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Subtleties in GPCR drug discovery: a medicinal chemistry perspective

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Therapeutic effects through G protein-coupled receptors (GPCRs) are promoted by a full agonist, partial agonist, neutral antagonist or inverse agonist. Dramatic change of function such as from a neutral antagonist to a full agonist with minimal variation of ligand structure is a phenomenon that medicinal chemists often encounter. This is also influenced by a change of assay format. The subtle nature of structure–function relationships is difficult to grasp unless carefully considered from both chemistry and assay perspectives. In this article we discuss the subtle aspects of GPCR drug discovery from the medicinal chemistry perspective.

Seven transmembrane receptors, commonly known as G protein-coupled receptors (GPCRs), are frequent targets for therapeutic intervention and approximately 26% of currently prescribed drugs target GPCRs [1]. Drugs are normally classified as agonists, neutral antagonists and inverse agonists according to the nature of their effect on basal activity.

The 'Similarity Principle', which states that structurally similar compounds possess similar biological properties, was proposed a decade ago [2]. This principle seems to be considered viable especially by computational chemists [3], but its validity may depend on how the chemical and biological similarities are defined and thus, it may not be a generalized concept. Medicinal chemists often encounter drastic changes in biological properties with minimal structural variations [4]. This serves as the basis of follower drugs and there are several cases in which atomic-level structural variation gave drugs with properties superior to the originals [5]. In the case of GPCR drug discovery, the Similarity Principle seems to be more complicated than for the other target families because not only do structurally similar compounds often display different functional responses (e.g. agonist and neutral antagonist), possibly due to structural flexibility of GPCRs, but so do assay formats such as receptor density, selection of readout and the metrics. For these reasons, the structure–activity relationships (SAR) of function differ from those of binding and are subtle and difficult to explore.

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For the agonist response, Emax is the common metric for medicinal chemistry and is translated into the compound design. Since Emax cannot be used to differentiate the full agonists, developing the SAR can be better accomplished by considering affinity and efficacy separately using an operational model of agonism. Likewise it is suggested that a neutral antagonist can be classified as either a partial agonist or an inverse agonist in a more sensitive assay format thus enabling better understanding of the SAR for efficacy. The importance of selecting suitable metrics with a suitable assay format to explore hidden SAR is discussed in this article.

Allosteric modulators for GPCRs that are difficult to target with an orthosteric ligand have been extensively studied recently [6]. There have been many reports on metabotropic glutamate receptors (mGluR), where the negative allosteric modulator (NAM), the positive allosteric modulator (PAM) and the silent allosteric modulator (SAM) can interconvert with minimal structural change of the modulators. Subtleties in allosteric modulation have been reviewed recently and thus are not covered here [7].

Subtleties in structure cellular functional response relationships

There are multiple examples of switching from agonist to antagonist or inverse agonist and vice versa. Using the chemical structure of an endogenous agonist is one of the design strategies for GPCR ligands [8]. This is what occurred with histamine H2 blockers. Burimamide, the first compound to be used in clinical trials, is a

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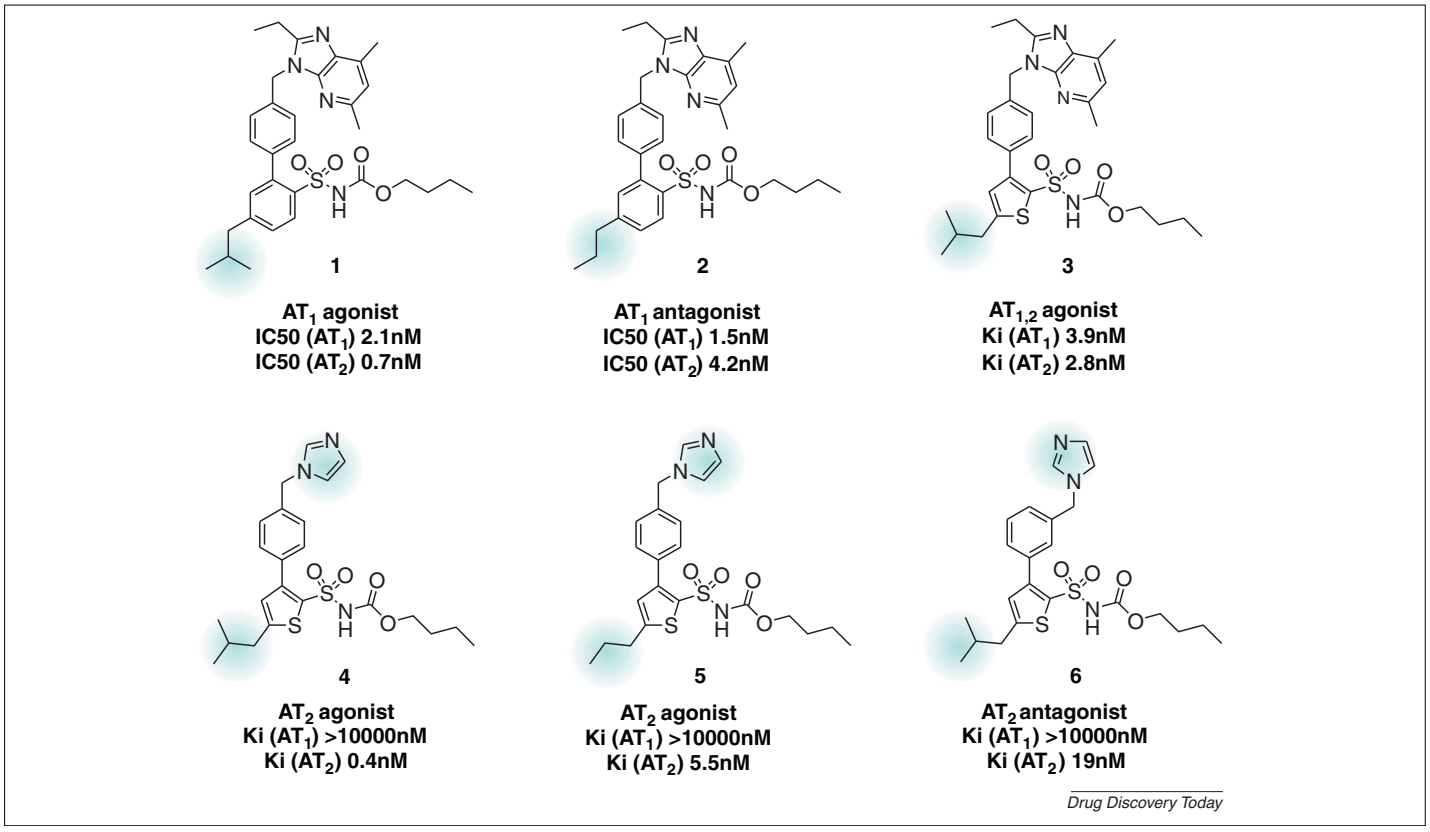

FIGURE 1

Switching in the functional response for AT_1 and AT_2 receptors was observed with minimal structural variation. Different fragments are responsible for switching the response for AT_1 and AT_2.
Biological activities were taken from Ref. [10].

thiourea derivative that was designed from the endogenous ligand, histamine. β2 adrenergic blockers are other examples of where the starting point for the medicinal chemistry was noradrenalin and adrenaline in which hydroxylamine represents the primary pharmacophore. Functional response varies depending on both the chemical transformation of ligands and assay formats. Here we highlight subtle SAR for the representative structural transformations that medicinal chemists often conduct, followed by the effect of assay format to functional response.

Change of the substituent and its position

Changing the substituent and its position is the basic strategy for designing compounds to develop SAR. One example can be seen from the angiotensin AT receptor [9]. Although antagonism of the AT_1 receptor is well known to be associated with regulation of blood pressure, the physiological role of the AT_2 receptor is less known. **1** (Fig. 1) was initially identified as an AT_1 receptor agonist in a phosphatidylinositol turnover assay. The agonist response of **1** is susceptible to a change in the alkyl substituent from iso-Bu to n-Pr to give a compound with an antagonistic property (**2**; Fig. 1). These ligands act differently on a series of point mutants, and thus the binding of **2** was affected by a mutation, such as T287A or N294A in human AT_1 receptors leading to a drop in affinity by eight- to ninefold, whereas that of **1** was unaffected by those mutations. The agonist response of **1** was susceptible to the mutation N295D rat AT_1 receptor giving **1** an antagonistic property whereas the antagonistic response of **2** remained intact with this mutation.

An interesting observation of the structure–function relationship on the AT_2 receptor was recently published [10]. Despite relatively low sequence homology (ca. 30%) between AT_1 and AT_2 receptors, **3** was discovered to be a dual AT_1 and AT_2 receptor agonist. To obtain a tool compound to elucidate the pharmacological role of the AT_2 receptor, modification of the imidazopyridine moiety to imidazole was performed giving a reduction in AT_1 affinity, which led to the first selective AT_2 receptor agonist **4**. To further address the substituent effect on binding activity and functional response to the AT_2 receptor, several derivatives based on this scaffold were obtained. Changing iso-Bu to n-Pr in this case did not cause a switch in the functional response as for the AT_2 receptor (**5**; Fig. 1). It turned out that the functional response is affected by a change of the position of imidazole methyl; a change from 'para' to 'meta' made the compound an AT_2 selective antagonist (**6**; Fig. 1) in the neurite outgrowth assay. Since the fragment having a role in the switching function is either lipophilic for the AT_1 receptor or hydrophilic for the AT_2 receptor, it is interesting to speculate whether there are different mechanisms operating for activation of these receptors.

Change of stereochemistry

Awareness of relationships between being successful in clinical trials and the three-dimensionality of the molecule denoted as a

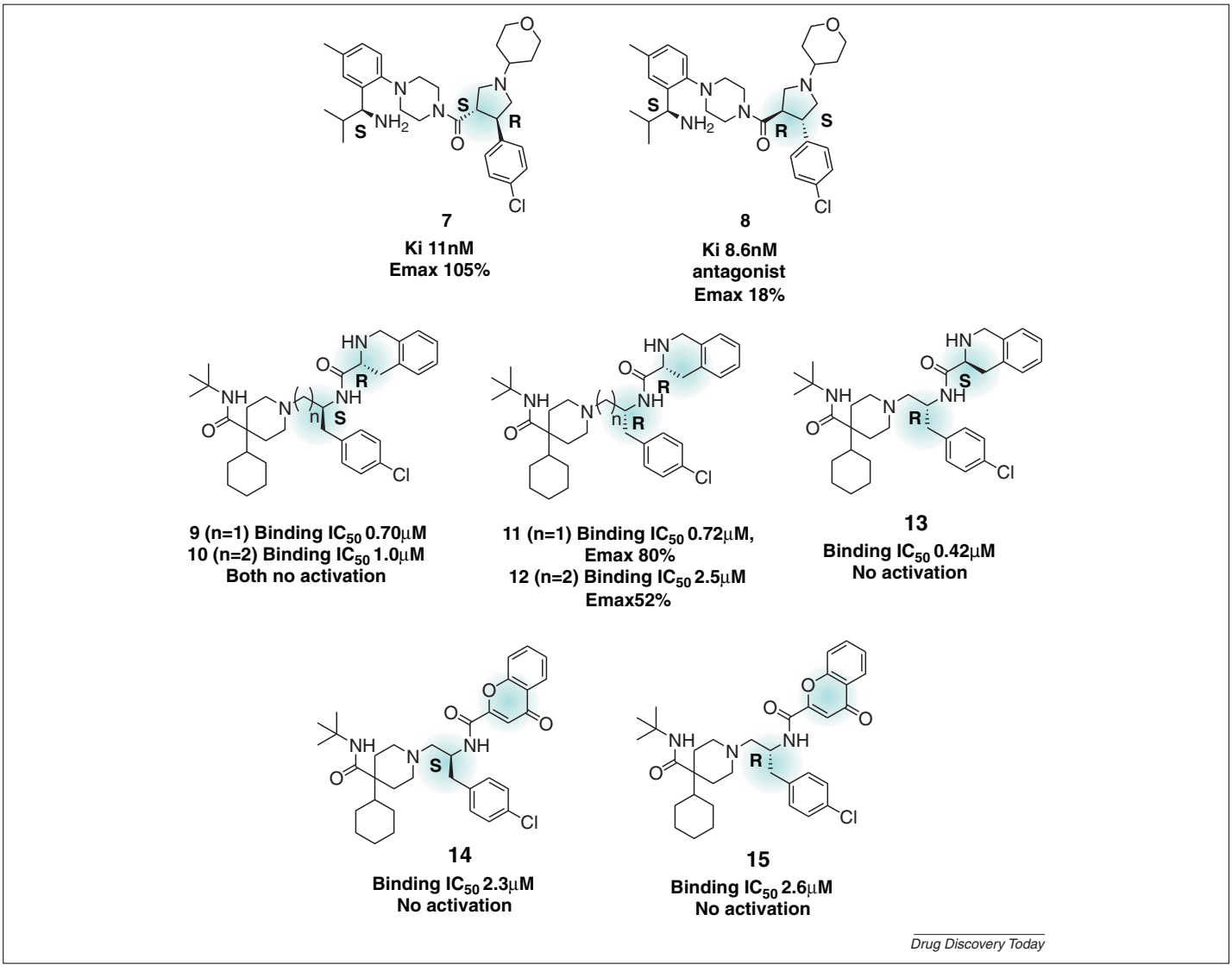

FIGURE 2

Switching from agonist to antagonist on the MC_4 receptor was observed with compounds with different chiralities. Both chiralities are responsible for functional responses.

Biological activities were taken from Refs. [14,15].

fraction of the SP_3 carbon led medicinal chemists to pursue compounds that are rich in SP_3 hybridized carbon centers [11]. As a result, controlling stereochemistry by synthesis and elucidation of the role of the stereocenter to biological response became crucial components in medicinal chemistry. Many examples of the stereochemical effect for functional response can be seen in ligands for melanocortin receptors (MCRs). MCRs having multiple roles such as energy homeostasis and sexual behavior have been targeted over the past decade [12]. In the discovery program for a selective MC_4 receptor agonist, a series of pyrrolidine-based compounds having different stereocenters was reported [13,14]. One kind of compound having (3S, 4R) stereochemistry in the pyrrolidine scaffold (**7**; Fig. 2) delivered an agonistic response whereas that with opposite stereochemistry (**8**; Fig. 2) gave an antagonistic response. This showed the dependence of stereochemistry on the functional response. Another example of the stereochemical effect can be seen in patent literature where researchers at Myo contract disclosed several piperidine-based MC_4 ligands [15]. In their study, both stereocenters on the benzyl substituted carbon and on the tetrahydroisoquinoline are important for defining the functional responses. Thus, both stereocenters with R stereochemistries (**11**, **12**; Fig. 3) are the only relevant stereocenters for an agonistic response in the cAMP production assay, otherwise the compounds would be nonagonists (**9**, **10**, **13**; Fig. 3) in this scaffold. The dependency of two stereocenters on the agonistic response was apparent since compounds having chromene carboxamide with no stereocenter in the upper part of the molecule (**14**, **15**; Fig. 3) displayed no potential as agonists.

Fixing the conformation of the molecule

Fixing the conformation of the molecule is one of the methods for designing compounds by which a chemist expects that the most stable conformation would resemble the bioactive conformation leading to entropic gain on binding affinity to the target protein. Recently, in explorations to discover the GPR119 agonist, researchers at Pfizer observed a switch between an agonist and

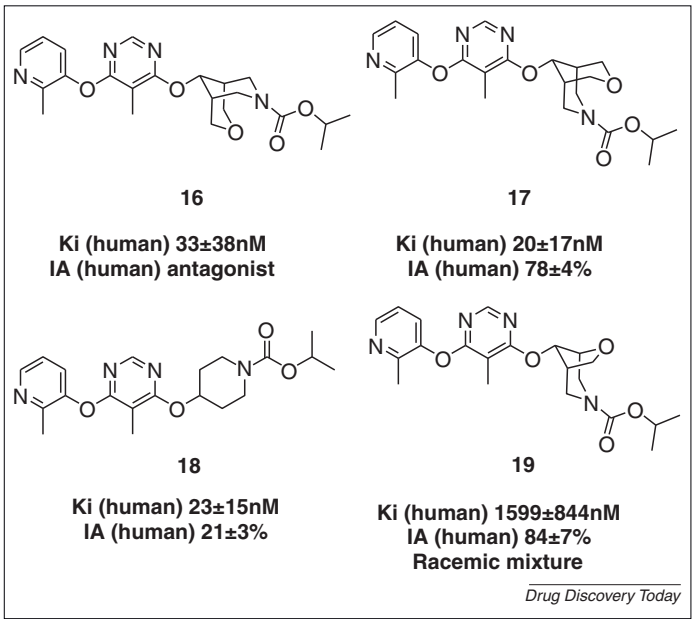

FIGURE 3

Switching functional responses on GPR119 receptor was observed with restriction of the conformation of the compound. Conformational restriction resulted in the compound being either an agonist or an antagonist. *Abbreviation*: IA: intrinsic activity.
Biological activities were taken from Ref. [16].

an antagonist on restricting the conformation of the piperidine ring [16]. Two compounds differing only in the relative stereochemistry of the ethereal linker bridging the piperidine ring showed completely different functional responses with comparable binding affinities for human GPR119 receptor. In this instance, the compound (**16**; Fig. 3) having an ether linker that was axially oriented was an antagonist in the cAMP functional assay, while that being equatorially oriented (**17**; Fig. 3) was an agonist. By conducting a conformational search, Pfizer researchers proposed that hydrogen bonding interaction involving the receptor and the carbomethoxy group seems to be essential for driving the compound to becoming either an agonist or an antagonist. Species difference was apparent, with both compounds being agonists to the rat GPR119 receptor. Interestingly, the compound without an ethereal linker (**18**; Fig. 3), which is apparently more flexible, has only partial agonistic activity. The authors suggested that a conformational ensemble of **18** buffers agonistic and antagonistic properties, leading to partial agonist activity. In addition to this, the effect of compounds having an ethereal linker that lacks one methylene (**19**; Fig. 3) was also examined to find the conformational requirement of agonistic and antagonistic responses. Although the binding affinity of **19** dropped, its intrinsic activity was the same as that of **17**, illustrating the role of relative stereochemistry of the ethereal linker to the functional response. This example nicely illustrates how fixing the conformation can be one of the design strategies for defining functional responses.

Influence of receptor density on agonist response

The system-dependent nature of the agonist response expressed by EC50 and Emax illustrate another subtlety in GPCR drug discovery which does not seem to be well acknowledged by medicinal chemists. In current drug discovery procedures, where the recombinant system for in vitro measurement is used, the agonist response using an actual cellular system in a human disease state would be variable. An old but notable example is the different behaviors of the muscarinic agonists carbachol and oxotremorine. Both behave as full agonists in normal pig ileum, but upon reduction of the receptor density, the maximal agonist response (Emax) of oxotremorine is reduced, whereas that of carbachol remains intact. The difference in behavior can be explained by considering the affinity (K_A) and coupling efficiency (denoted as efficacy term τ) separately by using an operational model of agonism [17,18]. Affinity is the term describing the interaction of receptor and ligand that is independent of functional readout; however, τ describes how efficiently ligand receptor interaction transmits the signal to the readout, thus being dependent both on the compound and the given assay system. Therefore, carbachol has low affinity and high efficacy, whereas oxotremoline has high affinity and low efficacy. The maximal agonist response of a compound having low efficacy is vulnerable to change of receptor density [19]. Because commonly adopted measures for agonist response such as EC50 and Emax are dependent on the cellular system, metrics less prone to be affected by system change should be employed by the medicinal chemist to avoid being misled when designing compounds. In this case, the relative efficacies calculated from τ were nearly the same regardless of receptor density, thus being less prone to be affected by system variation.

Influence of readout on agonist response

Classification of compounds in their function also depends on the readout because of signal amplification. Cellular assay is usually carried out by measuring cAMP, Ca^{2+} or GTPγS. For some GPCRs, correlation of those metrics to in vivo pharmacology is not well understood as it necessitates various in vitro readouts with several detection techniques [20,21].

An example of the effect of readout can be seen in the agonistic response of the muscarinic receptor [22]. In an effort to explore the mechanism of agonist efficacy, Sykes *et al.* investigated the

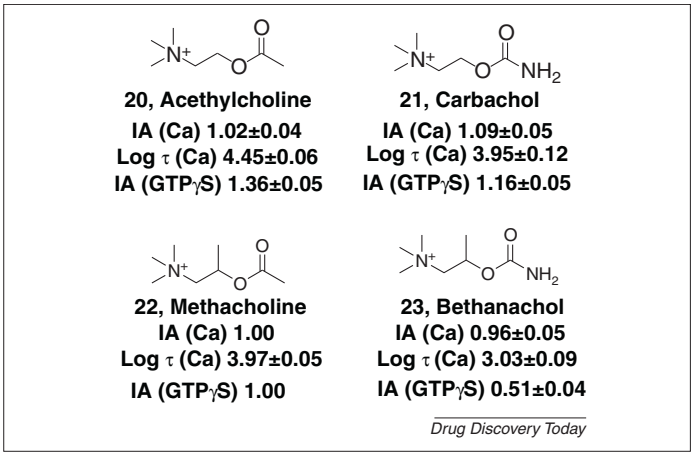

FIGURE 4

Muscarinic agonist response defined by measurement with Ca release and GTPγS. The use of τ shows a better correlation with IA from GTPγS.
Abbreviation: IA: intrinsic activity.
Biological activities were taken from Ref. [22].

dissociation rate of several muscarinic M_3 receptor agonists and sought the correlation between dissociation rate and agonist efficacy. For a series of agonists in which some differ in chemical structure at the atomic level (Fig. 4), agonist responses with GTPγS and calcium mobilization as readouts were measured. With the GTPγS assay, all agonists showed responses of different efficacies, however, with the calcium experiment, all agonists showed maximal efficacy comparable to the referencing compound and were thus classified as full agonists. Because of signal amplification, the calcium experiment was too sensitive to differentiate efficacies, making the readout from the GTPγS assay more suitable. Although the calcium experiment could not differentiate agonists when the SAR was developed with Emax, it could be done by employing τ from an operational model of agonism, and the result showed better agreement with the intrinsic efficacies from the GTPγS assay. Interestingly, the efficacy showed better correlation with the dissociation rate, thus implying that the residence time of the agonist is a decisive factor for efficacy with this receptor. In terms of SAR, a small change in structure is enough to change the dissociation kinetics and thus enhance efficacy. This reminds us of the importance of selecting suitable readouts and suitable metrics to develop a clear understanding of SAR.

Consideration of assay format to develop SAR of function

The definition of full agonist, partial agonist and antagonist has been argued to depend on the preparation used to study the receptor pharmacology [23]. It is reported that 85% of reported neutral antagonists are actually inverse agonists [24,25]. The relatively smaller proportion of neutral antagonists is well understood given the nature of the neutral antagonist having equal affinity for every conformation of GPCR that is considered to be less probable. For this reason, there has even been a report that neutral antagonists do not exist and can be classified as either partial or inverse agonists with more sensitive assay formats [26,27]. If the antagonist is classified as a partial or inverse agonist in the alternative assay format, it may provide a chance to explore hidden SAR because it can provide additional metrics in terms of efficacy [28]. In the example here, **2**, which is classified as an antagonist, is actually a partial agonist with Emax of 5.8% and **8**, which is classified as a functional antagonist, is a partial agonist with Emax of 18%.

Likewise for the agonist program, the assay may need to be supplemented with one in the antagonist mode. In the Pfizer GPR119 program, the fact that **16** had binding affinity comparable to **17** prompted them to conduct an assay with an antagonist mode giving Kb as 25 nM. Without the result from the antagonist mode, the structural determinant of function in this compound series would have been overlooked.

For a full agonist that cannot be efficiently differentiated like the partial agonist, the use of metrics such as a τ from an operational model or a less sensitive assay format (different readout or reducing the receptor density) should be considered other than using Emax as can be seen from the muscarinic agonist case described here. There are a limited number of papers describing the development of SAR with affinity and efficacy separately by using an operational model of agonism. Medicinal chemists should pay more attention to metrics other than the simple EC50 and Emax [29] to better develop structure–function relationship, although additional assay consideration may be labor-intensive to a biologist. If it is possible to differentiate responses with additional assay formats, SAR that appears bumpy initially would become clearly understandable as subtle SAR.

Influence of subtlety on the drug discovery process

GPCRs are considered to be disordered allosteric proteins where ligands and effectors such as the G protein affect each other through receptor proteins [30]. Given the nature of allosteric modulators in class C GPCRs in which PAM, NAM, SAM interconvert with small structural variations of allosteric modulators, the subtleties in the switch of function exemplified here are also considered to be consequences of allostery causing different SAR from the binding.

Difficulty in exploring the SAR of function resulting from the subtlety more or less influences the drug discovery process and one of them is hit selection from high throughput screening (HTS), which is important in the drug discovery process [31]. Although the definition of success varies with the company, the success rate of HTS for GPCR is relatively low compared to other target families. There are several reports discussing the factors that influence the success of HTS [32–34] from various aspects (assay type, target type, readout, library, among others). Where the functional response is primary metrics to generate primary SAR, such as orphan GPCRs, the probability to generate a singleton that may not be considered as a tractable hit series can be high if the hidden SAR of function is not well explored at this stage. One of the strategies to overcome the lack of success rate can be seen in the GPR119 agonist program of Arena. To search for novel hit series of GPR119 agonists, they performed HTS that could detect both agonists and inverse agonists and identified a pyrimidine-based hit compound as an inverse agonist. In the subsequent exploratory SAR study, they converted the inverse agonist to agonists in which the compounds differed only in the side chains [35]. As in the previous section, even in an agonist program, an assay format that captures inverse agonists can help explore hidden SAR and identify

potential hit series. One caveat in this strategy is that it may consume much time and resources if modification of the peripheral functional group does not lead to a switching function and necessitates modification of the scaffold due to difficulties in synthesis [31].

Concluding remarks

The subtlety of structure–function relationships inherent to GPCR drug discovery has been informed by past experiences in which the agonist–antagonist switch is a well-known strategy for designing GPCR ligands. By contrast, it may adversely affect drug discovery by necessitating the development of detailed SAR that can retard rapid and efficient decision making in the early stages of drug discovery. To tackle subtle SAR, a computational tool to elucidate structure determinants for the desired functional response was introduced recently [36]. Even with such a tool, unless the assay conditions are carefully selected, controlled and if necessary, supplemented with alternatives, the chance to explore hidden SAR will be missed.

If a pair of structurally similar compounds, which may possess similar in vivo Pharmacokinetics (PK) profiles [14], with opposite functional responses were explored, not only would this provide useful insights into SAR, but may also be useful for elucidating the pharmacological role of the receptor.

Although subtlety in SAR of function in GPCR drug discovery is considered to be of an intrinsic nature from the allosterism of the ligand, the influence of the assay format should also be well acknowledged by the medicinal chemist because alternative metrics and assay formats may make it possible to better understand the SAR of function and lead to better differentiation of compounds.

Conflict of interest

The authors are employed by Shionogi & Co., Ltd.

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