits that exhibited close to tenfold selectivity on $A_{2A}R$ versus $A_{1A}R$ systems [53]. Among currently available GPCR structures, the β-adrenergic system also provides an excellent set-up for fully assessing the capabilities of a structure-based approach because several crystal structures of two different subtypes, β_1AR and β_2AR, have been determined in complex with multiple agonists and antagonists.

As the structural coverage of GPCRs has increased, significant diversity in the binding pockets has become apparent. For example, CP-376395, an antagonist of corticotropin-releasing factor 1 (CRF_1R), binds deep within the transmembrane region close to the intracellular face of the receptor [57]. Crystal structures of purinergic receptors $P2Y_{12}$ and $P2Y_1$ exhibit multiple binding pockets and display significant differences in binding orientation of antagonists versus agonists [58–60]. Interestingly, a non-nucleotide antagonist (BPTU) of the $P2Y_1$ receptor binds on the outside surface of the receptor at the lipid interface [60]. Furthermore, an unusual binding site of a lipophilic agonist (TAK875) to GPR40 [61] and binding of a sphingolipid mimic (ML056) to sphingosine-1-phosphate receptor 1 ($S1P_1$) [62] suggest the possibility of ligand entry from the lipid membrane rather than from the extracellular face of the receptor. These recent

developments open up new frontiers in structure-based GPCR ligand discovery by providing significantly different templates when compared with aminergic receptors, which pushes the boundaries of virtual screening.

Overall, the examples discussed above establish the generality and feasibility of a structure-based approach for GPCR ligand discovery. Binding affinities of some of the hits in the low nM range underscore the inherent promise of this approach and put it on a par with HTS in this context. Although crystal structures of GPCRs are becoming available at a staggering rate and some receptors have been crystallized in complex with many different ligands (e.g., β_2AR and A_{2A}R), it is too early to evaluate the full potential of this approach for GPCRs. Nevertheless, the emerging trends and examples indeed show significant promise for the coming years.

Sensor-based Screening of GPCR Ligands

A second approach for ligand screening that has emerged recently is immobilization of purified GPCRs on SPR surfaces and subsequent screening of fragment libraries [63–68]. Given that SPR reports the direct interaction of target and ligand based on a change in refractive index at the surface, it is particularly appealing for identifying ligands that bind outside the conventional orthosteric and allosteric binding pockets. The development of SPR has benefited from the recent advances in recombinant GPCR expression, affinity tagging, new detergents and lipids, as well as optimized purification protocols [69–72]. The functional solubilization and purification of stable GPCRs has allowed flexibility in immobilizing the receptors through N- or C-terminal affinity tags and subsequent validation by measuring the affinities of well-known ligands for the target receptors. In SPR screening carried out so far, immobilized GPCRs are incubated with relatively smaller fragment libraries followed by a direct receptor–fragment interaction as readout for identification of potential hits (Figure 1C). Similar to HTS and structure-based strategies, initial candidate hits are subsequently characterized in detail using radioligand binding and cell-based functional assays (Figure 1C). However, in some cases, SPR-based candidate hits may not be potent enough to be directly tested in cell-based functional assays and, therefore, the hits must undergo several rounds of optimization to allow subsequent detail characterization.

The first successful SPR-based ligand screening for a GPCR was carried out on a thermostabilized adenosine A_{2A} receptor and yielded approximately half a dozen hits with an affinity range from 10 μM to 5 mM [67]. Subsequently, a chemokine receptor, chemokine receptor subtype 5 (CCR_5), which is involved in the entry of HIV to white blood cells and is a target for the anti-HIV drug maraviroc, was subjected to SPR-based screening [63]. A relatively small fragment library screen of solubilized and immobilized CCR_5 resulted in five hits with affinities in the low μM range. Although these ligands were not validated by subsequent radioligand binding or functional assays, they appeared to prefer the maraviroc-bound receptor conformation, which suggests that they are allosteric in nature. Recently, a more elaborate SPR-based screening was carried out on purified human β_2AR, resulting in half a dozen fragment-based ligands with affinities ranging from low nM to low μM [66] (Table 2). Comprehensive profiling based on radioligand binding and functional assay revealed that the hits were competitive antagonists with reasonable selectivity for β_2AR over β_1AR.

So far, SPR-based screening has utilized either detergent-solubilized or affinity-purified receptor. In some cases, solubilized GPCRs display relatively lower ligand-binding affinities compared with the receptor in the membrane; therefore, some concerns involve the possibility of missing low-affinity hits or underestimation of affinities. Thus, whenever feasible, even lower-affinity hits identified from SPR screens should be tested in cell-based validation assays. It is also important to validate SPR hits in both orthosteric and allosteric modes using cell-based functional assays considering the direct interaction as SPR readout. Furthermore, many purified GPCRs have been successfully reconstituted in well-defined lipid vesicles and in nanodiscs; this also offers the

Table 2. Summary of Sensor-based GPCR Ligand Discovery[a]

GPCR	Capture	Ligands Screened	Hits	Efficacy of Hits	Affinity Range	Remarks	Refs
CCR_5	$Rho^{C\text{-}term}$	~200	~5	Not tested	~8 μM–49 μM	Also bind to closely related CXCR4 suggesting low subtype selectivity	[63]
β_2AR	$His^{C\text{-}term}$	~650	~5	Competitive antagonists (radioligand binding and functional assays)	~15 nM–20 μM	One of the hits displayed about tenfold selectivity for β_2AR over β_1AR	[66]
$A_{2A}R$	$His^{C\text{-}term}$	~80	~8	Not tested	~10 μM–5 Mm	Thermostabilized $A_{2A}R$ was used	[67]
β_1AR	$His^{C\text{-}term}$	~650	~12	Not tested	~5–20 μM	Subsequent SAR studies identified more potent analogs	[68]
$A_{2A}R$	$His^{C\text{-}term}$	~500	~5 (+7)	Five competitive antagonists and seven weak allosteric modulators (radioligand binding and functional assays)	~70 μM–2 mM	NMR-based approach was used for screening	[100]

[a]Abbreviations: $His^{C\text{-}term}$, carboxyl-terminus histidine affinity tag; NMR, nuclear magnetic resonance; $Rho^{C\text{-}term}$, carboxyl terminus rhodopsin affinity tag.

possibility of indirect immobilization of purified receptor (e.g., through lipid biotinylation or scaffold protein biotinylation) in a more native-like surrounding. A potential drawback of this approach is the relatively low throughput of the screening protocol, because of stability issues of purified GPCRs and the extensive time frame involved. Tailored instrumentation with more automation and perhaps multichannel recording might increase the throughput of this approach in the future. Furthermore, similar to many other technologies, the expertise currently lies in selected laboratories and might restrict frequent usage of this approach, limiting the realization of its full potential. It is important to note that the SPR-based approach has the unique advantage of reporting candidate hits that bind new allosteric binding patches outside of conventional orthosteric and allosteric pockets, including the intracellular surface of the receptors. Although HTS can also identify such hits outside of conventional binding pockets, radioligand competition binding assays may not be utilized to confirm such candidate hits. Furthermore, SPR can be potentially utilized for screening on preformed and highly stabilized GPCR signaling complexes (e.g., receptor–G protein or receptor–β-arrestin, to target their interaction interface), an avenue that remains challenging in the cell-based HTS set-up. However, the feasibility of such a screen, even with SPR, remains to be documented.

An interesting path with significant promise is the use of hybrid approaches that combine sensor- and structure-based approaches for initial hit identification followed by extensive search for structurally similar ligands in available chemical space. This may help reduce the frequency of false-positives and provide indirect lead optimization. In fact, for thermostabilized β_1AR (Figure 4A), SPR-based screening identified low μM hits and a database search for similar compounds yielded similar structures with better affinities (Figure 4B) [68]. Two of these hits were potent enough to be used in crystallography efforts, which revealed their binding pattern in the β_1AR orthosteric ligand-binding pocket (Figure 4C,D). Another recent study combined

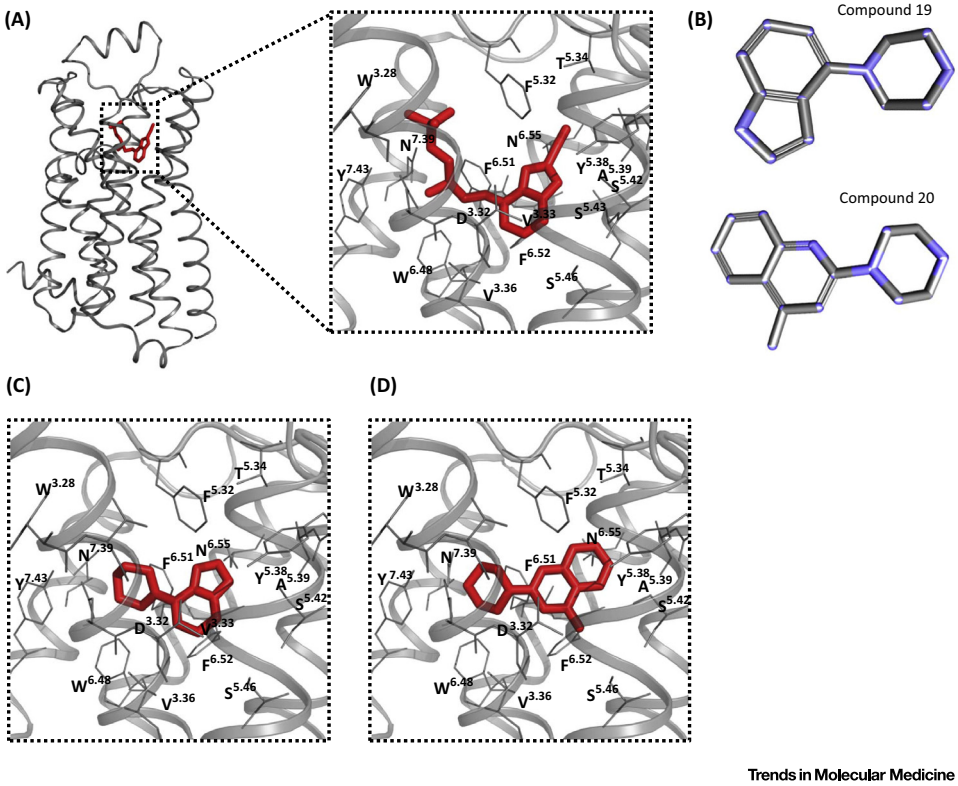

Figure 4. Sensor-based Hit Identification and Guided Discovery of β_1 Adrenergic Receptor (β_1AR) Ligands. (A) Thermostabilized β_1AR was immobilized on a surface plasmon resonance (SPR) chip surface followed by screening of a library containing approximately 600 fragments. (B) Two of the hits were used for structure–activity relationship studies that resulted in the identification of two new fragments of similar structural scaffolds with much better affinity. (C,D) Subsequently, β_1AR was co-crystallized with these two hits and crystal structures [Protein Data Bank (PDB) ID 3ZPQ and 3ZPR] revealed an overall binding mode that was similar to that of the classical inverse agonist cyanopindolol (PDB ID 2VT4).

fragment screening followed by structure-guided optimization to identify a novel negative allosteric modulator of class C GPCR, metabotropic glutamate receptor 5 (mGluR5) [73]. These studies highlight the power of a multipronged approach that encompasses both sensor-based and structure-based strategies together with an orthogonal approach of structure-based similarity searches, once a few hits are identified.

Emerging Frontiers

Biased Ligands

The concept of biased agonism (i.e., the ability of ligands to selectively trigger one or the other signaling cascade downstream of GPCRs), has added a new dimension to the fundamental GPCR signaling paradigm [74–77]. This has also led to excitement around the possibility of improving currently existing clinical drugs as well as designing more effective drugs targeting existing GPCR targets. For many different GPCRs, it has been proposed that biased ligand-based drugs might potentially have fewer adverse effects compared with currently existing conventional agonists or antagonists. Therefore, structure- and sensor-based approaches should be tested to explore the possibility of discovering biased ligands. Considering a strong correlation between the type of ligand present in the docking template and efficacy of hits generated, the structure-based identification of biased ligands will require GPCR crystal structures bound to different types of strongly biased ligand (e.g., G protein biased versus β-arrestin biased) as well as structures of biased ligand–receptor–effector complexes. Crystal structures of

β_1AR bound to carvedilol, a β-arrestin-biased ligand [78], and 5-HT receptor subtypes [55,56] represent the first step in this direction, although their true potential for yielding biased ligands through a structure-based approach remains to be explored.

Allosteric Ligands

Another emerging area in GPCR research is the structure-based discovery of allosteric modulators. Among the crystal structures that are currently determined, the human M_3R contains an agonist and a negative allosteric modulator bound to the receptor [30]. A more recent crystal structure of the nucleotide-binding receptor, P_2Y_1 also contains an agonist and an allosteric ligand [60]. Furthermore, the crystal structure of a class B receptor, corticotropin-releasing factor receptor subtype 1 (CRF1R) bound to a small molecule allosteric antagonist CP-376395 has also been determined [57]. In addition, crystal structures of Class C GPCRs (metabotropic glutamate receptors) in complex with allosteric modulators have also started to emerge [79,80]. These structures now provide optimal templates for virtual screening of chemical libraries to identify allosteric ligands to GPCRs. In fact, virtual screening on the dopamine D_3 receptor yielded noncompetitive allosteric modulators [44]. Furthermore, this structural visualization might also allow better design of SPR-based approaches to search for allosteric GPCR ligands. However, the functional validation of potential allosteric hits, especially those having relatively low affinity, is not straightforward and should be carefully designed.

Intracellular Modulators and Combinatorial Approaches

As mentioned above, GPCR ligand discovery has primarily focused on extracellular surface and small molecule ligands. However, recent examples of a few antagonists that are proposed to bind at the intracellular surface of a chemokine receptor, $CXCR_2$ provide a novel framework to look outside the box [81,82]. As mentioned earlier, a sensor-based approach can be leveraged to directly screen for small-molecule allosteric modulators that target the intracellular surface of GPCRs, followed by testing of their cell permeability, optimization, and subsequent cell-based functional testing. There is also considerable evidence that GPCRs having small-molecule endogenous ligands can also bind to, and get activated by, peptide ligands [83,84]. In fact, a synthetic peptide agonist velcalcetide (AMG 416) of the calcium-sensing receptor (CaSR) has shown promising results in clinical trials for the treatment of hyperthyroidism in patients undergoing hemodialysis [85,86]. In addition, there are several examples of agonistic and antagonistic GPCR antibodies in the literature, both in natural systems as well as through protein design and selection approaches [87–91]. Considering the recent progress and development of new combinatorial biology tools, it is plausible that new synthetic peptide- and protein (e.g., antibody fragments)-based GPCR ligands might be discovered in near future.

Signaling Complexes as Targets

As mentioned above, conventional GPCR ligand discovery has focused on extracellular orthosteric or allosteric ligand-binding pockets. Whether a receptor–effector interface can be targeted by designing cell-permeable small-molecule compounds remains unexplored. So far, the crystal structure of only two GPCR signaling complexes (i.e., β_2AR–G protein and rhodopsin–visual arrestin) have been determined [50,92]; this limits the availability of docking templates for structure-based identification of interface binders. A sensor-based approach might be tested as a possible strategy for screening ligands against signaling complexes, because one could envisage the possibility of immobilizing stable preformed complexes on sensor surfaces or even assembling signaling complexes on sensor surfaces for direct screening of ligand libraries. However, such screening efforts are likely to be challenging due to stability issues of signaling complexes. Moreover, the functional validation of candidate hits will also be difficult and will require innovative cellular assay designs.

Expanding the Scope through Homology Modeling

Considering the focus of this review, we have not discussed efforts related to GPCR ligand screening through homology modeling. With an increasing number of GPCR crystal structures, we are better equipped to build homology models than before and use such models with higher confidence for novel ligand discovery. This area has gained momentum over the past couple of years, with some evidence of success [43,93–95].

Concluding Remarks

Rapid developments in the area of GPCR crystallography have opened a previously untapped avenue of novel ligand discovery. However, it is too early to assess its impact on the development of drugs targeting GPCRs. In particular, it is unclear how soon structure-based 'ligand' discovery of GPCRs will translate into structure-based 'drug' discovery. With continuously increasing coverage of GPCR crystal structures, the stage is set to fully leverage structural information to make the long-standing dream of structure-based GPCR drug discovery a reality. Still, incorporating a step of structure-based lead optimization through co-crystallography of hits remains a challenging endeavor and requires further streamlining of GPCR crystallography efforts. Sensor-based ligand screening is also emerging as a powerful approach and now provides a handle on targets, especially signaling complexes, that cannot be directly used for screening in cell-based systems. However, adopting the sensor-based approach to larger-scale screening is likely to require further process optimization and innovative instrumental design. In addition to being primary ligand discovery tools, both these approaches can also serve to further characterize, optimize, and develop potential hits identified through HTS. Therefore, at this point, structure- and sensor-based GPCR ligand discovery can be appreciated as complementary approaches to conventional HTS screening, while offering some unique advantages, such as better cost effectiveness, typically higher hit rates, covering relatively larger chemical space, and potentially targeting novel interfaces on the receptors. Going forward, structure- and sensor-based approaches in combination with the conventional HTS, are likely to result in much excitement in the GPCR drug discovery field (see Outstanding Questions). These methods may significantly broaden the scope of potential targeting interfaces, and offer novel possibilities for the therapeutic design of more precise drugs with fewer adverse effects.

Outstanding questions

Can we begin comparing whether structure-based identified GPCR ligands display an advantage over conventionally discovered ligands?

Can structure-based GPCR drug discovery overcome the limitations of conventional approaches relative to receptor subtype selectivity?

Can the structure-based approach yield allosteric ligands that target the intracellular sites of GPCRs or coupled proteins from signaling complexes?

Can the structure-based approach facilitate the deorphanization of orphan GPCRs when more crystal structures become available?

Can the structure-based approach provide a better repertoire and atomic view of orthosteric ligand-binding pockets?

Can sensor-based approaches be developed and/or adopted into a relatively higher throughput format to screen larger libraries?

Can the sensor-based approach be leveraged to facilitate ligand screening of signaling complexes?

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References

1. Pierce, K.L. *et al.* (2002) Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol.* 3, 639–650
2. Bockaert, J. and Pin, J.P. (1999) Molecular tinkering of G protein-coupled receptors: an evolutionary success. *EMBO J.* 18, 1723–1729
3. Heng, B.C. *et al.* (2013) An overview of the diverse roles of G-protein coupled receptors (GPCRs) in the pathophysiology of various human diseases. *Biotechnol. Adv.* 31, 1676–1694
4. Pierce, K.L. and Lefkowitz, R.J. (2001) Classical and new roles of beta-arrestins in the regulation of G-protein-coupled receptors. *Nat. Rev. Neurosci.* 2, 727–733
5. Kang, D.S. *et al.* (2014) Role of beta-arrestins and arrestin domain-containing proteins in G protein-coupled receptor trafficking. *Curr. Opin. Cell Biol.* 27, 63–71
6. DeWire, S.M. *et al.* (2007) Beta-arrestins and cell signaling. *Annu. Rev. Physiol.* 69, 483–510
7. Ma, P. and Zemmel, R. (2002) Value of novelty? *Nat. Rev. Drug Discov.* 1, 571–572
8. Overington, J.P. *et al.* (2006) How many drug targets are there? *Nat. Rev. Drug Discov.* 5, 993–996
9. Violin, J.D. and Lefkowitz, R.J. (2007) Beta-arrestin-biased ligands at seven-transmembrane receptors. *Trends Pharmacol. Sci.* 28, 416–422
10. Whalen, E.J. *et al.* (2011) Therapeutic potential of beta-arrestin- and G protein-biased agonists. *Trends Mol. Med.* 17, 126–139
11. Takakura, H. *et al.* (2015) Cell-based assays and animal models for GPCR drug screening. *Methods Mol. Biol.* 1272, 257–270
12. Lin, C. *et al.* (2006) Discovery and development of VX-950, a novel, covalent, and reversible inhibitor of hepatitis C virus NS3.4A serine protease. *Infect. Disord. Drug Targets* 6, 3–16

13. Rahuel, J. *et al.* (2000) Structure-based drug design: the discovery of novel nonpeptide orally active inhibitors of human renin. *Chem. Biol.* 7, 493–504
14. Palczewski, K. *et al.* (2000) Crystal structure of rhodopsin: a G protein-coupled receptor. *Science* 289, 739–745
15. Evers, A. and Klebe, G. (2004) Successful virtual screening for a submicromolar antagonist of the neurokinin-1 receptor based on a ligand-supported homology model. *J. Med. Chem.* 47, 5381–5392
16. Evers, A. and Klabunde, T. (2005) Structure-based drug discovery using GPCR homology modeling: successful virtual screening for antagonists of the alpha1A adrenergic receptor. *J. Med. Chem.* 48, 1088–1097
17. Evers, A. and Klebe, G. (2004) Ligand-supported homology modeling of G-protein-coupled receptor sites: models sufficient for successful virtual screening. *Angew. Chem. Int. Ed. Engl.* 43, 248–251
18. Cherezov, V. *et al.* (2007) High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. *Science* 318, 1258–1265
19. Jazayeri, A. *et al.* (2015) From G protein-coupled receptor structure resolution to rational drug design. *J. Biol. Chem.* 290, 19489–19495
20. Piscitelli, C.L. *et al.* (2015) A molecular pharmacologist's guide to GPCR crystallography. *Mol. Pharmacol.* 88, 536–551
21. Ghosh, E. *et al.* (2015) Methodological advances: the unsung heroes of the GPCR structural revolution. *Nat. Rev. Mol. Cell Biol.* 16, 69–81
22. Lee, S.M. *et al.* (2015) Structural insights into ligand recognition and selectivity for classes A, B, and C GPCRs. *Eur. J. Pharmacol.* Published online May 14, 2105. http://dx.doi.org/10.1016/j.ejphar.2015.05.013
23. Katritch, V. *et al.* (2013) Structure-function of the G protein-coupled receptor superfamily. *Annu. Rev. Pharmacol. Toxicol.* 53, 531–556
24. Hollenstein, K. *et al.* (2014) Insights into the structure of class B GPCRs. *Trends Pharmacol. Sci.* 35, 12–22
25. Bennett, K.A. *et al.* (2015) Structures of mGluRs shed light on the challenges of drug development of allosteric modulators. *Curr. Opin. Pharmacol.* 20, 1–7
26. Ghosh, E. *et al.* (2014) SnapShot: GPCR–ligand interactions. *Cell* 159, 1712
27. Xu, F. *et al.* (2011) Structure of an agonist-bound human A2A adenosine receptor. *Science* 332, 322–327
28. Jaakola, V.P. *et al.* (2008) The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist. *Science* 322, 1211–1217
29. Rasmussen, S.G. *et al.* (2011) Structure of a nanobody-stabilized active state of the beta(2) adrenoceptor. *Nature* 469, 175–180
30. Kruse, A.C. *et al.* (2013) Activation and allosteric modulation of a muscarinic acetylcholine receptor. *Nature* 504, 101–106
31. Haga, K. *et al.* (2012) Structure of the human M2 muscarinic acetylcholine receptor bound to an antagonist. *Nature* 482, 547–551
32. Huang, W. *et al.* (2015) Structural insights into micro-opioid receptor activation. *Nature* 524, 315–321
33. Manglik, A. *et al.* (2012) Crystal structure of the micro-opioid receptor bound to a morphinan antagonist. *Nature* 485, 321–326
34. Shoichet, B.K. and Kobilka, B.K. (2012) Structure-based drug screening for G-protein-coupled receptors. *Trends Pharmacol. Sci.* 33, 268–272
35. Congreve, M. *et al.* (2014) Structure-based drug design for G protein-coupled receptors. *Prog. Med. Chem.* 53, 1–63
36. Kolb, P. *et al.* (2009) Structure-based discovery of beta2-adrenergic receptor ligands. *Proc. Natl. Acad. Sci. U.S.A.* 106, 6843–6848
37. Wacker, D. *et al.* (2010) Conserved binding mode of human beta2 adrenergic receptor inverse agonists and antagonist revealed by X-ray crystallography. *J. Am. Chem. Soc.* 132, 11443–11445
38. Rodriguez, D. *et al.* (2015) Molecular docking screening using agonist-bound GPCR structures: probing the A2A adenosine receptor. *J. Chem. Inf. Model.* 55, 550–563
39. Katritch, V. *et al.* (2010) Structure-based discovery of novel chemotypes for adenosine A(2A) receptor antagonists. *J. Med. Chem.* 53, 1799–1809
40. Langmead, C.J. *et al.* (2012) Identification of novel adenosine A (2A) receptor antagonists by virtual screening. *J. Med. Chem.* 55, 1904–1909
41. Congreve, M. *et al.* (2012) Discovery of 1,2,4-triazine derivatives as adenosine A(2A) antagonists using structure-based drug design. *J. Med. Chem.* 55, 1898–1903
42. de Graaf, C. *et al.* (2011) Crystal structure-based virtual screening for fragment-like ligands of the human histamine H(1) receptor. *J. Med. Chem.* 54, 8195–8206
43. Vass, M. *et al.* (2014) Virtual fragment screening on GPCRs: a case study on dopamine D3 and histamine H4 receptors. *Eur. J. Med. Chem.* 77, 38–46
44. Lane, J.R. *et al.* (2013) Structure-based ligand discovery targeting orthosteric and allosteric pockets of dopamine receptors. *Mol. Pharmacol.* 84, 794–807
45. Carlsson, J. *et al.* (2011) Ligand discovery from a dopamine D3 receptor homology model and crystal structure. *Nat. Chem. Biol.* 7, 769–778
46. Kruse, A.C. *et al.* (2013) Muscarinic receptors as model targets and antitargets for structure-based ligand discovery. *Mol. Pharmacol.* 84, 528–540
47. Rodriguez, D. *et al.* (2014) Structure-based discovery of selective serotonin 5-HT(1B) receptor ligands. *Structure* 22, 1140–1151
48. Negri, A. *et al.* (2013) Discovery of a novel selective kappa-opioid receptor agonist using crystal structure-based virtual screening. *J. Chem. Inf. Model.* 53, 521–526
49. Mysinger, M.M. *et al.* (2012) Structure-based ligand discovery for the protein-protein interface of chemokine receptor CXCR4. *Proc. Natl. Acad. Sci. U.S.A.* 109, 5517–5522
50. Rasmussen, S.G. *et al.* (2011) Crystal structure of the beta2 adrenergic receptor-Gs protein complex. *Nature* 477, 549–555
51. Weiss, D.R. *et al.* (2013) Conformation guides molecular efficacy in docking screens of activated beta-2 adrenergic G protein coupled receptor. *ACS Chem. Biol.* 8, 1018–1026
52. Reynolds, K.A. *et al.* (2009) Identifying conformational changes of the beta(2) adrenoceptor that enable accurate prediction of ligand/receptor interactions and screening for GPCR modulators. *J. Comput. Aided Mol. Des.* 23, 273–288
53. Tosh, D.K. *et al.* (2012) Optimization of adenosine 5′-carboxamide derivatives as adenosine receptor agonists using structure-based ligand design and fragment screening. *J. Med. Chem.* 55, 4297–4308
54. Kruse, A.C. *et al.* (2012) Structure and dynamics of the M3 muscarinic acetylcholine receptor. *Nature* 482, 552–556
55. Wacker, D. *et al.* (2013) Structural features for functional selectivity at serotonin receptors. *Science* 340, 615–619
56. Wang, C. *et al.* (2013) Structural basis for molecular recognition at serotonin receptors. *Science* 340, 610–614
57. Hollenstein, K. *et al.* (2013) Structure of class B GPCR corticotropin-releasing factor receptor 1. *Nature* 499, 438–443
58. Zhang, J. *et al.* (2014) Agonist-bound structure of the human P2Y12 receptor. *Nature* 509, 119–122
59. Zhang, K. *et al.* (2014) Structure of the human P2Y12 receptor in complex with an antithrombotic drug. *Nature* 509, 115–118
60. Zhang, D. *et al.* (2015) Two disparate ligand-binding sites in the human P2Y1 receptor. *Nature* 520, 317–321
61. Srivastava, A. *et al.* (2014) High-resolution structure of the human GPR40 receptor bound to allosteric agonist TAK-875. *Nature* 513, 124–127
62. Hanson, M.A. *et al.* (2012) Crystal structure of a lipid G protein-coupled receptor. *Science* 335, 851–855
63. Navratilova, I. *et al.* (2011) Screening for GPCR ligands using surface plasmon resonance. *ACS Med. Chem. Lett.* 2, 549–554
64. Navratilova, I. and Hopkins, A.L. (2011) Emerging role of surface plasmon resonance in fragment-based drug discovery. *Future Med. Chem.* 3, 1809–1820

65. Aristotelous, T. *et al.* (2015) Surface plasmon resonance analysis of seven-transmembrane receptors. *Methods Enzymol.* 556, 499–525
66. Aristotelous, T. *et al.* (2013) Discovery of beta2 adrenergic receptor ligands using biosensor fragment screening of tagged wild-type receptor. *ACS Med. Chem. Lett.* 4, 1005–1010
67. Congreve, M. *et al.* (2011) Fragment screening of stabilized G-protein-coupled receptors using biophysical methods. *Methods Enzymol.* 493, 115–136
68. Christopher, J.A. *et al.* (2013) Biophysical fragment screening of the beta1-adrenergic receptor: identification of high affinity arylpiperazine leads using structure-based drug design. *J. Med. Chem.* 56, 3446–3455
69. Lundstrom, K. *et al.* (2006) Structural genomics on membrane proteins: comparison of more than 100 GPCRs in 3 expression systems. *J. Struct. Funct. Genomics* 7, 77–91
70. Shukla, A.K. *et al.* (2006) Dimethylsulphoxide as a tool to increase functional expression of heterologously produced GPCRs in mammalian cells. *FEBS Lett.* 580, 4261–4265
71. Kobilka, B.K. (1995) Amino and carboxyl terminal modifications to facilitate the production and purification of a G protein-coupled receptor. *Anal. Biochem.* 231, 269–271
72. Chae, P.S. *et al.* (2010) Maltose-neopentyl glycol (MNG) amphiphiles for solubilization, stabilization and crystallization of membrane proteins. *Nat. Methods* 7, 1003–1008
73. Christopher, J. *et al.* (2015) Fragment and structure-based drug discovery for a class C GPCR: discovery of the mGlu5 negative allosteric modulator HTL14242 (3-chloro-5-[6-(5-fluoropyridin-2-yl)pyrimidin-4-yl]benzonitrile). *J. Med. Chem.* 58, 6653–6664
74. Wisler, J.W. *et al.* (2014) Recent developments in biased agonism. *Curr. Opin. Cell Biol.* 27, 18–24
75. Luttrell, L.M. and Minireview: (2014) More than just a hammer: ligand 'bias' and pharmaceutical discovery. *Mol. Endocrinol.* 28, 281–294
76. Kenakin, T. and Christopoulos, A. (2013) Signalling bias in new drug discovery: detection, quantification and therapeutic impact. *Nat. Rev. Drug Discov.* 12, 205–216
77. Shukla, A.K. *et al.* (2014) Emerging structural insights into biased GPCR signaling. *Trends Biochem. Sci.* 39, 594–602
78. Warne, T. *et al.* (2012) Crystal structures of a stabilized beta1-adrenoceptor bound to the biased agonists bucindolol and carvedilol. *Structure* 20, 841–849
79. Wu, H. *et al.* (2014) Structure of a class C GPCR metabotropic glutamate receptor 1 bound to an allosteric modulator. *Science* 344, 58–64
80. Dore, A.S. *et al.* (2014) Structure of class C GPCR metabotropic glutamate receptor 5 transmembrane domain. *Nature* 511, 557–562
81. Salchow, K. *et al.* (2010) A common intracellular allosteric binding site for antagonists of the CXCR2 receptor. *Br. J. Pharmacol.* 159, 1429–1439
82. Bradley, M.E. *et al.* (2009) SB265610 is an allosteric, inverse agonist at the human CXCR2 receptor. *Br. J. Pharmacol.* 158, 328–338
83. Wang, D. *et al.* (2011) Amyloid beta peptide-(1-42) induces internalization and degradation of beta2 adrenergic receptors in prefrontal cortical neurons. *J. Biol. Chem.* 286, 31852–31863
84. Coureuil, M. *et al.* (2010) Meningococcus Hijacks a beta2-adrenoceptor/beta-Arrestin pathway to cross brain microvasculature endothelium. *Cell* 143, 1149–1160
85. Walter, S. *et al.* (2013) Pharmacology of AMG 416 (Velcalcetide), a novel peptide agonist of the calcium-sensing receptor, for the treatment of secondary hyperparathyroidism in hemodialysis patients. *J. Pharmacol. Exp. Ther.* 346, 229–240
86. Bell, G. *et al.* (2015) A randomized, double-blind, phase 2 study evaluating the safety and efficacy of AMG 416 for the treatment of secondary hyperparathyroidism in hemodialysis patients. *Curr. Med. Res. Opin.* 31, 943–952
87. Webb, D.R. *et al.* (2013) Opportunities for functional selectivity in GPCR antibodies. *Biochem. Pharmacol.* 85, 147–152
88. Wallukat, G. and Schimke, I. (2014) Agonistic autoantibodies directed against G-protein-coupled receptors and their relationship to cardiovascular diseases. *Semin. Immunopathol.* 36, 351–363
89. Rossant, C.J. *et al.* (2014) Phage display and hybridoma generation of antibodies to human CXCR2 yields antibodies with distinct mechanisms and epitopes. *MAbs* 6, 1425–1438
90. Hino, T. *et al.* (2012) G-protein-coupled receptor inactivation by an allosteric inverse-agonist antibody. *Nature* 482, 237–240
91. Shukla, A.K. *et al.* (2015) Antibody fragments for stabilization and crystallization of G protein-coupled receptors and their signaling complexes. *Methods Enzymol.* 557, 247–258
92. Kang, Y. *et al.* (2015) Crystal structure of rhodopsin bound to arrestin by femtosecond X-ray laser. *Nature* 523, 561–567
93. Szollosi, E. *et al.* (2015) Cell-based and virtual fragment screening for adrenergic alpha2C receptor agonists. *Bioorg. Med. Chem.* 23, 3991–3999
94. Perry, S.R. *et al.* (2015) Three homology models of PAR2 derived from different templates: application to antagonist discovery. *J. Chem. Inf. Model.* 55, 1181–1191
95. Congreve, M. *et al.* (2011) The use of GPCR structures in drug design. *Adv. Pharmacol.* 62, 1–36
96. Sabio, M. *et al.* (2008) Use of the X-ray structure of the beta2-adrenergic receptor for drug discovery. Part 2: Identification of active compounds. *Bioorg. Med. Chem. Lett.* 18, 5391–5395
97. Sanders, M.P. *et al.* (2012) A prospective cross-screening study on G-protein-coupled receptors: lessons learned in virtual compound library design. *J. Med. Chem.* 55, 5311–5325
98. Carlsson, J. *et al.* (2010) Structure-based discovery of A2A adenosine receptor ligands. *J. Med. Chem.* 53, 3748–3755
99. van Westen, G.J. *et al.* (2012) Identifying novel adenosine receptor ligands by simultaneous proteochemometric modeling of rat and human bioactivity data. *J. Med. Chem.* 55, 7010–7020
100. Chen, D. *et al.* (2012) Fragment screening of GPCRs using biophysical methods: identification of ligands of the adenosine A(2A) receptor with novel biological activity. *ACS Chem. Biol.* 7, 2064–2073