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Molecular pharmacology of metabotropic receptors targeted by neuropsychiatric drugs

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Abstract

Metabotropic receptors are responsible for so-called slow synaptic transmission and mediate the effects of 100's of peptide and non-peptide neurotransmitters and neuromodulators. Over the past decade or so a revolution in membrane protein structural determination has clarified the molecular determinants responsible for the actions of these receptors. Here I focus on the G protein-coupled receptors (GPCRs) which are targets for neuropsychiatric drugs and show how insights into the structure and function of these important synaptic proteins are accelerating our understanding of their actions. Importantly, illuminating GPCR structure and function should enhance the structure-guided discovery of novel chemical tools to manipulate and understand these synaptic proteins.

MAIN

Neurotransmitter receptors are essential for mediating the effects of neurotransmitters in the brain and peripheral nervous system. There are generally considered to be two types of neurotransmitter receptors: ionotropic and metabotropic. While ionotropic receptors are typically ligand-gated ion channels, through which ions pass in response to a neurotransmitter, metabotropic receptors require G proteins and second messengers to indirectly modulate ionic activity in neurons. G protein-coupled receptors (GPCRs) represent the largest family of metabotropic receptors, although receptor-tyrosine kinases¹ and guanylate cyclase receptors^{2,3} can also be considered to be metabotropic receptors⁴. GPCRs also constitute the largest family of druggable targets^{5,6} in the human genome, and 34% of FDA-approved medications have GPCRs as their main therapeutic target^{7,8}—especially those used for neuropsychiatric disorders^{6,9,10}(Table 1).

There are many distinct chemical classes of neurotransmitters including: (1) small molecules (e.g. glutamate, norepinephrine, serotonin); (2) neuropeptides (e.g. enkephalins, endorphins, neurokinins); and (3) others, including metabolites (e.g. endocannabinoids, ATP, ADP, adenosine) and gases (e.g. nitric oxide). Although the precise number of brain neurotransmitters is not known with certainty, it is likely that 100's of distinct neurotransmitters exist, including many orphan neuropeptides¹¹. Each neurotransmitter system has distinct cellular and region-specific distribution patterns in the brain; these can be visualized at the mRNA level using the Allen Brain Atlas¹² or with genetically-encoded

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markers using the GENSAT resource¹³ Virtually all neurotransmitters transduce their signals at least in part by activating GPCRs, and the regional and cellular distributions of brain GPCR mRNAs are well described^{12,14}. As is the case with neurotransmitters, each neurotransmitter metabotropic receptor has a distinct regional and cellular distribution in the brain^{12,14} and elsewhere¹⁴.

GPCRs modulate synaptic transmission via so-called 'slow synaptic transmission'¹⁵ which occurs in the seconds-minutes time frame in the central nervous system. Metabotropic receptors like GPCRs may be found pre- and post-synaptically. Presynaptic Gi-coupled GPCRs, such as the 5-HT1A and 5-HT1B serotonin receptors^{16,17}, inhibit neurotransmitter release via Gβ/γ-mediated activation of inhibitory channels like G-protein inwardly rectifying potassium channels¹⁸ and by inhibition of vesicle docking SNARE-like proteins^{19,20} (Fig.1). Post-synaptically GPCRs can enhance neuronal excitability via Gs- and Gq-coupled GPCRs which have complex actions mediated by second messengers and protein kinases^{21,22} (Fig 1).

Here I will discuss how structural insights into neurotransmitter GPCRs transforms our understanding of them, focusing on example GPCRs that are targets of approved drugs used in neuropsychiatric disorders. The past decade has witnessed remarkable progress in elucidating the structure and function of GPCR family, with perhaps 60 or so GPCRs having their structures determined by x-ray crystallography⁷ or cryo-EM⁷. Additionally, various intermediate signaling states ranging from inactive to active have been elucidated for exemplar GPCRs⁶. Finally, progress has been made on utilizing this structural information for structure-guided and structure-inspired neuropsychiatric drug discovery⁸. Here I will provide an overview of our understanding of how structure informs the function of representative neurotransmitter GPCRs. I will also show how an understanding of structure illuminates neuropharmacology and I will highlight therapeutic challenges and opportunities for neurotransmitter-targeted GPCRs.

TOWARDS A STRUCTURAL GENOMICS OF NEUROTRANSMITTER TARGETED GPCRs

Structural genomics has been defined as the approach which proposes “determining protein structures on a genome-wide scale”²³—which boils down to ultimately determining the three-dimensional structure of every protein in the human genome. Obtaining GPCR structures has been historically challenging. Thus, although low-resolution structures of rhodopsin were available as early as 1993²⁴ and useful models of helical arrangements were generated using this information²⁵, the first bona fide high resolution GPCR structure was not achieved until 2000 with rhodopsin²⁶. The first non-opsin GPCR structures were obtained in 2007^{27,28,29} and since then structures, mainly via x-ray crystallography, have been obtained for around 60 distinct human GPCRs (Fig 2a and ref⁷). To obtain useful structures, GPCR crystallography typically requires high affinity ligands which serve to stabilize the receptor in a distinct state suitable for crystallography. Such compounds not only need to be high affinity (e.g. low nM to pM) but also to have slow dissociation rates³⁰. Ideally, then to obtain complete structural coverage of all GPCRs in the human genome, one

would need high affinity, slowly dissociating ligands for each of them. Such ligands do not necessarily need to be specific as, for instance, the non-selective drug ergotamine has been used to obtain three different serotonin receptor subtype structures: 5-HT1B³¹, 5-HT2B³² and 5-HT2C³³. Once structures are obtained—even with non-selective ligands—the structural features for distinct pharmacological properties can be elucidated (see for instance 3233). Furthermore, obtaining structures of related GPCRs frequently facilitates the structure-guided discovery and design of subtype-selective drugs³⁴.

An analysis of available GPCR structures and cognate ligands, utilizing open-source databases of small molecules binding known protein targets along with databases of GPCR structural information can be the first step towards determining the feasibility of a comprehensive structural elucidation of the GPCR-ome. Chembl³⁵, the KiDatabase³⁶ or PubChem³⁷ are databases matching compounds and their targets. Fig 2A shows the number of ligands with Chembl -annotated activity against a total of 393 non-olfactory GPCRs and, simultaneously, the number of structures available for each of these receptors (as of February 2019). Similar to our previous analyses³⁸³⁹⁶⁸ and those of others⁷, around half of all human non-olfactory GPCRs are well-annotated with respect to chemical matter, while the rest have few to no compounds associated with them in openly available databases³⁵. With one exception—the Frizzled 4 (FZD4) receptor—for which the structure of the seven transmembrane domain (7-TM) in the ligand-free state was recently solved⁴⁰—those GPCRs for which there are reported structures typically have 100's to 1000's of annotated ligands (Fig 2a). No clear relationship exists between the number of annotated ligands in Chembl and the number of distinct structures (Fig 2b), though as mentioned above, high affinity, slowly dissociating ligands are typically key for obtaining structures. Thus, to comprehensively illuminate the structural genomics of GPCRs, suitable ligands will likely need to be obtained for a large number. Efforts are now ongoing to obtain these ligands including the 'Illuminating the druggable genome' initiative (<https://commonfund.nih.gov/idg>), which has those GPCRs for which there is little chemical matter a top priority.

Taking into account this wealth of data, and with new GPCR structures now appearing every week or so, what are the opportunities and challenges for completing a structural genomics survey for neurotransmitter GPCRs? Even with respect to the 240 Class A (Rhodopsin-like) GPCRs—comprising most neurotransmitter receptors and for which there is the most structural coverage—fewer than 50 distinct members have structures elucidated. Several classes—B2 (Adhesion), C (Glutamate), F (Frizzled) and T (Tastant 2), for instance—have two (Glutamate and Frizzled) to zero (Adhesion and Tastant) structures reported.

Even among the thoroughly structurally annotated families of neurotransmitter receptors—for instance the biogenic amine receptor family—structural annotation is still quite sparse. Thus, only one⁴¹ (the H1-histamine) of 4 histamine receptors has a reported structure, and there are no publicly available structures for $\alpha 1$ or $\alpha 2$ adrenergic receptors. Finally, only 3 (5-HT1B, 5-HT2B and 5-HT2C)^{31,3233} of 14¹⁷ serotonin receptors have published structures. Thus, considerable opportunities exist for obtaining structures of representative members of each neurotransmitter GPCR subfamily. Additionally, even for those neurotransmitter families such as the muscarinic receptor family, which has acetylcholine as its neurotransmitter, and at which 4 (M1, M2, M3 and M4)^{42,4344} of 5 members have

structures reported, the structural determinants for subtype selectivity are still to be elucidated and fully exploited for the creation of selective muscarinic agonists and antagonists⁴⁵. For muscarinic receptors, in particular, it is clear that drugs targeting M1 and M4-muscarinic receptors may have special utility for enhancing cognition in Alzheimer's Disease and Schizophrenia, while interactions with M3-muscarinic are associated with debilitating side-effects^{46,47}. In terms of creating subtype-selective drugs, a variety of structure-guided approaches can be used including targeting allosteric and orthosteric sites (see refs^{86,48}). As the foregoing makes clear, although significant progress has been made towards a comprehensive understanding of the structural genomics of human neurotransmitter GPCRs, a vast amount of work remains which will require the integrated and coordinated efforts of structural biologists, pharmacologists, chemists and computational biologists.

STRUCTURAL INSIGHTS INTO NEUROTRANSMITTER-BASED GPCR ACTIVATION AND BIASED SIGNALING

As is the case with GPCR structural genomics, considerable insights into the structural features of neurotransmitter-targeted GPCR activation and biased signaling have been obtained. With regard to GPCR activation, the first structure of a GPCR in complex with heterotrimeric G proteins was obtained in 2011⁴⁹ and since then several structures of nanobody (e.g. β 2-adrenergic⁵⁰ M2-muscarinic^{51,52} and μ -opioid⁵³ receptors) and heterotrimeric G protein-stabilized active states (e.g. calcitonin⁵⁴ calcitonin gene related peptide^{55,56} 5-HT1B-serotonin^{57,58} μ -opioid⁵⁹ and glucagon-like peptide-1 (GLP-1)⁶⁰ receptors) have appeared—essentially all with neurotransmitter-targeted GPCRs. Further, structures of arrestin-biased agonists at 5-HT2B receptors^{32,61,62}, arrestin-bound rhodopsin⁶³ and G-protein biased agonists at GLP-1 receptors⁶⁴ have been reported. Finally, there are inactive-state structures stabilized by sodium (e.g. A2A-adenosine⁶⁵ δ -opioid⁶⁶ and D4-dopamine³⁴ receptors), negatively modulating allosteric nanobodies at the β 2-adrenergic receptor⁶⁷ and the ligand-free basal structure of the FZD4-frizzled receptor⁴⁰. Fig 3 depicts representative structures of these various states along with a graphical representation of an extended ternary complex model of receptor activation that includes both G protein-⁶⁸ and β -arrestin-[see ref⁶⁹] stabilized complexes. The outward movement of TM6 is the hallmark for GPCR activation along with several other canonical rearrangements^{49,53}. These include rearrangements of several micro-switches³² as follows: (1) residues P^{5.50}, I^{3.40} and F^{6.44} which form the P-I-F motif. Here there is an inward shift of P^{5.50} along with a rotamer switch of I^{3.40} and a large inward movement of F^{6.44}. Additional microswitches which undergo rearrangement include the D(E)/RY motif in TM III and the NPxxY motif in TM VII. The salt bridge between D^{3.49} and R^{3.50} is typically broken in active-state structures^{49,53}. At the NPxxY motif activation reveals a rotation of Y^{7.53}. Additionally the sodium site collapses due to rearrangements of key residues coordinating sodium⁵³. Finally there is typically a large outward movement (10-15 Å) of the cytoplasmic end of TMVI which facilitates interactions with G proteins⁴⁹ and other transducers⁵³. In general, the 'active-like' receptor states in Fig 3 show the various transitions mentioned above without the large outward movement of TMVI.

Currently, there are two published structures of GPCRs complexed with β -arrestin (β -ARR) biased agonists including LSD⁷⁰ and ergotamine³² with the 5-HT_{2B} serotonin receptor and one structure of a G-protein biased ligand complexed with heterotrimeric G-protein for the GLP-1 receptor⁶⁴. The β -ARR-biased agonist structures (Figs 3 and 4) appear to represent an ‘intermediate’ state between the signaling complex states and the inactive states. Notably, the sodium site is collapsed, with rearrangements of key residues that stabilize the bound sodium ion including, using the Ballesteros-Weinstein numbering convention⁷¹, Ser^{3.39} and Asp^{2.50} (Fig 4A). Notably Ser^{7.45} rotates in to occlude the sodium pocket (Fig 4a). Additionally, a key extracellular residue in loop 2–L209^{EL2} appears to form a ‘lid’ over LSD, retarding its dissociation and being essential for arrestin recruitment. A similar role of the same EL2 residue was verified in studies of the D₂-dopamine receptor and several other biogenic amine receptors^{61,72,62}. Notably the ‘P-I-F’ and NPXXY motifs along with conformational rearrangements in TM6 and TM7 are consistent with an intermediate activated state⁶¹ (Fig 4b). Taken together, these rearrangements suggest that concerted conformational changes involving residues throughout the receptor, albeit not fully mimicking the changes in the rhodopsin–arrestin complex (ref⁶³), are responsible for the arrestin-biased conformation which favors arrestin binding (Fig 4b). By contrast, the GLP-1 complex with the G-protein biased agonist extendin-P5 shows the most pronounced difference in TM1, along with extracellular regions of TM6, TM7 and extracellular loop 3 (EL3), for which there is support by extensive mutagenesis (see ref⁶⁴ for details).

The inactive states typically show a conserved sodium site with sodium visualized in the highest resolution structures^{34,65,66}. Additionally a conserved ‘ionic lock’ between D/E^{3.49} (of the D(E)/RY motif and N^{6.30} which stabilizes the ground state of several GPCRs^{73,74} is occasionally seen. Remarkably in an inactive state stabilized by Nb60 and carazolol⁶⁷ this ionic lock is recapitulated by the nanobody. Additionally in many inactive-state structures the P-I-F, NPxxY and D(E)/RY motifs are all in a generally conserved ‘inactive-like’ state.

The above studies demonstrate clear progress in the understanding of the structural features required for different modalities of receptor activation and signaling. Clearly, much remains to be done in this direction, and the discovery of many novel permutations of these features is eagerly anticipated.

CHALLENGES AND OPPORTUNITIES FOR STRUCTURE-GUIDED DRUG DESIGN AND DISCOVERY FOR NEUROTRANSMITTER TARGETED GPCRS

Selectivity:

Given the large number of GPCR structures and reasonable coverage of some GPCR families, these structures should, at least in theory, be useful for the *de novo* design of selective drugs. This is particularly true when new ‘pockets’ are discovered, as was the case for the D₂⁷⁵, D₃⁷⁶ and D₄³⁴ dopamine receptors. As shown in Fig 5a-c, these structures revealed potentially unique binding surfaces that could be exploited for the design of selective ligands. Fig 5d shows a more detailed view of the D₄-selectivity filter while 5e shows how this is occluded in the D₂ receptor³⁴. Given that all three structures were obtained with chemically distinct and non-selective ligands, however, it is conceivable that

the different binding pockets might simply reflect the fact that different ligands with distinct chemical scaffolds (e.g. different chemotypes) engage different residues in the receptors. One way to test the hypothesis that these different binding pockets are pharmacologically relevant is to create new ligands that are designed to engage these ‘selectivity filters’. For the D3 receptor, remarkable success was achieved⁷⁷ with a compound with 1700-fold selectivity for the D3 receptor, and minimal off-target activity (Fig 5). Then, starting with the seed compound eticlopride (5f), which is a potent and non-selective D2 and D3 antagonist with weaker activity at D4, the group used a combination of docking and medicinal chemistry to identify a potential modified scaffold predicted to target a putative selectivity region within the D3 receptor previously identified by mutagenesis and molecular modeling⁷⁶ (Fig 5g). Via this structure-inspired design approach, **Compound 19** (5g) was eventually synthesized and found to be a potent and selective D3 antagonist.

An alternative approach is to target these ‘selectivity filters’ via automated docking (5c-e). In an initial proof-of-concept study, the D4-dopamine receptor selectivity filter was targeted by a docking campaign of 600,000 commercially-available compounds³⁴ from the ZINC database⁷⁸. A set of ‘seed’ compounds discovered from the initial docking campaign was further optimized by docking and subsequent identification of analogs, which ultimately led to the highly selective D4 partial agonist **Compound 9-6-24**³⁴ which was >1000-fold selective for the D4 receptor and lacked activity at 320 other GPCRs.

More recently the computational approach has been enhanced by automated docking-based screening of ultra-large libraries in a campaign with 138 million drug-like compounds. This *in silico* screening campaign, wherein each compound was docked in >100,000 conformations, required the analysis and scoring of 70 trillion docking events⁷⁹ (Fig 5h). In this study, the previously identified D4 selectivity filter³⁴ comprised of the pocket formed by residues Leu^{3.28} and Phe^{2.61} (see Fig 5c-e) was again computationally targeted. This docking campaign led to the discovery of a large number of chemically novel and highly selective D4 ligands. Of these, **ZINC621433144** (5h) was the most potent of the agonists tested, with an EC₅₀ of 180 pM and selectivity for D4 over D2 and D3 (Fig 5h). Based on the rate of true positives among the predicted actives, the authors estimate that there could exist as many as 400,000 distinct D4-active compounds having more than 70,000 diverse chemotypes⁷⁹ in the library of 138 million compounds. Similar, albeit less computationally intensive, docking-based approaches have yielded selective ligands for the 5-HT_{1B}-serotonin⁸⁰, κ -opioid⁸¹, D2-dopamine⁸¹ and other receptors [see refs^{8,82} for reviews]

Structure-guided design of biased ligands for neurotransmitter receptors

Functional selectivity, also known as biased signaling⁸³, has been defined as the process by which “ligands induce (or stabilize) unique, ligand-specific receptor conformations... result(ing) in differential activation of signal transduction pathways associated with that particular receptor”⁸³. The phenomenon of functional selectivity occurs when different ligands at the same receptor lead to preferential activation of different G proteins (e.g. G-protein subtype bias), a preference for G-protein signaling over arrestin (e.g. G-protein bias) or a preference for arrestin signaling (e.g. arrestin bias) over G-proteins. Reports of functional selectivity at GPCRs have appeared for many decades (see Ref⁸⁴ for example)

and such bias is now recognized as a nearly universal phenomenon for GPCRs⁶. Indeed there, are now multiple reports of the discovery and optimization of arrestin^{85,86}- and G-protein⁸⁷⁻⁸⁹ biased ligands for many GPCRs [see refs^{6,90,91} for recent reviews]. As mentioned above, to date there is only one structure of a GPCR (a GLP-1 receptor) in complex with G-protein biased ligand (peptide exendin-P5) and heterotrimeric G proteins⁶⁴, a handful of structures of arrestin-biased ligands complexed with the 5-HT_{2B} serotonin receptor^{32,62,70} and no structures of arrestin-biased ligands in a GPCR-arrestin complex. Thus, although there is a paucity of structural information regarding GPCR functional selectivity, there have been several reports in which structure-informed and structure-inspired design of neurotransmitter targeted G-protein and arrestin-biased ligands have been achieved.

For example, Manglik et al⁹² reported the discovery of G-protein biased agonists for the μ opioid receptor although this discovery was accomplished without knowledge of any of the structural features responsible for biased signaling. In that study, molecular docking of 3 million compounds was performed against the inactive conformation of the μ opioid receptor targeting a highly conserved aspartic acid (Asp^{3.32}) and engaging a conserved water network and tyrosine (Tyr^{3.33}) putatively involved in selectivity and agonist activity. Compounds with predicted activity were tested for functional activity at G-protein and arrestin signaling and, after several rounds of medicinal chemistry optimization, PZM21 was identified as a potent and efficacious G-protein biased agonist⁹². Similar G-protein biased ligands have been discovered for μ -^{93,94} and κ -^{89,95} opioid receptors, albeit without being informed by structural determinants. On the other hand, via a combination of molecular dynamics and synthetic studies, a Salvinorin A⁹⁶ -derivative was discovered for μ -opioid receptors⁹⁷ that was G-protein biased, and thus likely has reduced abuse potential. Similarly, in a recent study of κ -opioid receptors, residues identified from structural studies as being essential for biased signaling ultimately led to the creation of novel structure-guided G-protein biased opioid agonists⁵².

Additionally, McCorvy et al⁷² provided a structure-inspired approach for creating biased ligands at aminergic GPCRs—all of which are essential targets for the neurotransmitters serotonin, norepinephrine, dopamine and histamine. For this study, they took advantage of prior studies indicating that interactions with TMV serine and other residues are essential for G-protein signaling⁶² at aminergic GPCRs, while interactions with EL2 residues can be essential for arrestin signaling⁶¹. Via a combination of molecular modeling, molecular dynamics simulations, automated docking and synthesis, the arrestin-biased Compound **7** was discovered for the D2-dopamine receptor⁷² (Fig 6a). As previous studies have shown that such arrestin-biased compounds may have efficacy in treating schizophrenia and related neuropsychiatric disorders^{85,98}, this could represent a useful approach for optimizing arrestin-biased medications for treating neuropsychiatric disorders. Finally, it is also worth mentioning that biased signaling might be influenced on the level of recruitment of and receptor phosphorylation by GPCR kinases (GRKs)⁹⁹ and this potentially can be influenced by GPCR ligands.

Orphan and understudied GPCRs

As previously mentioned, nearly 50% of GPCRs—most of which are highly expressed in the brain¹⁴--have a paucity of known ligands. Even for those neurotransmitter receptors that have been the subject of intensive investigation, such as muscarinic and dopamine receptors, the identification of suitably selective ligands for the various subtypes continues to be challenging. Thus, for instance, there are no truly selective D5-dopamine or M5-muscarinic receptor agonists, although a modestly selective D5¹⁰⁰ – and a fairly selective M5¹⁰¹ antagonist have been reported. Unfortunately, off-target pharmacology has not been reported for these compounds, so their selectivity over other GPCRs is unknown.

Although there are no published structures of orphan or understudied brain GPCRs (oGPCRs³⁸), the use of homology models and automated docking provides a structure-inspired approach for ligand discovery. Thus for instance, an integrated approach using parallel screening, homology modeling, docking and analog synthesis led to ogerin—a selective positive allosteric modulator for GPR68—and selective negative allosteric modulators for GPR65¹⁰² (Fig 6b). The allosteric modulator ogerin was demonstrated to affect learning and behavior in mice, indicating that GPR68 is a potentially important GPCR in the brain¹⁰². A similar approach led to the discovery of selective agonists for the oGPCR MRGPRX2 which is involved in pain and itch¹⁰³ and binds many important neurotransmitters including substance P and other peptides¹⁰³. These approaches are facilitated by parallel-screening approaches in which 100's of oGPCRs can be screened simultaneously^{102,104}; active compounds are used to inform homology models for docking and subsequent discovery of new ligands^{102,103,105-107}.

Genetic and model organism studies are also facilitating the understanding of the potential therapeutic importance of oGPCRs for neuropsychiatric disease. Thus, a very recent study indicates that the orphan GPCR MRGPRX4 is involved in the pain and itch sensations mediated by bile acids¹⁰⁸. Another recent study shows that MRGPRX4 is also essential for the preference for menthol cigarettes in certain ethnic populations¹⁰⁹. Given the distribution of MRGPRX4 in peripheral nerves, it is likely that MRGPRX4 and related receptors are neurotransmitter receptors involved in peripheral sensations like pain and itch¹¹⁰. Databases devoted to oGPCR brain distribution^{39,111,112} should facilitate discovering their endogenous neurotransmitters, function and neuropsychiatric implications for pathogenesis and treatment.

Polypharmacology

Historically, the most effective drugs for many complex neuropsychiatric diseases have targeted multiple GPCRs and other molecular targets^{9,113}. It is now understood that this is likely because of the exceedingly complex genetic landscape of common diseases in which 100's-1000's of genes might exert small effects¹¹⁴. This has led to the understanding that complex diseases are omnigenic rather than polygenic¹¹⁴—meaning that nearly every gene may ultimately exert a small effect on core disease pathways. Perhaps not surprisingly, the desire to discover increasingly selective drugs has resulted in lower overall success rates of drug discovery campaigns¹¹⁵. This lack of success has led to the hypothesis that generating polypharmacologic drugs with designated multiple targets represents a useful approach for

treating complex diseases^{113,116}. For aminergic GPCRs, there has been some recent success in discovering the structural features responsible for polypharmacological activities³³ which comprises a series of 9 semi-conserved residues (Asp^{3.32}, Ile/Val^{3.33}; Cys^{3.36}, Thr^{3.37}, Ala/Ser/Thr^{5.46}, Trp^{6.48}, Phe^{6.51}, Phe^{6.52}, EL2Val/Ile/Leu). Although in theory structure-guided approaches should be helpful for the discovery and design of polypharmacological drugs, to date, this has not been entirely successful^{117,118}.

THERAPEUTIC AND COMMERCIAL OPPORTUNITIES FOR STRUCTURE-GUIDED GPCR DRUG DISCOVERY

Heptares (acquired by Sosei in 2016)¹¹⁹, and Receptos (acquired by Celgene in 2015¹²⁰) along with Conformetrix¹²¹ have invested considerable resources to bring GPCR structure-guided drug discovery to fruition. To date, publicly available information indicates that the exemplar compounds are targeted mainly to well-studied GPCRs for which selective ligands are difficult to obtain. Thus, for instance, Heptares has advanced an M4-selective agonist to Phase I trials, presumably for cognition enhancement (<https://clinicaltrials.gov/ct2/show/NCT03244228?term=heptares&rank=4>). Heptares has also brought a structure-guided mGlu5 negative allosteric modulator¹²² for metabotropic glutamate receptors to initial Phase I trials, presumably for a central nervous system application (<https://clinicaltrials.gov/ct2/show/NCT03785054?term=heptares&rank=2>). Receptos, in partnership with Celgene, has advanced RPC1063 (an S1P receptor agonist for treating multiple sclerosis <https://www.accessdata.fda.gov/scripts/opdlisting/oopd/detailedIndex.cfm?cfgridkey=611517>) to Phase III clinical trials.

FUTURE DIRECTIONS

Given the wealth of structural information now available for many GPCRs, it is anticipated that structure-guided approaches will eventually lead to neuropsychiatric medications with greater efficacy and fewer side-effects. As on- and off-target side-effects, along with clinical effectiveness and safety, continue to be major drivers of drug failures in clinical trials¹²³, insights into the structural basis of ligand engagement could provide new opportunities for GPCR-based neuropsychiatric drug discovery. Thus, where a G-protein biased ligand might show greater efficacy and fewer side-effects, insights into the structural basis of such bias could accelerate the discovery of novel chemotypes with biased signaling patterns. Indeed, this approach has been successful in generating biased tool compounds for many neurotransmitter receptors, including D4-dopamine^{34,124}, μ -opioid⁹² and others^{62,72}. Although there are currently no structure-guided biased drugs in clinical trials, encouraging results have appeared for TRV-130 as a G protein-biased opioid receptor agonist for pain¹²⁵, albeit with some abuse liabilities as manifested in preclinical studies^{126,127}.

As is clear from the foregoing, the past decade has witnessed astounding progress in understanding the structure and function of metabotropic neurotransmitter receptors. This increasing wealth of structural information, and advances in structure guided and inspired drug discovery, promise to accelerate the discovery of drugs targeting metabotropic neurotransmitter receptors with improved efficacies and fewer side-effects.

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FIGURE 1. Metabotropic receptors such as GPCRs modulate synaptic transmission. Shown is a cartoon depicting several of the mechanisms by which metabotropic receptors like GPCRs can modify synaptic transmission. As is depicted, Gi-coupled GPCRs can attenuate presynaptic release via activating various channels including inhibitory G-protein inwardly rectifying potassium channels (GIRKS) ¹⁸¹³⁸ and by inhibiting vesicle release machinery including SNARE proteins ¹⁹. Postsynaptic Gq and Gs-coupled GPCRs can induce or potentiate neuronal firing via a number of intracellular second messengers ¹³⁹ and secondary modulation of ion channel activity ²¹.

FIGURE 2. The availability of chemical matter is useful for obtaining GPCR structures. (a) shows a graph with the number of compounds annotated in Chembl against a particular human GPCR (blue dots) versus the number of X-ray or Cryo-EM structures (red squares) deposited for each particular GPCR (effective date Feb 1, 2019). (b) shows that there is no direct linear relationship comparing the number of compounds annotated and the number of structures currently available.

FIGURE 3. Structural validation of the extended ternary complex model of GPCR action. The extended ternary complex model^{68,69,6} predicts the existence of multiple interconvertible GPCR states stabilized by ligands (agonists, neutral antagonists and inverse agonists) and transducers (G proteins, arrestins and other transducers). The figure depicts various high resolution structures which have provided validation for this schema. These states include: inactive states stabilized by allosteric nanobodies (RL_2)⁶⁷, active (R^*L ;⁷⁰) and inactive sodium-bound⁶⁶ states stabilized by agonists and inverse agonists, respectively; as well as coupled states (R^*GL and $R^{**}L\beta ARR$). The model also provides for the possibility of biased signaling due to agonist-induced stabilization of distinct active and coupled states^{32,31} (e.g. R^*L and $R^{**}L$). Finally, spontaneously active (R^*) and coupled (R^*G) states which have been demonstrated in many systems⁶⁸ are predicted to exist although structures of these are not yet available.

FIGURE 4. Structural rearrangements associated with distinct GPCR states.

(a) shows rearrangements within the sodium pocket associated with stabilization of an arrestin-biased state of the 5-HT2B serotonin receptor. Green depicts the inactive sodium-stabilized state of the D4 dopamine receptor while cyan shows the LSD-bound 5-HT2B receptor. As can be seen, key residues involved in stabilizing sodium (Ser3.39 and Asp2.59) along with Ser 7.45 collapse to occlude the sodium site. (b) shows rearrangements in the P-I-F motif associated with inactive (green), G-protein (magenta) and arrestin-biased (cyan) conformations.

FIGURE 5. Structure-guided design of selective GPCR ligands for dopaminergic modulating neurotransmission.

As shown, structures of the three members of the D2-family dopamine receptor family have been solved: (a) D2⁷⁵ shown in magenta, (b) D3⁷⁶ shown in cyan and (c) D4³⁴ in green. (d) shows the location of the D4-selectivity filter with nemonapride bound in green while (e) shows that this is occluded in the D2 receptor (D2 residues magenta). As described in the text, the D3 receptor structure was used to modify the non-selective D3 inverse agonist eticlopride (f) to **Compound 19** (g) which has high affinity and selectivity. Alternatively, ultra-large scale docking led directly to **ZINC621433144** (h) which emerged as a pM selective D4 agonist⁷⁹

FIGURE 6. Structure-inspired design of ligands for GPCRs to modulate synaptic transmission.

(a) depicts strategy (see text for details) to design ligands with arrestin bias for biogenic amine neurotransmitter receptors⁷². Here using a combination of molecular dynamics simulations, docking and medicinal chemistry, compounds were created which were predicted to interact with a conserved EL2 residue which can impart arrestin bias (b) depicts computational strategy to discovery small molecule probes to modulate orphan synaptic GPCRs such as GPR68¹⁰². Here, 3.1 million compounds were docked to a model of GPR68 and then via iterative docking and testing of analogs ogerin was identified as a selective GPR68 positive allosteric modulator.

Representative metabotropic neurotransmitter receptors as targets for neuropsychiatric disorders and drugs of abuse

Table 1.

Target class	Neurotransmitter	Receptor	Representative medication	Therapeutic indications or abuse	Pharmacological action	Structure determined
GPCR	Dopamine	D2-dopamine	Ropinirole	Parkinson's Disease	Agonist	75
GPCR	Dopamine	D2-dopamine	Nemonapride	Schizophrenia	Antagonist/inverse agonist	75
GPCR	Serotonin	5-HT _{2C} serotonin	Lorcaserin	Obesity	Agonist	33
GPCR	Serotonin	5-HT _{1A} serotonin	Buspirone	Anxiety and depression	Partial agonist	None reported
GPCR	Serotonin	5-HT _{1B}	Ergotamine	Migraine headaches	Antagonist	31,32
GPCR	Adenosine	A _{2A} -adenosine	Caffeine	Alertness	Antagonist	128
GPCR	Norepinephrine	β ₁ and β ₂ -adrenergic	Propranolol	Post-traumatic stress disorder; anxiety disorders	Inverse agonist	129,130
GPCR	Serotonin	5-HT _{2A} serotonin	Pimavanserin	Psychosis related to Parkinson's Disease	Inverse agonist	131
GPCR	Serotonin	Many 5-HT receptors	LSD	Hallucinogen use and abuse	Agonist	70
GPCR	Endocannabinoids	CB ₁ cannabinoid	Tetrahydrocannabinol	Anorexia related to cancer chemotherapy; cannabis abuse	Agonist	132
GPCR	γ-amino butyric acid (GABA)	GABA-B	Baclofen	Spasticity-related movement disorders	Agonist	None reported
GPCR	Histamine	H ₁ -histamine	Diphenhydramine	Sedative	Antagonist	41
GPCR	Endorphins; endomorphans	μ opioid	Morphine	Pain; abused opioid	Agonist	59,133
GPCR	Dynorphin	κ opioid	Nalturfine	Itch	Agonist	52,134
GPCR	Dynorphin	κ opioid	Salvinorin A	Hallucinogen use and abuse	Agonist	52,134
GPCR	Orexin	OX ₁ -orexin	Suvorexant	Insomnia	Antagonist	135
GPCR	Norepinephrine	α ₂ -adrenergic	Clonidine	Anxiety; opioid withdrawal	Agonist	None reported
GPCR		S1P ₁ sphingosine antagonist	Fingolimod	Multiple sclerosis	Down-regulates receptor	136
Recept or tyrosine kinase	Nerve growth factors	TRK	Entrectinib	Cancers including astrocytoma	Antagonist	No full length structure reported
Guanylate cyclases	Atrial natriuretic peptide	NPRA-atrial natriuretic receptor	Atrial natriuretic peptide	None (heart failure)	Agonist	137