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Discovery of the First Selective, Nonpeptidic Orexin 2 Receptor Agonists

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ABSTRACT: In this issue, Nagase and colleagues report the discovery of the first selective nonpeptidic orexin 2 receptor (OX_2R) agonists. The discovery of these OX_2R selective agonists opens up new avenues for therapies related to the activation of the orexin system, especially with respect to the treatment of sleep disorders such as narcolepsy.

Through an extensive synthesis and screening program, Nagahara et al., 2015,[1] report the discovery of the first selective nonpeptidic orexin 2 receptor (OX_2R) agonists culminating in compound **26** (OX_2R EC_{50} = 0.023 μM, E_{max} = 98%; OX_1R EC_{50} = 1.616 μM, E_{max} = 100%). The design of small-molecule agonists (activators) rather than antagonists (inhibitors) of such receptors, and especially of peptide-activated G-protein-coupled receptors (GPCRs), is considered as one of the current challenges in drug discovery. Such ligands hold high therapeutic potential especially for neuropeptide-activated GPCRs like the orexin receptors, where natural peptides are frequently nonselective and are often inefficient for in vivo studies due to lack of ability to penetrate the blood−brain barrier (BBB). The selectivity profile of the agonists reported here is also attractive, and as such, they will serve as good tool compounds for exploration of OX_2R function that was not previously feasible with nonselective peptide agonists. Furthermore, given the recent appearance of a crystal structure of an antagonist (suvorexant) in complex with OX_2R, it would seem likely that X-ray crystallography can now also be used to guide further drug-design efforts for this important target for several different conditions.

OX_1R and OX_2R, which are class A GPCRS, are located predominantly in the brain and are linked to a range of different physiological functions, including the control of feeding, energy metabolism, modulation of neuroendocrine function, and the regulation of the sleep−wake cycle. The natural agonists for OX_1R and OX_2R are two nonselective neuropeptides, orexin A (OxA) and orexin B (OxB), which have dual activity, at both receptors. This phenomenon has limited their use as probe compounds to dissect out precise contributions but has enabled several conditions to be related to OXR activity. Years of research have suggested that OXR agonists could be useful for the treatment of sleep disorders, narcolepsy, cataplexy, obesity, hypophagia, as well as attention deficit hyperactivity, depression, and related bipolar disorders. Furthermore, the discovery of OX_2R agonists will provide an excellent start point for the design of agonists for the related orexin-1 receptor (OX_1R). OX_1R has been shown to drive apoptosis in human colon cancer cells, and treatment with orexins dramatically slowed the growth and even reversed the development of established tumors. OX_1R agonists are therefore also prime candidates for colon cancer therapy. OXR agonists could also be useful for the treatment of Parkinson's disease, which is characterized by massive loss of hypocretin neurons. The design of selective OXR agonists has been a challenging problem, despite extensive mutagenesis[2] and modeling work.[3] This new chemical screening information along with the recently solved OX_2R crystal structure[4] (PDB entry 4S0V) should create a step change in the development of drugs against this important family.

It is known that for effective antagonism, it is sufficient for small-molecule ligands to occupy a relevant receptor site in order to inhibit the binding of agonists. However, for the discovery of agonists, there is the additional complication and requirement that the ligand is able to activate the receptor. Peptide-activated GPCRs are considered especially challenging in this respect because of the potential for a large number of specific and nonspecific interactions that could be involved in binding and activation. The OXRs are particularly difficult because the natural agonist peptides, orexin A and orexin B, are quite large at 33 and 28 amino acids, respectively. Directly trying to mimic the effects of these large peptides with small molecules has been viewed as extremely challenging.

Although peptides or small molecule agonists activate the vast majority of GPCRs, a spontaneous self-activation can also often be observed. Full agonists are traditionally defined as ligands that maximally activate the receptor, partial agonists induce submaximal activation, inverse agonists inhibit basal activity, and neutral antagonists have no effect on basal activity but competitively block access of other ligands.

Ligand binding is translated into conformational changes in the receptor that result in activation of intracellular G-proteins and/or β-arrestins, which in turn modulate the activity of downstream effectors inside the cell. GPCRs are in a dynamic structural equilibrium between multiple distinct conformations that can include more than one active or inactive conformation[5] (see Figure 1). The mechanism and structural changes associated with the activation of GPCRs remain a challenge. It was observed that agonists are able to break and mediate key interactions between transmembrane helices (TMs) and by doing so enable them to move closer to each other, push them further apart, or rotate one relative to the other. These structural changes are

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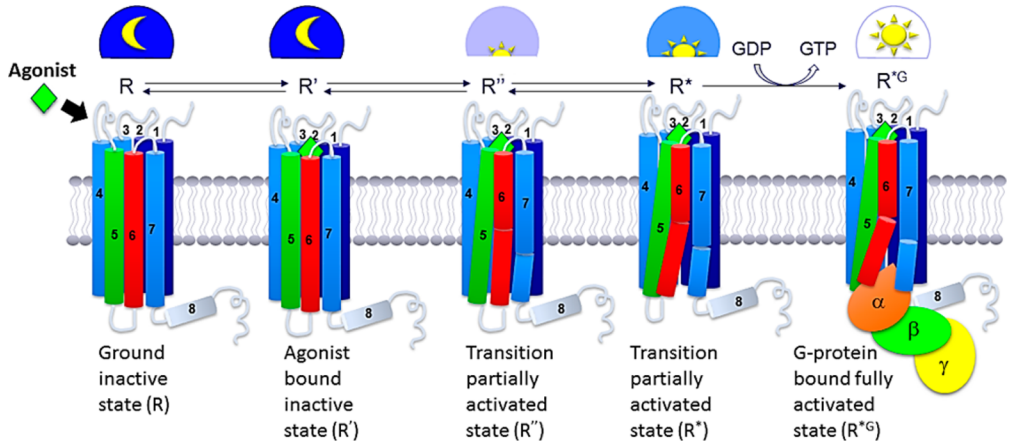

Figure 1. Scheme describing the agonist promoted activation process from inactive ground state to fully activated G-protein bound state. Evidence for such activation processes has been provided by GPCR X-ray crystallography for β1- and β2-adrenergic and adenosine A_{2a} receptors and others.

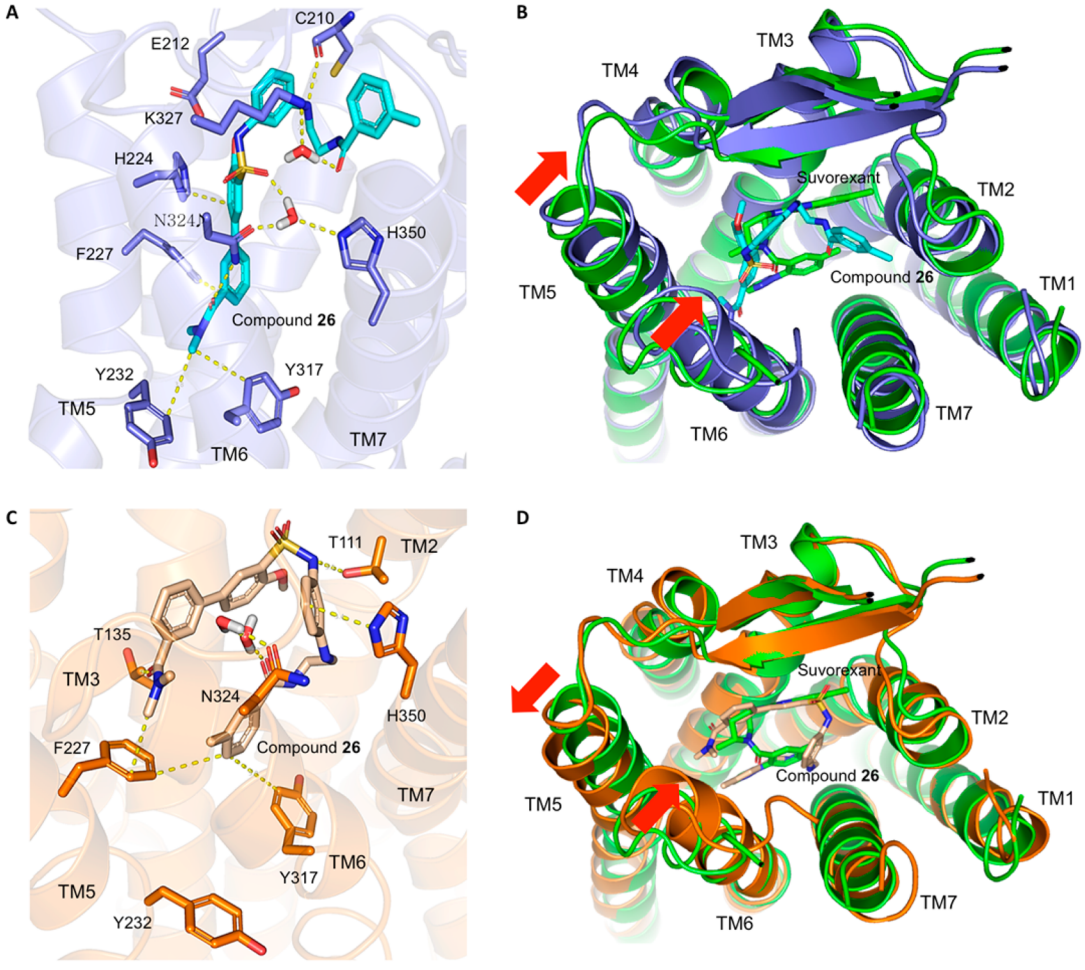

Figure 2. Two binding modes of compound **26** with OX_2R produced by hierarchical GPCR modeling protocol (HGMP)[3a] that included postdocking OX_2R-**26** complex optimization. (A) Literature-like "L" shape docking pose as reported by Nagahara et al., 2015.[1] The carbon atoms of compound **26** are shown in cyan and for the receptor in blue. Nitrogen atoms are shown in blue, oxygen in red, sulfur in yellow, and chlorine in light green. The key interactions are shown as yellow dashed line. (B) Superposition of OX_2R-suvorexant crystal structure (PDB entry 4S0V) (backbone and suvorexant colored green) with the OX_2R-**26** complex (backbone colored blue). The key differences in the TM conformations are shown as red arrows. (C) Alternative "U" shape docking pose. The carbon atoms of the **26** are shown in light pink and for the receptor are orange. (D) Superposition of OX_2R-suvorexant crystal structure (PDB entry 4S0V) (backbone and suvorexant colored green) with the OX_2R-**26** complex (backbone colored orange) in "U" docking pose. The key differences in the TM conformations are shown as red arrows.

known as "molecular switches".[5b] In recent years, it has become apparent that these molecular switches can induce an ensemble of activated conformations which trigger different activation pathways.

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So key a question that arises now is the following: Do we know how these new OX_2R agonists, and compound **26** in particular, work as agonists? The answer is not yet, but we are in a position to generate testable hypotheses. By combining these new agonists with the antagonist-bound crystal structure, we can begin to postulate likely binding modes. Two such example poses are shown in Figure 2. In the first pose (Figure 2A) residues $T111^{2.61}$, $Q134^{3.32}$, $T135^{3.33}$, $C210^{ECL2}$, $E212^{ECL2}$, $H224^{5.39}$, $F227^{5.42}$, $Y317^{6.48}$, $N324^{6.55}$, $K327^{6.58}$, $H350^{7.39}$, and two water molecules are involved in binding of compound **26** (superscript represents residue indices according to the Ballesteros and Weinstein[6] numbering scheme). Furthermore, these modeling observations are directly supported by the published SDM data.[2,3] In the SDM studies the alanine mutations of $T111^{2.61}$, $Q134^{3.32}$, $D211^{45.51}$, $W214^{5.54}$, $Y223^{5.38}$, $F227^{5.42}$, $F346^{7.35}$, and $H350^{7.39}$ caused a large (>50) fold decrease in the potency of OxA without affecting the efficacy compared to WT.

The mutations $Y232A^{5.47}$ and $Y317A^{6.48}$ resulted in a reduction of both EC_{50} (by 28.4- and 17.7 -fold, respectively) and E_{max} of 44.9% and 49.6%, respectively, of OxA. These mutations caused a moderate decrease in potency of OxA (by 22.3-fold) without affecting its efficacy. These SDM data suggest that there is no clear correlation between the importance of residues for potency and for efficacy. However, $Y232^{5.47}$ and $Y317^{6.48}$ are involved in OX_2R activation. The direct interaction of **26** with these key residues might explain to some extent its agonist activity. Recently solved crystal structures propose that the movement of TM5 and TM6 is made possible through rearrangement of the TM3−5−6 interface and it is potentially the most commonly conserved switch among class A GPCRs.[5b] This agonist-bound switch was recently proposed to be part of a larger "transmission switch" that accounts for the relocation of conserved residues $W^{6.48}$ ($Y317^{6.48}$ in OX_2R) and $F^{6.44}$ toward $P^{5.50}$.[5b] On the other hand the role of $F/Y^{5.47}$ ($Y232^{5.47}$ in OX_2R) is not clear, but it is frequently engaged in interaction with agonists.[5b] This typical inward movement of TM5 and TM6 with respect to the inactive state is also observed in compound **26** binding to the OX_2R (see Figure 2B).

It is also possible to model compound **26** in a suvorexant-like flipped "U" shape (see Figure 2C). According to this pose, the residues $T111^{2.61}$ (Ser in OX_1R), $T135^{3.33}$, $E212^{ECL2}$, $H224^{5.39}$, $F227^{5.42}$, $Y317^{6.48}$, $N324^{6.55}$, $H350^{7.39}$, and two water molecules are involved in **26** binding (see Figure 2D). The interactions with the toggle switch residue $Y317^{6.48}$ and with the aromatic cluster residue $F227^{5.42}$ support the activation switch mechanism that allows compound **26** to have OX_2R agonism function. For this pose, outward movements of TM5 and inward movement of TM6 (with respect to the antagonist-bound conformation) were observed to occur as part of the docking protocol (see Figure 2D). A potential explanation for OX_1R selectivity arises from potential interactions with the nonconserved residues $T111^{2.61}$ ($S102^{2.61}$ in OX_1R) and $T135^{3.33}$ ($A135^{3.33}$ in OX1R). Further structural and structure−activity relationship (SAR) exploration will be required to explore these hypotheses.

The excellent work of Nagese and colleagues suggests that with further application of X-ray crystallography, mutagenesis and modeling approaches we can look forward to the future development of agonists against these important targets for the treatment of multiple different conditions.

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ABBREVIATIONS USED

OXR, orexin receptors 1 and 2; BBB, blood−brain barrier; HGMP, hierarchical G-protein-coupled-receptor modeling protocol; TM, transmembrane helix; ECL, extracellular loop

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