

NUCLEAR RECEPTORS

A new mode of inhibition

Complementary structural biology approaches reveal how an agonist and a covalent inhibitor simultaneously bind to a nuclear receptor.

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Related research article Shang J, Kojetin DJ. 2024. Unanticipated mechanisms of covalent inhibitor and synthetic ligand cobinding to PPAR γ . *eLife* **13**:RP99782. doi: [10.7554/eLife.99782](https://doi.org/10.7554/eLife.99782)

Shuttling between a cell's cytoplasm and nucleus are nuclear receptor proteins, which are involved in a diverse number of physiological and pathological roles. These nuclear receptors can be activated by a range of ligands – such as vitamins, hormones and fatty acids – which trigger the receptor to upregulate the transcription of certain genes (*Nagy and Schwabe, 2004*). Due to their broad biological functions, nuclear receptors are a major drug target in pharmacology (*Santos et al., 2017*). These medicines can either activate the receptor (agonists), inhibit receptor activity (inverse agonists), or occupy the ligand-binding pocket so that other molecules cannot access it (antagonists).

One such nuclear receptor, peroxisome proliferator-activated receptor gamma (or PPAR γ for short), regulates genes involved in producing fatty tissue and controlling blood glucose levels. Various ligands can manipulate PPAR γ activity, including the body's endogenous lipids and fatty acids, as well as anti-diabetic drugs, such as rosiglitazone. Although rosiglitazone and its related synthetic compounds have been approved by the Federal Drug Administration (FDA) in the United States, their global use has been limited due to the adverse effects they can cause (*Kroker and Bruning, 2015*). As a result, there is growing interest in finding other drugs that can target PPAR γ . However, to

achieve this, researchers need a better understanding of how molecules interact with PPAR γ and modulate its activity, including compounds used in basic scientific studies, as these experiments will inform the development of pharmaceutical drugs.

Two covalent inhibitors, called GW9662 and T0070907, are commonly used in the laboratory to study the potency of synthetic ligands for the PPAR γ receptor. Initially, these two compounds were thought to be antagonists that block the activity of PPAR γ by occupying the ligand-binding site to prevent agonists binding. However, previous studies found that these covalent inhibitors do not stop all agonists from activating PPAR γ (*Hughes et al., 2014*). In addition, it has been shown that the ligand-binding pocket of PPAR γ can bind to multiple ligands at a time, including co-binding with an agonist and an antagonist simultaneously (*Shang et al., 2018*). Now, in eLife, Jinsai Shang (Scripps Research and Guangzhou Medical University) and Douglas Kojetin (Scripps and Vanderbilt University) report new insights into the inhibitory properties of GW9662 and T0070907 (*Shang and Kojetin, 2024*).

First, Shang and Kojetin profiled how co-activators and co-repressor proteins – which help to promote and inhibit gene expression, respectively – are recruited to PPAR γ when GW9662 or T0070907 are present. Typically, agonists make it easier for co-activators but harder for co-repressors to bind to nuclear receptors, while inverse agonists have the opposite effect, and antagonists are somewhere between the two. Shang and Kojetin found that some agonists could still induce PPAR γ to interact with co-activators, even when its ligand-binding pocket was also covalently bound to GW9662 or T0070907. This

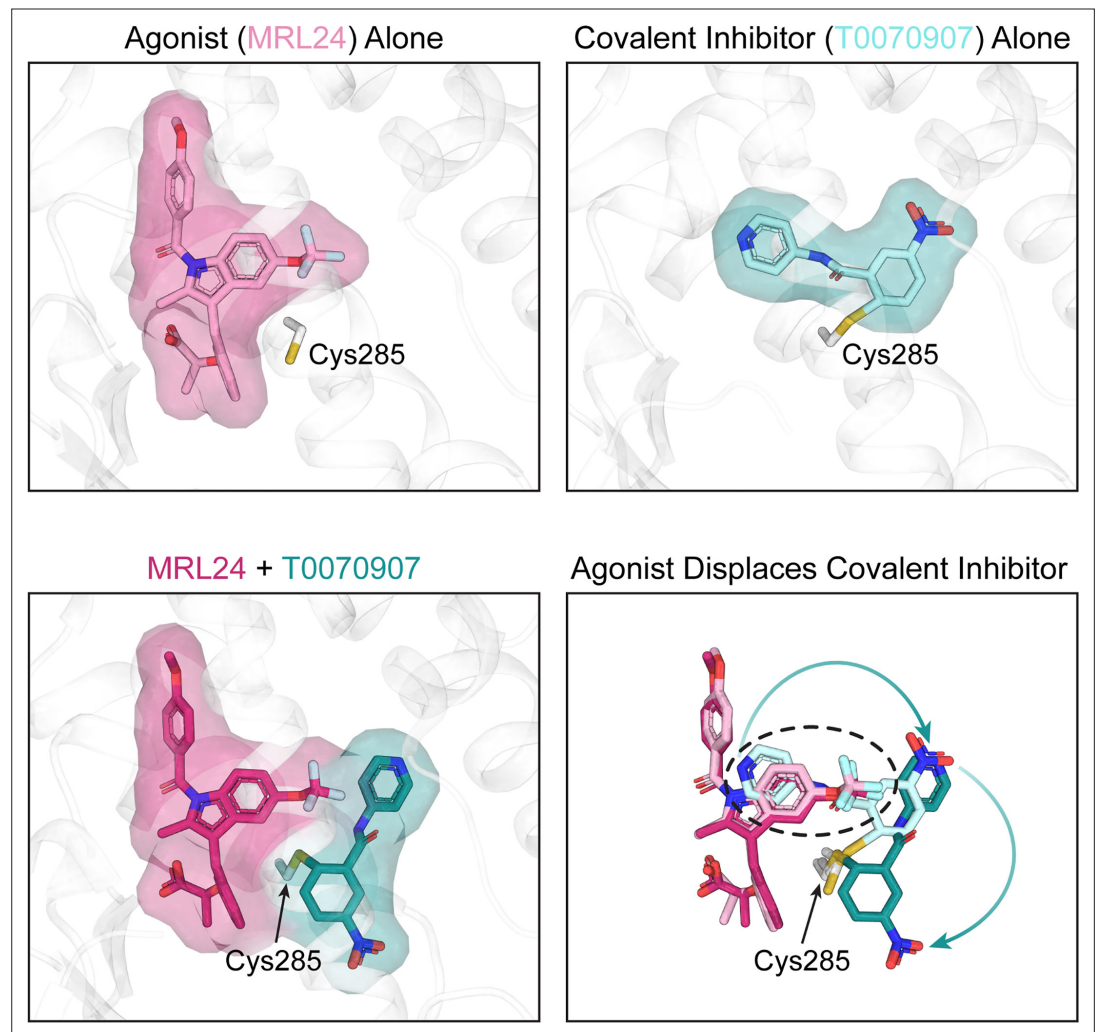


Figure 1. Crystal structures of the PPAR γ ligand-binding pocket. When an agonist or a covalent inhibitor binds to PPAR γ , it alters the structure of the ligand-binding pocket in PPAR γ . The top left image shows the crystal structure of the PPAR γ ligand-binding pocket (white) when bound to an agonist called MRL24 (light pink). The top right image shows the crystal structure of the PPAR γ ligand-binding pocket when bound to T0070907 (light cyan), a covalent inhibitor that attaches via the amino acid Cys285. When MRL24 and T0070907 simultaneously bind to PPAR γ , T0070907 is displaced (bottom left). The site where MRL24 sits in the PPAR γ ligand-binding pocket overlaps with the site that T0070907 occupies (bottom right, black dashed ring). This clash causes the agonist (ML24) to displace the covalent inhibitor (T0070907) and shift it to a different position.

Image credit: Andrew D Huber; structure of MRL24 alone (PDB ID 2Q5P), T0070907 alone (PDB ID 6C11), and MRL24/T0070907 together (Shang and Kojetin, 2024).

suggests that neither GW9662-bound PPAR γ or T0070907-bound PPAR γ is fully inhibited.

This finding was corroborated by studying the structure of PPAR γ using NMR spectroscopy, which revealed that agonists could induce an active PPAR γ conformation even when GW9662 or T0070907 was present. To investigate how this happens, Shang and Kojetin solved crystal structures of the ligand-binding pocket of PPAR γ that were covalently bound to GW9662 or T0070907 and had been subsequently incubated with an agonist. Surprisingly, they found that agonists

only slightly adjusted their binding modes when GW9662 or T0070907 were present. Instead, it was the covalent inhibitors that underwent pronounced conformational changes, which allowed agonists to be incorporated into the ligand-binding pocket (Figure 1).

The classical model of receptor antagonism is simple: an agonist cannot bind to a pocket that is plugged by an antagonist. However, the findings of Shang and Kojetin suggest that the commonly used covalent inhibitors GW9662 and T0070907 act by a different mechanism. The covalent

inhibitor favors an inactive PPAR γ state when attached to the receptor. However, rather than preventing agonists from binding to the receptor, these compounds allow agonists to bind alongside the inhibitor, which leads to an inhibitor/agonist co-bound state. As higher amounts of agonists are added to the system, the inhibitor/agonist co-bound state may become more dominant and lead to PPAR γ activation.

These findings provide a structural explanation for why GW9662 and T0070907 do not block the activity of all PPAR γ agonists (*Hughes et al., 2014*). They also demonstrate that PPAR γ antagonists that are completely inhibitory are needed to fully understand the biology of this nuclear receptor. More generally, the results demonstrate why it is important to fully characterize how ligands bind to receptors before using them as tools for studying biological functions.

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Competing interests: The authors declare that no competing interests exist.

Published 19 August 2024

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