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Computational studies to predict or explain GPCR polypharmacology

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Abstract

Since G protein-coupled receptors (GPCRs) belong to a very large superfamily of evolutionarily related receptors (>800 members in the human) and due to the rapid progress on their structural biology, they are ideal candidates for polypharmacology studies. Broad screening and bioinformatics/chemoinformatics have been applied to understanding off-target effects of GPCR ligands. It is now feasible to approach the question of GPCR polypharmacology using molecular modeling and the available X-ray GPCR structures. As an example, large and sterically constrained adenosine derivatives (potent adenosine receptor ligands with low conformational freedom and multiple extended substituents) were screened in binding at diverse receptors. Unanticipated off-target interactions, including at biogenic amine receptors, were then modeled using a structure-based approach to provide a consistent understanding of recognition. A conserved Asp in TM3 changed its role from counterion for biogenic amines to characteristic H-bonding to adenosine. The same systematic approach could potentially be applied to many GPCRs or other receptors using other sets of congeneric ligands.

Keywords

G protein-coupled receptors; molecular modeling; bioinformatics; chemoinformatics

Polypharmacology of GPCRs

In the course of pharmaceutical development of new small molecules that act at G protein-coupled receptors (GPCRs), the off-target effects of a candidate molecule are routinely determined in broad screening at other receptors, ion channels, enzymes and transporters.¹ The early identification of off-target activity of a mechanism-based novel experimental drug for disease treatment is important in the de-risking process when that activity is considered

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undesirable. Given the high number of cellular targets, most pharmacologically active small molecules are likely to modulate the activity of more than one of them, thus rendering complete selectivity an unrealistic goal. Of note, this set of multiple interactions, also known as polypharmacology (Glossary), is not necessarily deleterious, since it may offer the potential of higher efficacy due to the beneficial combination of multiple desired biological outcomes. Nevertheless, in order to achieve a tighter control over pharmacological therapies and to better understand the consequences of drug administration, a thorough knowledge of the cellular targets affected by a given chemical is certainly desirable. Moreover, detection of off-target effects offers the opportunity of repurposing existing drugs for a different indication, or further engineering molecules in order to potentiate the interactions with a newly discovered target.²⁻⁴

Since GPCRs belong to a very large superfamily of evolutionarily related receptors (>800 members in the human)⁵ and due to the rapid progress on their structural biology, they are ideal candidates for polypharmacology studies.⁶⁻⁸ Moreover, the polypharmacology of GPCR ligands is not necessarily confined within the scope of the superfamily, but can extend well beyond its boundaries.

Computational approaches to predict GPCR polypharmacology

As recently reviewed by Rognan,⁹ computational research can facilitate the identification of off-target effects through three different approaches (Figure 1): a) a molecular modeling strategy, intended to predict the likelihood of interaction of the studied molecule with a set of target candidates; b) a bioinformatic strategy, based on the sequence similarity between the known target candidates; c) a chemoinformatic strategy, based on the chemical similarity between the ligands of the known target and those of the target candidates. Each of these three strategies offers specific advantages, but also presents inherent shortcomings, and can be applied using a number of alternative methodologies. Importantly, while bioinformatic approaches are confined within the GPCRs superfamily, molecular modeling and chemoinformatic approaches are applicable to identification of off-target effects of GPCR ligands within and beyond the GPCR superfamily.

Molecular modeling strategy

With the growing list of GPCRs and other membrane-bound receptors that have been determined structurally by X-ray crystallography, some in both agonist-bound and antagonist-bound states, it is now feasible to analyze GPCR polypharmacology structurally.⁶⁻⁸ The identification of off-target effects of GPCR ligands within the superfamily can be assisted by structure-based molecular modeling strategies that rely on knowledge of the three-dimensional structure of the primary and potential alternative targets. Importantly, the experimentally elucidated GPCR structures, beyond providing a direct means to study the likelihood of interaction of GPCR ligands with the solved receptors, also provide a platform for the construction of theoretical models of other members of the superfamily through the homology modeling technique.¹⁰⁻¹⁶ As blind assessments of GPCR modeling accuracy suggest, these homology models will become increasingly more accurate as novel templates become available through experimental structural biology.^{13, 14}

Molecular docking, i.e. the computational generation of models of protein-ligand complexes and their subsequent scoring, is one of the techniques through which molecular modeling can help identify off-target effects. Modeling strategies were used to study the polypharmacology of protein families where the structural biology is much more extensive (e.g. kinases).³⁵ As has been demonstrated through controlled experiments and actual ligand discovery campaigns, targeting GPCR crystal structures through docking-based computational screening, also known as virtual screening, is a very effective way of identifying candidate molecules that are likely to bind to a given receptor.^{17–30} Hence, one could select the receptors for which the compound of interest is scored better than decoy compounds and test the suggested biological activity experimentally. In the absence of crystal structures, well-validated homology models have proven satisfactory for virtual screening (Box 1).^{19, 24, 31–33} However, in several cases homology modeling has not led to meaningful structural results applicable to virtual screening.³⁴ Hence, the use of docking for polypharmacology studies is likely confined to the receptors endowed with crystal structures and their closely related homologs. As explained below, molecular docking, can also be used to rationalize the experimentally observed polypharmacology of compounds of interest, thus providing the basis for accentuating or diminishing the off-target effects.³⁶

Box 1

Example of the utility of GPCR homology models in virtual screening

A virtual screening campaign targeting the crystal structure of the dopamine D₃ receptor and a homology model constructed on the basis of the closely related β_2 adrenergic receptor yielded comparable hit rates.²⁴ Moreover, the two campaigns led to the identification of two non-overlapping hit sets, revealing an unexpected synergy between the screening that targeted the experimental structure and the one targeting the model.²⁴

Bioinformatic strategy

Since GPCRs are a large superfamily of evolutionarily related proteins, clusters of phylogenetically related receptors can be identified through comparisons of their amino acid sequences.^{5, 37–41} Of note, such comparisons can be restricted to the residues that line the ligand-binding cavity (Box 2).³⁹

Box 2

Example of a bioinformatic approach focused on the ligand binding cavity

A large-scale sequence comparison was based on the residues lining interhelical ligand-binding cavities of GPCRs.³⁹ The study was restricted to a set of 30 specific position of the alignment of TMs identified by analyzing the structure of bovine rhodopsin, which was the first GPCR to be solved crystallographically⁴² and, up until 2007, remained the only GPCR endowed with experimentally elucidated structures.⁴³ In particular, the residues were selected on the basis of their distance from retinal, which is the covalently bound ligand co-crystallized with rhodopsin, their solvent accessible surface area, and the orientation of their side chains. The study revealed that the tree obtained by comparing

the 30 positions only overlapped very well with the one resulting from the comparison of the entire seven TM bundle.³⁹

For GPCRs, these analyses focused on the binding cavity are facilitated by the fact that most class A receptors, i.e. the rhodopsin-like family, bind their orthosteric ligands to a common cavity at the opening of the helical bundle toward the extracellular space. Different receptors bind their ligands with different orientations and exploit nonidentical regions of the cavity.⁸ Some GPCRs, e.g. within class C (glutamate family) or the glycoprotein hormone members of class A, do not bind their natural ligand within this interhelical cavity, rather through a large extracellular N-terminal domain. However, even in those cases, the interhelical cavity can be exploited to bind synthetic allosteric ligands.^{44–46} Of note, the parallelism between overall sequence similarity and similarity within the ligand-binding cavity of receptors closely related to the primary target of a given molecule may provide alternative targets for the same molecule.

Cheminformatic strategy

While the sequence comparison approach described above is necessarily confined to the study of phylogenetically related targets, cheminformatics-based approaches have the potential of expanding the cross-reactivity analysis to virtually any targets with known ligands. Such cheminformatic methods, rather than assessing target similarity through sequence comparisons, look at the chemical similarity between the ligands that are known to bind to them. Networks based on target similarity differ substantially from networks based on ligand similarity.⁴⁷ Hence, comparing ligands facilitates the identification of cross-reactivity patterns that would not be discovered on the basis of sequence similarity analyses (Box 3). Such chemical similarity is typically assessed through molecular fingerprinting, a computational technique based on the codification of molecular structures in the form of strings that reflect their topology and chemical characteristics.⁴⁷

Box 3

Examples of cheminformatic approaches to study polypharmacology

An analysis of chemical similarities between FDA-approved and investigational drugs against hundreds of targets revealed many unanticipated associations.⁴ Several of these drug-target associations were confirmed experimentally, and some of them linked together phylogenetically unrelated targets. For instance, an antagonist of an NMDA (N-methyl-D-aspartate) ion channel was found to bind also to the μ -opioid receptor, a GPCR, and a serotonin transporter.⁴ Similarly, a cheminformatics-based approach identified the targets of compounds that were found to be active in a zebrafish behavioral assay.⁵⁴ The testing of 20 predictions led to the identification of targets for 11 compounds, several of which were found to be active at one or more GPCRs.

While cheminformatic analyses are traditionally based on the comparison of the molecular fingerprint of ligands, researchers at Novartis recently developed a novel type of fingerprint based on the biological activity profiles of ligands, which they dubbed HTS-FPs, for 'high-throughput screening fingerprints'.⁴⁸ The name derives from the fact

that these fingerprints translate biological data from high-throughput screenings (HTS) into a vector of normalized activities that can be readily interrogated by computational methods. In a separate article, the ability of this method to identify new targets for existing drugs and natural products was tested.⁴⁹ Notably, of 65 predicted drug-target interactions that were experimentally tested, 48 were confirmed by the experimental results. In analogy with what was found with the screenings based on chemical fingerprints, this method yielded the identification of cross-reactivity profiles that spanned across phylogenetically diverse targets. Not surprisingly, frequent cross-reactivity between GPCRs and transporters for chemically related compounds was detected.

A computational approach to explain polypharmacology: Docking rigid congeners

Beyond predicting possible unknown off-target effects of the compounds of interest, when these off-target effects are found experimentally, molecular modeling techniques can be used to explain the structural basis of the observed polypharmacology.

Homology modeling and ligand docking are powerful techniques when informed by multiple data sources, such as site-directed mutagenesis, bioinformatics, biophysical studies and molecular dynamics.^{6, 50, 51} Molecular modeling of GPCRs based on homology to closely related receptors has a proven predictive value for probing sites of interaction and for drug screening. The location of agonist and antagonist ligands in orthosteric binding sites of family A GPCRs often involves residues at the same positions within the helical transmembrane domains (TMs).⁵² Even though the nature of the particular residues varies greatly from receptor to receptor, there are some positions that tend to have a conserved important role in ligand recognition, such as 3.32, 6.48 and 6.52 (using the Ballesteros-Weinstein notation⁵³). Currently, binding of GPCR ligands to allosteric sites and secondary binding pockets, typically around the variable extracellular regions, is less readily modeled than at orthosteric sites.

We have used for comparison of polypharmacology a set of congeners of adenosine that were originally designed in the context of the A₃ adenosine receptor (AR).³⁶ ARs are related to the biogenic amine family of GPCRs, both residing on the α -branch of family A GPCRs. This branch of the GPCR sequence dendrogram is the location of the targets for many commonly used pharmaceuticals: anti-depressant, antinociceptive, antipsychotic, antihypertensive and antiasthmatic drugs, etc. Thus, the pharmacological overlap between these varied GPCRs and a new anti-inflammatory target, the A₃AR, is important. Many of these targets already benefit from polypharmacology, i.e. agents that combine activity at various biogenic amine GPCRs and other receptors.⁵⁵ One such example is the antipsychotic agent clozapine,⁵⁵ which is more effective because it acts as a mixed D₂/D₃ dopamine antagonist, 5HT_{1A} partial agonist and agonist at the glycine site of the NMDA receptor, etc. Is there a means of rationally dialing up or down these activities by structural modification?

Ten members of a structurally rigidified family of adenine nucleosides (Figure 2) that act as selective, nanomolar A₃AR agonists and antagonists were examined by broad screening at 41 off-target sites by the NIMH Psychoactive Drug Screening Program (PDSP).¹ A low degree of conformational freedom within this series (due to a bicyclic ribose equivalent and a linear alkyne) increased the reliability of the docking by limiting the possible ligand conformations. Sterically bulky substituents were optionally present at the C2 and N⁶ positions. A large group at the C2 position was found essential for binding to the β_3 adrenergic receptor, and a large N⁶ group was found conducive to affinity at the 5HT₂ and α_2 receptors. The maximum affinity attained at the 5HT₂ (a compound truncated at **c**) and α_2 (a compound truncated at **c** and **d**) receptors were 75 and 61 nM, respectively. Asp3.32, conserved among the biogenic amine receptors, is a common point of electrostatic interaction with the 'essential' charged amino groups of the biogenic amine ligands, but it was predicted to play a role in H-bonding, with the exocyclic NH or with the 2',3'-hydroxyl groups of the pseudo-sugar ring system, when adenosine congeners bind to the same receptors (Figure 3). In some cases, the docked orientation of the nucleoside was coincident with its position in the primary target site, i.e. with the hydrophilic ribose moiety pointed downward as in the A₃AR. However, in most cases hydrophobic groups were predicted to lodge deep in the binding cleft anchoring the ligand, and when they were omitted that off-target biochemical effect disappeared. Sometimes, at a particular receptor two possible orientations of the adenosine derivatives were found, with a preference depending on the substituents present on the ligand scaffold (Figure 3). Thus, the off-target activities could be modulated by exclusion of various substituents, which also had variable effects on the efficacy and selectivity at the ARs. So, it was possible to combine or eliminate pharmacological components in a predictive manner, and subsequent analogues have confirmed these profile predictions. Moreover, examination of additional analogues with different substituents can validate the modeling predictions and help to define a unique binding mode when more than one possible orientation is found.

Although the starting point for this investigation was the structure activity relationship (SAR) of a class of A₃AR agonists, antagonist activity appears to predominate in the off-target hits determined. We have demonstrated moderately potent antagonism by various members of the series at α_2 and 5HT_{2C} receptors. A feature of the nucleosides that promotes agonist action at the ARs, i.e. a ribose 5'-alkylamide moiety, was present in five of the ten congeners (i.e. the structure was truncated at **a** or **d** in the other five members). At the off-target interaction with the 5HT₂ receptors, this group is defined as part of the pharmacophore for binding at moderate affinity, but it did not maintain its agonist-promoting role from the target A₃AR. It is now possible to explore therapeutic profiles within this structural series that might have beneficial multi-target selectivity. Specific examples will be the subject of future studies.

It would be desirable to use a common core structure, such as a nucleoside, to hit various targets and eventually, through an optimization process to eliminate the original activity of the core structure in favor of a new desired activity. Similarly, this was accomplished empirically for several GPCR targets using 1,4-dihydropyridines (DHPs) as a core structure. The L-type Ca²⁺ channel blocker nifedipine was chemically modified to serve as an

antagonist at α -adrenergic receptor,⁵⁷ A₃AR⁵⁸ and other GPCRs, without any significant residual Ca²⁺ channel activity. Thus, DHPs became known as a privileged structure for drug design. By comparing the off-target effects in conjunction with docking within a family of rigid congeners, we have treated adenosine as a privileged structure. Potentially, many core structures will display this versatility when probed systematically. This analysis³⁶ is also relevant to the concept of scaffold-hopping, which aims to apply cores that have improved properties, such as fewer off-target effects, at a given target.⁵⁹ Now with the possibility of achieving structure-based drug design in the GPCR superfamily,⁶ it is feasible to perform scaffold-hopping by a rational design process.

Conclusions

As discussed, structure-based molecular modeling methods are becoming increasingly applicable to GPCRs, due to the rapid advances in structural biology. These methods are indeed applicable to assist in identifying ligands for a specific target of interest.^{9, 17–34,56} They are also potentially applicable to the identification of cross-reactivity profiles,³⁶ with the advantage over other methods that they do not require close evolutionary relationships like bioinformatic methods or prior knowledge of common ligands like chemoinformatic methods. However, a limitation of structure-based molecular modeling methods lies in the fact that they are not ideal for high-throughput target identification. In fact, for a thorough study of the cross-reactivity of a GPCR ligand with all the member of the superfamily, docking studies directed at the experimental structures or models of each and every receptor would have to be conducted. Other challenges remaining are: accounting for protein flexibility (particularly true when considering extracellular loops of GPCRs) and induced fit caused by ligand binding; predicting atypical binding sites and the possible role of water molecules in binding; calibrating the scoring functions.⁵⁶ Moreover, homology modeling in case of receptors with low similarity is still not very accurate, therefore there is still a huge need for more crystallographic data for GPCRs.

Bioinformatic methods are also very much applicable to the study of GPCRs, for the very fact that they belong to a large superfamily of evolutionarily related ligands.^{9, 39} One caveat is that in order to study potential cross-reactivity profiles through sequence comparisons, the analyses should be extended to relatively large branches of the superfamily tree rather than focusing on small clusters of closely related receptors.

Chemoinformatic methods are indeed applicable to all the GPCRs having known ligands.^{4, 47–49} In particular they become increasingly applicable to a given receptor as novel ligands for that receptor are identified. Notably, by their nature these methods have the potential of revealing cross-reactivity across a wide range of classes of targets. A limitation of chemoinformatics-based methods is given by the fact that only targets for which ligands are known can be subjected to this kind of analysis, which renders it inapplicable to orphan GPCRs. Moreover, for chemoinformatic studies a target is defined only through the ligands that are currently known to bind to that particular target. In other words, if a specific target binds different classes of molecules, but only a few of the classes have been identified, cross-reactivity profiles based on the unknown classes of ligands would be omitted.

With these caveats in mind, while structure-based molecular modeling screenings seem more suitable to study off-target effects within a limited pool of receptors than for a wide-spectrum identification of cross-reactivity profiles, they also appear to be applicable in conjunction with bioinformatic or chemoinformatic methods in order to provide more robust results. For instance, whenever experimental structures or accurate models are available, molecular docking could be used to provide further support to potential novel targets for a given ligand hypothesized on the basis of sequence relatedness with the primary target or chemoinformatic analyses or to structurally rationalize pharmacological data of off-target activity.

We have discovered unanticipated ligand cross-reactivity of large and sterically constrained adenosine derivatives, which all lack a charged amine, to many aminergic GPCRs in the micromolar range of binding affinity.³⁶ We have no indication that such promiscuity is a particular feature of this class of adenosine congeners; rather, it is likely typical of many GPCR ligand series. Docking was performed at the X-ray structures of the ‘hit’ receptors, or where unavailable to homology models based on closely related structures. The docking at 5HT₂ serotonergic and α_2 and β_3 adrenergic receptors predicted pharmacological patterns suggesting binding orientation. The consistency of docking and pharmacology likely benefits from the comparison of closely related ligand structures, especially because they are rigid and have multiple extended substituents that protrude in different directions. Even though this study provided as an example is based on a limited set of compounds, the coordinated application of broad screening and modeling has led to a more complete understanding of off-target effects and to predict the modes of binding, which could be applied toward crafting more effective drug molecules to dial in or out secondary effects. Initial, extensive cross-GPCR screening is still needed during drug discovery, but this analysis can help guide the design of new compounds with the aim of reducing or improving specific off-target effects. Thus, a detailed structural understanding of the polypharmacology of this interrelated set of structurally rigidified GPCR ligands is now attainable, and the same approach could potentially be applied to other sets of congeneric ligands at many GPCRs.

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Glossary Box

Bioinformatics	use of computational methods to analyze biological data, including sequence data
Chemoinformatics	use of computational methods to correlate chemical structures of small molecules with their properties or biological activity
Orthosteric site	site on a receptor to which a native or principal ligand binds

Docking	computational prediction of an energetically favorable orientation of a ligand in complex with a protein or other biopolymer
Homology model	computationally predicted three-dimensional structure of a protein based on the experimental structure of a related homologous protein, which serves as a template
Molecular fingerprint	descriptors of molecular structure containing 2D or 3D information used for comparing similarity
Polypharmacology	multiple pharmacological effects of a single molecule or ligand due to interaction with different targets
Structure activity relationship	relationship between the molecular structure of a bioactive molecule, including specific chemical groups or conformations, and its degree of biological activity

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Highlights

- Bioinformatic and chemoinformatic approaches have been applied to GPCR polypharmacology.
- X-ray structures and molecular modeling enable precise understanding of GPCR interactions.
- A set of rigidified adenosine receptor ligands was screened at many off-target proteins.
- These analogues dock in consistent orientations at off-target hit GPCR structures/models.
- This analysis will guide future drug design to combine or eliminate multiple actions.

Figure 1. Three different computational approaches useful in the identification of off-target effects. Ligand-based pharmacophores refer to pharmacophoric hypotheses, i.e. a minimum ensemble of molecular features in a ligand or a receptor binding site that are needed for molecular recognition; however, this method is most effectively applied in combination with other approaches.

Figure 2.

General structure of adenosine congeners that were developed as potent and selective ligands of the A₃AR. Truncation points (**a** – **d**) to form the full set of 10 congeners studied in Paoletta et al.³⁶ are shown. Pharmacophores of the adenosine congeners that correspond to moderate (mostly μM) affinity at five off-target GPCRs (5HT₂ serotonergic, α₂ and β₃ adrenergic receptors) are shown in colored polyhedra. Arrows indicate the direction of binding in the TM region (from the extracellular toward the cytosolic side) for each specific off-target receptor. In the A₃AR, the ribose moiety is embedded in the deepest region of the binding pocket. At β₃ receptors, the rigid C2-arylalkynyl substituent was predicted to be buried deep in a hydrophobic region. At 5HT₂ and α₂ receptors, the N⁶ substituent was more likely to bind in the deep hydrophobic region.

Figure 3.

Transformed role of Asp3.32 that is important as a negative counterion in binding at aminergic receptors. Superposition of the X-ray crystallographic pose of (+)-charged agonist ergotamine (green carbons) at the human 5HT_{2B} serotonergic receptor with two alternative docking poses of an adenosine congener (2-Cl variant of the structure shown in Figure 2, i.e. truncated at **c**) at the same receptor. One docking mode (orange carbons, which is the only docking possible when an extended C2 substituent is present) shows the proposed role of Asp3.32 in the recognition of the adenine exocyclic NH group. This mode was similar to the predicted binding of various adenosine congeners at 5HT₇, α_{2B} and α_{2C} receptor models.³⁶ A second, alternative docking mode (pink carbons, coincident with the position of A₃ agonists at the A₃AR, possible only when the extended C2 substituent is absent) shows the proposed role of Asp3.32 in the recognition of the pseudo-sugar 2' and 3' hydroxyl groups.

Similarly, both poses were predicted for the homology-modeled 5HT_{2C} serotonergic receptor.