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Review article

Modes of action insights from the crystallographic structures of retinoic acid receptor-related orphan receptor-γt (RORγt)

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ABSTRACT

RORγt plays an important role in mediating IL-17 production and some tumor cells. It has four functional domains, of which the ligand-binding domain (LBD) is responsible for binding agonists to recruit co-activators or inverse agonists to prevent co-activator recruiting the agonists. Thus, potent ligands targeting the LBD of this protein could provide novel treatments for cancer and autoimmune diseases. In this perspective, we summarized and discussed various modes of action (MOA) of RORγt-ligand binding structures. The ligands can bind with RORγt at either orthosteric site or the allosteric site, and the binding modes at these two sites are different for agonists and inverse agonist. At the orthosteric site, the binding of agonist is to stabilize the H479-Y502-F506 triplet interaction network of RORγt. The binding of inverse agonist features as these four apparent ways: (1) blocking the entrance of the agonist pocket in RORγt; (2) directly breaking the H479-Y502 pair interactions; (3) destabilizing the triplet H479-Y502-F506 interaction network through perturbing the conformation of the side chain in M358 at the bottom of the binding pocket; (4) and destabilizing the triplet H479-Y502-F506 through changing the conformation of the side chain of residue W317 side chain. At the allosteric site of RORγt, the binding of inverse agonist was found recently to inhibit the activation of protein by interacting directly with H12, which results in unfolding of helix 11′ and orientation of H12 to directly block cofactor peptide binding. This overview of recent advances in the RORγt structures is expected to provide a guidance of designing more potent drugs to treat RORγt-related diseases.

1. Introduction

Nuclear receptors (NRs) are ligand-modulated transcription factors [1]. Different from other members of NRs, the retinoic acid receptor-related orphan receptor (ROR) γt (also called NR1F3) is only expressed in lymphoid organs [2]. The RORγt binds to the retinoid-related orphan receptor response element (RORE) within the nuclear DNA and then activates gene transcription (Fig. 1). As observed, this receptor could be constitutively active even without binding to a ligand [3]. So far, the RORγt is recognized to play indispensable role in the production of the pro-inflammatory cytokine interleukin IL-17, which is expressed from T-helper (Th)17 cells [3,4]. The IL-17 is actively involved in immunology processes, and is found as a critical driver of the pathogenesis of autoimmune diseases such as cancer, rheumatoid arthritis (RA), multiple sclerosis (MS), psoriasis and inflammatory bowel diseases [5–8]. Lots of studies have found that the RORγt is very important in multiple inflammatory and immunological pathways. Therefore, the receptor has been established as a suitable drug target for the design and discovery of novel treatments for these diseases [1,7,9–15]. Besides, recent studies focused on the over-expression of RORγt in tumor cells and found that the receptor plays an important role in metastatic castration-resistant prostate cancer (mCRPC) and pancreatic cancer [16–20].

Given the prominent role of RORγt in autoimmunity and cancer, the discovery of small molecules targeting the RORγt have been intensely pursued in the past decade [8–12,15,21–35]. We have sorted out the reported active and calculated drug-likeness values of some small molecules (Tables S1–S17). Among these endeavors, the latest chemical language models have been used to automatically and intelligently design novel inverse agonists against RORγt [35]. Basically, an agonist

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of RORγt can promote the formation of a coactivator complex, then enhance the gene transcription relative to the constitutive level of transcriptional activity, and an agonist could provide an unique approach to cancer immunotherapy [8,25]. On the other hand, an inverse agonist of RORγt represses the formation of a coactivator complex, and then decreases the gene transcription relative to the constitutive level of transcription activity [9]. A designed small molecule which meets such repressing effect on RORγt could be ideally used as novel treatments for autoimmune diseases [15,23,26]. Interestingly, in tumor cell, an inverse agonist of RORγt can strongly inhibit expression of androgen receptor (AR) [16] and cell autophagy *via* increasing the expression of autophagy adaptor SQSTM [18], which shows robust antitumor activity against mCRPC (Fig. 1). Furthermore, an inverse agonist of RORγt in tumor cell has been reported to treat pancreatic cancer as well (Fig. 1) [20].

RORγt is composed of four distinct domains: the amino-terminal domain (A/B), the DNA-binding domain (DBD), the hinge region, and the ligand-binding domain (LBD) (Fig. 1) [7]. The DBD of RORγt binds to the ROR response elements (RORE) on DNA via two zinc-finger motifs [2]. The LBD region binds with an agonist to recruit a coactivator as well as an inverse agonist to stop the recruitment of a coactivator, and therefore affects the gene transcription [8,9,15,26]. During the past decades, numerous crystal structures of the LBD domain of RORγt in apo-state (without a bound ligand) and in complexes binding with agonist and inverse agonist have been published from different research labs and the R&D groups of pharmaceutical companies [11,14,36–54]. These x-ray structures revealed that the LBD domain of RORγt (Fig. 2) is featured as a three-layered fold of approximately 12 α-helices and 2–3 β-strands. Among the core of its structure, there is a cavity with volume of around 940 $Å^3$, forming a highly conserved hydrophobic orthosteric-binding pocket (Fig. 2A) [47]. For the apo- and agonist-bound state of RORγt-LBD, the binding site near the helix 12 (H12) is ready to bind with a cofactor like steroid receptor coactivator 2 (SRC2) [11,14,36–45]. Upon the binding of RORγt-LBD with an inverse agonist, the pocket is distorted through re-orientating H12 to a totally different position and pose (as shown in the right panel of Fig. 2) [46–52, 54]. Recent published structures revealed a new allosteric-binding site at the LBD [55–60], which is located distal to the orthosteric site, and formed by helices 4, 5, 11 and 12 (Fig. 2B). Based on the published x-ray structures and the general information of how the transcriptional activity of RORγt is influenced by agonists and inverse agonists, it is really the time to explore the insights into the modes of action from the structural details of the RORγt LBD-ligand binding, which will be invaluable to help the design of novel small molecules as innovative therapeutics. This review is devoted to analyze the MOA of RORγt-ligand binding at both orthosteric and allosteric sites, with the eventual aim to trigger new ideas for the rational design of more optimal drugs in the near future.

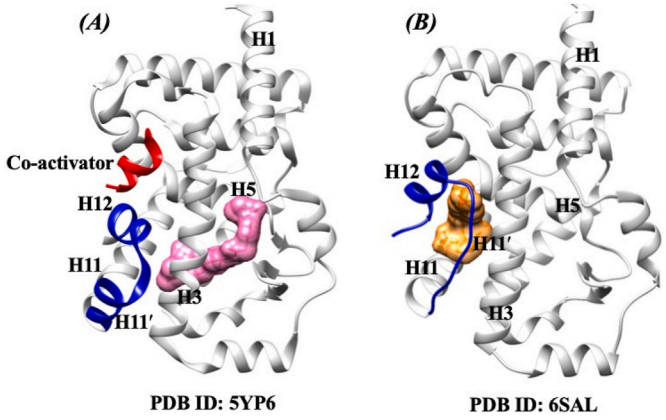

Fig. 2. Ribbon representative of RORγt LBD with its ligands. (A): RORγt LBD-agonist complex [43]. The agonist is bound at the orthosteric site, and shown in pink-colored space-filled style, while the H11 and H12 in blue ribbon, and the co-activator SRC2 in red. (B): RORγt LBD bound with an inverse agonist [57]. The inverse agonist is bound at the allosteric site, and is shown in gold-colored space-filled style. The distorted H11 and H12 are shown in blue ribbon.

2. Mode of action in agonists of RORγt

Early studies [14,32,43] like cell-based reporter gene assays, and solution $^{13}C/^{15}N$-labeled NMR sampling revealed that the RORγt is able to stimulate gene transcription in the absence of an exogenously added agonist, and its LBD in physiological solution is capable to recruit coactivator SRC2, indicating that the native RORγt is constitutively active. Cholesterol biosynthetic pathway study [14] found that the sterol lipids are sufficient to drive RORγt-dependent transcription, leading to the report that 25-hydroxycholesterol (25-HC) and other cholesterol biosynthetic intermediates are the natural ligands of this receptor. X-ray structures on both the apo-state RORγt LBD and its binding complex with agonists including 25-HC showed that the highly conserved orthosteric-binding pocket is predominantly hydrophobic with 61% of the total cavity volume, while the hydrophilic, basic and acidic compositions have the volume percentage as 17%, 19% and 3%, respectively (Fig. 3A and B). As observed from these x-ray structures [11,37–45], the binding pocket is mainly composed of helices H1, H2, H3, H5 and H6, it extended to helices H11 and H12. At the entrance of the pocket, it sits mainly the hydrophilic and/or charged residues Q286, H323, R364 and

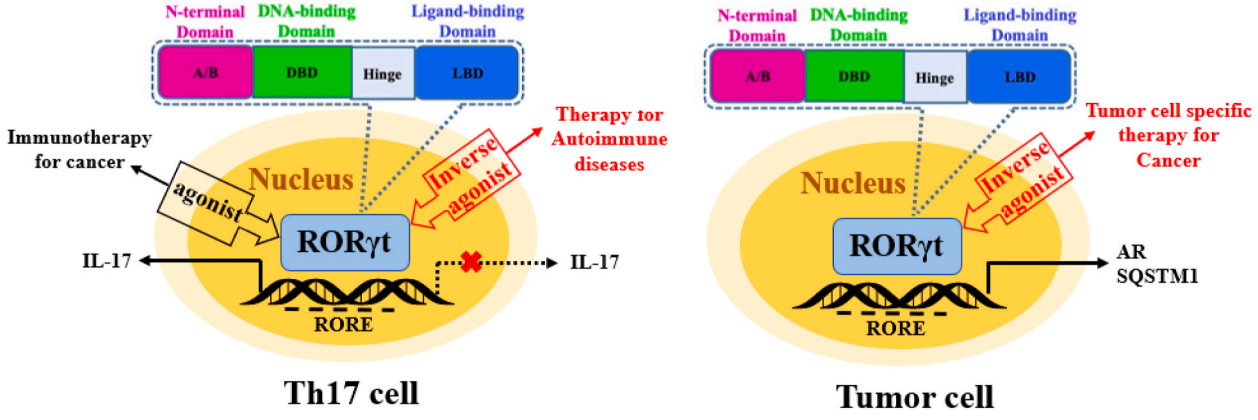

Fig. 1. Schematic representation of RORγt receptor with its different domains and its nuclear binder RORE. In Th17 cell, the agonist of this receptor can be used for cancer immunotherapy, and its inverse agonist for potential treatment of autoimmune diseases. The inverse agonist of RORγt in tumor cell can be used for cancer therapy by tumor cell specific pathways.

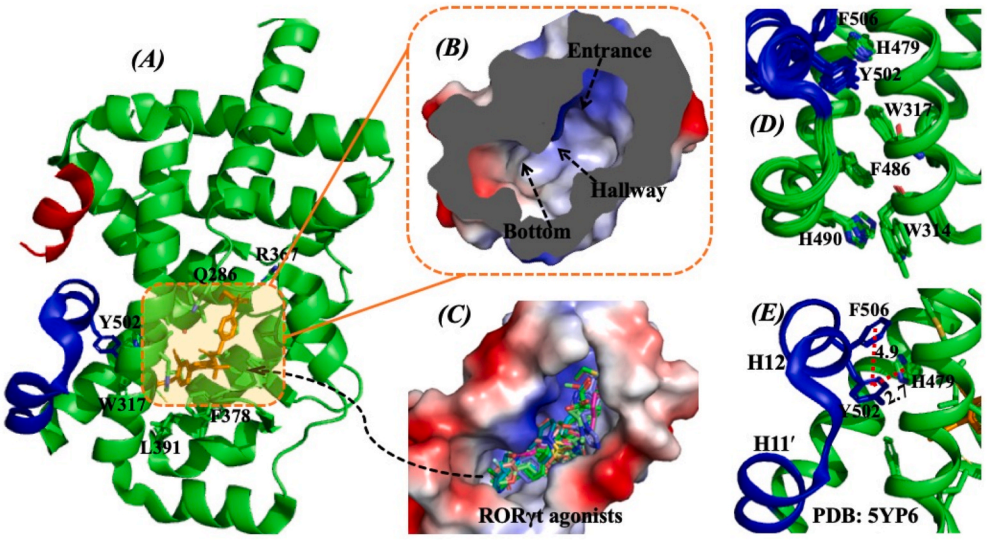

Fig. 3. Cartoon representation for orthosteric binding site at the RORγt LBD-agonists. (A) Colored ribbon for RORγt LBD showing residues important for agonist binding, rendered from x-ray structure (PDB code 5YP6). The recruited coactivator SRC2 is shown as red ribbon. (B) The molecular surface of the binding pocket was colored by the electrostatic potentials, and sliced through the pocket entrance. (C) Typical agonists bound inside the orthosteric pocket, overlapped from x-ray structures (PDB codes as: 3KYT, 4XT9, 4WPF, 5APH, 5IZ0, 5UFO, 5YP6, 6E3G, 6NWU). (D) A hydrophobic π-π cluster interactions were formed by F506, Y502, H479, W317, F486, H490 and W314 in the agonist-bound RORγt LBD structures. (E) Local representative for the enhanced triplet H479-Y502-F506 interactions (dashed lines with labeled distances in Å) and the stabilized helix H12 (PDB code 5YP6).

R367, with surrounding water molecules to form a network of electrostatic and hydrogen bonding interactions (Fig. 3A and B). As it goes deeper along the hydrophobic hallway of the pocket, the hydrophobic residues V361, M365, A368, V376, F377, F378, F388, L391 and I400 form intra-molecular hydrophobic packing and strong inter-molecular hydrophobic interactions with an agonist. At the bottom of the pocket, hydrophobic residues L324, M358, L362, L396 and W317 act as not only a host for the embrace of the coming agonist, but also as a π-π stacking paw with residues H479, F486, H490, Y502 and F506 from the surrounding helices H11, H11′ and H12 (Fig. 3A, B, 3D). Such hydrophobic π-π packing is pivotal for the local intra-molecular interactions, making the H11 tightly packing around H3 and H5. This hydrophobic packing also makes residue H479 properly orientated toward H12, leading to the hydrophilic and hydrogen bonding interactions with residue Y502 of helix H12 (Fig. 3D and E). Once the H479-Y502 pair interactions are formed, the helix H12 is pulled to a position to help residue Y502 packing with M358, it forms a protein binding site ready to recruit a coactivator SRC2 (Fig. 3E). It has been predicted that the triplet H479-Y502-F506 interactions contributed −12.90 kcal/mol energy to stabilize the local structures between H11, H11′, H12 and the neighboring helices [47]. His479-Tyr502-Phe506 cluster is thought to act as an agonist lock in RORγt, which is critical for the agonism and inverse agonism of RORγt binding in both the orthosteric site and the allosteric site [61].

Based on these general features of orthosteric binding site at the RORγt LBD structure, an agonist is expected to form hydrophilic/charged interactions at the entrance of the pocket, then fills the hydrophobic cavity along the hallway of the pocket. More importantly, the bound agonist is supposed to match very well with the packing paw at the bottom of the pocket, it enhances the hydrophobic cluster as shown in Fig. 3D, and greatly to stabilize the triplet H479-Y502-F506 interactions (Fig. 3D and E). As shown in Fig. 3, the reported typical

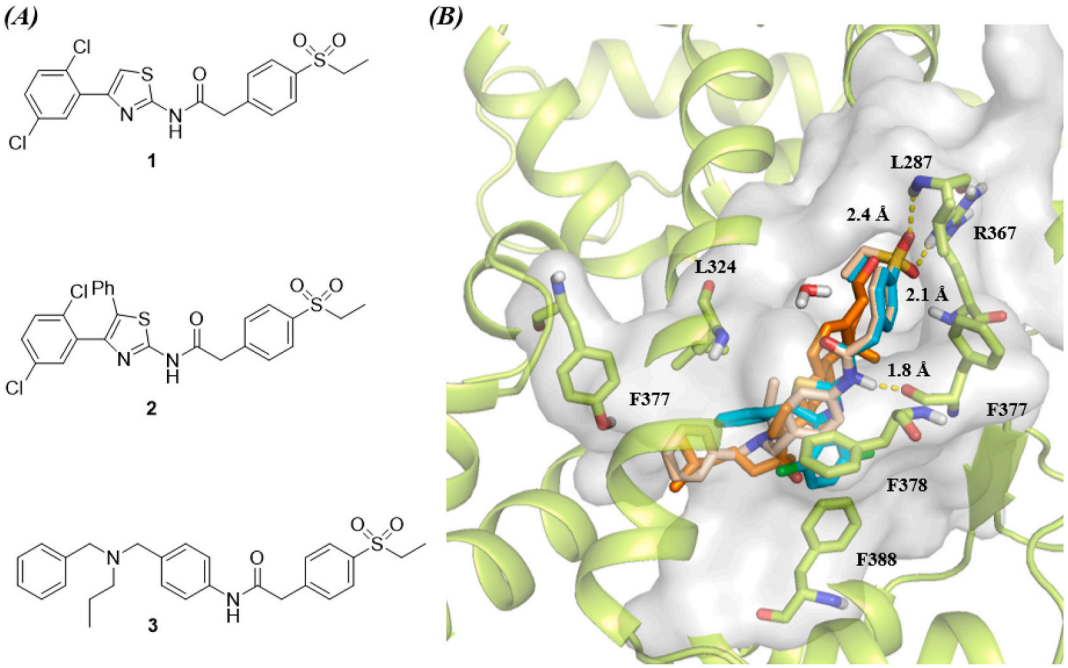

Fig. 4. Chemical structures of compounds **1**–**3** and the cocrystal structures with RORγt-LBD. (A) Structures of agonist **1**–**3**. (B) Structural overlay of **2** (PDB code 4XT9, in cyan stick), **3** (PDB code 4NIE, in wheat stick) and 20-hydroxycholesterol (PDB code 3KYT, in orange stick).

agonists [11,14,36,38–41,44,45] fit comfortably inside the orthosteric binding pocket (Figs. 2A and 3C), with minor adaptive movements of side-chain in residues H323, A324 and M365 around the pocket entrance of the receptor (Fig. 3A). The enhanced RORγt-dependent gene transcription after agonist binding is mostly due to the hydrogen bonding between the agonist and the residues around the pocket entrance, the hydrophobic stacking with the residues in hydrophobic hallway of pocket, it further stabilized triplet H479-Y502-F506 (Fig. 3A, D, 3E).

As an typical example, the thiazole amide RORγt agonists defines the binding mode of RORγt agonists. Starting from the novel RORγt inverse agonist hit (**1**) (Fig. 4A) (inhibitory pIC_{50} of 6 in a FRET assay), the substitution at 5-position of the thiazole ring with a bulky phenyl group led to the discovery of compound **2** (Fig. 4A), and compound **2** structurally occupies the hydrophobic pocket near AF2 domain (H12) and thus activates RORγt by stabilizing the AF2 domain toward steroid receptor co-activator (SRC) recruitment. Correspondingly, Compound **2** is transformed to be a partial agonist, which activates RORγt with a pEC_{50} of 5.5 in dual FRET assay and decreases much of maximum percentage of inhibition in FRET assay. Compounds **2** exhibits partial agonistic activity because its 5-phenyl moiety only partially occupies the hydrophobic pocket. The derivative compound **3** and the 20-hydroxycholesterol exhibit agonistic activity due to almost complete occupancy at the binding site (Fig. 4A and B). Besides, the hydrogen bonding interactions with R367 and π-π stacking interactions with L287, F377 and F388 enhance the binding of both compounds **2** and **3** with RORγt LBD (Fig. 4B) [11,37,61].

Molecular dynamics (MD) simulations [21,22,31] on the RORγt LBD revealed how the apo-state could be constitutively active and how an agonist could enhance the RORγt-dependent transcription activity. In the apo-state, the structure of RORγt LBD is dynamically stable, particularly the helix H12. Only the helix 2 around the entrance of the binding pocket, and the helix H11′ are flexible to some extent. At the time scale of ~500 ns, the bound agonist could form a quite stable hydrophobic cluster with residues H479 and W317, and a local hydrophobic network among helices H11, H11′ and H12. The H479-Y502 pair interaction survives 98.2% during the whole simulation, which is ever stronger than that in the apo-state structure with survival percentage of 85.2%. A recent reported [44] 500 ns MD simulations also confirmed that a bound agonist could stabilize the H479-Y502 hydrogen bonding. These MD simulation results [21,22,31,44] at atomic level helped pretty better understanding of mode of action for RORγt agonism and are consistent with the reported structural indications for the RORγt-dependent transcription activity [14,36,39,40,43,45].

3. Mode of action for inverse agonists at orthosteric site

As previous studies [4–7,9,13,14,16] showed that an inverse agonist of RORγt is more interesting and potent to repress high production of IL-17 and AR expression, thus could be used as direct treatment for some autoimmune diseases and cancers. Such pharmaceutical potential has been driving huge amounts of endeavor in designing novel and effective inverse agonist toward RORγt receptor [17,38–42,44,46–52,54,62–87]. Based on the above-described structural features of RORγt, an inverse agonist is supposed to be big enough to fill the binding pocket, and perturb the local packing paw with helices H11, H11′ and H12 (Fig. 3A). Most importantly, the inverse agonist should be able to weaken the hydrophobic cluster and destabilize the triplet H479-Y502-F506 interactions (Fig. 3D), of which is the point of Achilles heel for RORγt--dependent gene transcription activity. As we explored from these reported x-ray structures, the binding of inverse agonists at orthosteric site is featured in these different MOAs: (1) the binding of inverse agonist blocks the entrance of the agonist pocket in RORγt; (2) the inverse agonist directly breaks the H479-Y502 pair interactions; (3) the inverse agonist destabilizes the triplet H479-Y502-F506 interaction network through perturbing the conformation of the side chain in M358 at the bottom of the binding pocket; (4) and the inverse agonist destabilizes the H479-Y502-F506 triplet through changing the conformation of the side chain of residue W317 side chain.

Blocking the pocket entrance. As shown in Fig. 5, these inverse agonists [44,46,49–54,63–68] do not penetrate very deep into the binding pocket, so the H479-Y502-F506 triplet can still survive but is obviously weakened. On the contrary, these inverse agonists interact very tightly and extensively with residues around the pocket entrance through hydrogen bonding, electrostatic and local hydrophilic network involving water molecules (Fig. 5). Typical inter-molecular interactions include the hydrogen bonding with residues Q286, H323, E379 and S404; directly or water mediated bridged; electrostatic interactions with residues R364 and R367; and hydrophobic packing with residues M365 and F388 (Fig. 5C, D, 5E). After these inverse agonists sealed the pocket entrance (Fig. 5A), the natural ligands like 25-HC [36] is not able to competitively bind with the RORγt LBD any more. Therefore, the native constitutive activity of the receptor is stopped.

The above MOA can be exemplified by the complex structure of compound **4** [62] with RORγt. This compound has the inhibitory IC_{50} of 31 nM from a SRC recruitment assay (Fig. 6A). Structurally, the left-side terminal indane ring of compound **4** is unable to reach the depth of binding pocket and perturb the conformation of triplet H479-Y502-F506. Compound **4** blocks the pocket entrance through hydrogen bonding with F377, E379 and water molecules (Fig. 6B). The derivative compound 5 turned out to be able to fully occupy the binding pocket at the original position of ninhydrin, which stabilizes the triplet, and thus exhibited agonistic activity with a EC_{50} of 53 nM in a SRC recruitment assay (Fig. 6A).

Directly breaking the H479-Y502 pair interactions. The inverse agonists [41,47,69–72] go through the hydrophobic hall of the binding pocket using their slim hydrophobic group in the structure, move the side chain of M365 to a pretty flat conformation, and finally reach the bottom of the binding site in RORγt LBD (Fig. 7A, C, 7D). The tail group of these inverse agonists push away residue H479 side chain and direct intercalate into the H479-Y502-F506 triplet (Fig. 7B, C, 7D), even some inverse agonists gain a hydrogen bond with H479 side chain (Fig. 7D). Such binding mode disrupts the triplet interactions, and therefore, destabilize the constitutively active conformation of RORγt (Fig. 7B). Such structural perturbation is also confirmed in our recent MD simulations [22] on the inverse agonist bound RORγt LBD (PDB code: 5VB5). We have found that the H479 side chain is forced by the bound inverse agonist to turn away from Y502 side chain, helix H11 moves outwards about 5.1 Å, and H11' and H12 are structurally uncoiled.

Compound **6** (digoxin) is a traditional reverse agonist of RORyt (Fig. 8A). Digoxin could displace SRC3-1b co-activator peptides (IC_{50} of 1.8 μM) from the RORγt LBD and facilitate its interaction with co-repressor NCOR2 peptides (IC_{50} of 3.9 μM) [88]. Inside the binding pocket, digoxin maintains hydrogen bonds with R367, F377 and H479 or indirect hydrogen bonds with V361 and E379. Wherein digoxin induced the significantly conformational change of the side chain of H479 in helix 11 of RORyt through the formation of a hydrogen bonding interaction with H479. Thus, digoxin disrupted the hydrogen bonding and π-π interactions of the H479-Y502-F506 triplet and inactivated the protein (Fig. 8B).

Perturbing the conformation of M358. As observed from the x-ray structure of apo-state and agonist-bound RORγt LBD [47], residues M358 and W317 are very important to act as the packing paw from helices H5 and H3, with the helices H11, H11′ and H12 (Fig. 3A, D, 3E). Among the inter-helical packing, residue M358 interacts directly with H479 and F506, and provides a solid support for the bottom of the binding pocket (Figs. 3A and 9B). Perturbing such local interaction network would directly affect the formation of the H479-Y502-F506 triplet (Fig. 9), and therefore disrupt the H12 to take part in the formation of coactivator binding site [40,42,46,54,74–76]. A recently reported [40] x-ray structure of RORγt LBD bound with a synthetic inverse agonist revealed that residue M358 is obviously a trigger to induce

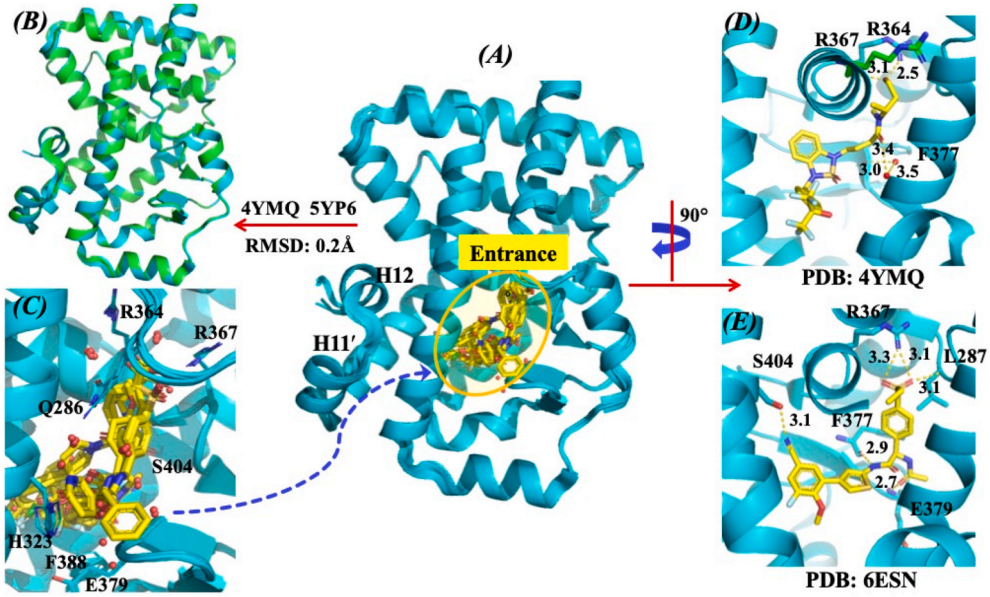

Fig. 5. Mechanism of RORγt inverse agonism through blocking the entrance of the binding pocket by a class of inverse agonists. (A) Typical binding structures of RORγt LBD with inverse agonists, retrieved from PDB codes 4YMQ, 5NI5, 5NI7, 5NI8, 6E3E, 6ESN, 6FGQ, 6R7A, 6R7J, 6R7K, 6P9F, 6U25, 6O3Z, 7JTW, 7JYM, 7KCO, 7KQJ, 7KXD, 7KXE, 7KXF, and 7LUK. The inverse agonists are shown as yellow sticks in the binding pocket. (B) Structural comparison between the RORγt LBD binding with an inverse agonist (PDB code 4YMQ, colored in cyan) and that of the same receptor binding with an agonist (PDB code 5YP6, green ribbon). The root-mean square deviation (RMSD) is only 0.2 Å, and the H12 is survived from the binding of the inverse agonist. (C) Local view of the inverse agonists blocking the pocket entrance, and the surrounding hydrophilic and charged residues of RORγt. Red spheres represent the water molecules from X-ray structures. (D) Atomic interactions between the RORγt LBD and the inverse agonist, derived from the x-ray structure (PDB code 4YMQ). Dashed lines represent the hydrogen bonds with labeled distances (Å). (E) Another example derived from the x-ray structure (PDB code 6ESN) showing details of inter-molecular interactions (with distances labeled in Å).

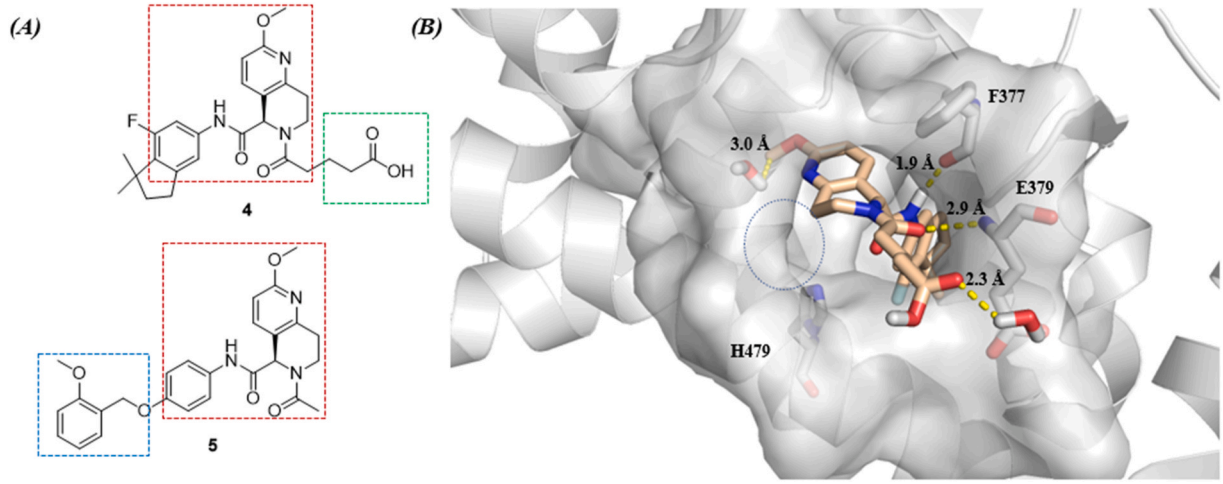

Fig. 6. Structures of compounds **4**–**5** and their cocrystal structures with RORγt-LBD. (A) Structures of inverse agonist **4** and agonist **5**. (B) Binding model of compound **4** to the RORγt LBD (PDB code 6E3E, in wheat stick). Protein structure and binding pocket are colored in gray and hydrogen bond is colored in yellow.

inverse agonism of the receptor (Fig. 9B). A specifically designed U-shaped inverse agonist [76] with a IC_{50} of 3.6 nM in a reporter gene assay pushed M358 outward from the bottom of the binding pocket (Fig. 9C), and destroyed the helical structures of H11′ and H12, although the inverse agonist maintains a hydrogen bond with the side chain of H479. As shown in Fig. 9B, after binding with an inverse agonist, the side chain of M358 is forced to change from *gauche*-to *trans*--conformation. The conformational change in M358 makes its side chain directly clash with the side chains of H479 and F506, and dramatically changes the orientation of H11 or even destroy the helical structures of H11 and H11' (Fig. 9A and B).

Interfering the conformation of W317. A series of x-ray structures of RORγt LBD reveal another mechanism of inverse agonism through interfering the conformation of the side chain of W317 [17,38,39,42,45,48,62,74,75,78–86]. These inverse agonists orientate inside the binding pocket in a way (Fig. 10) that is different to some extent from those inverse agonism through perturbing the side chain of M358 (Fig. 9). That is, taking more advantage of hydrophilic interactions with Q286, R364 and R367, but not E379; packing better with hydrophobic side chains of F378, F388, F401, C320 and L324 along the hydrophobic hall way; and pushing away the side chain of W317 from its *gauche*--conformation to mostly the *trans*-conformation (Fig. 10B). Meanwhile, due to the leftover space around M358, the side chains of H479 and L483 are pulled closer to the bottom of the binding pocket (Fig. 10B). Originally in the apo-state and agonist-bound RORγt LBD (Fig. 3D), the W317 is parallelly packed with the side chain of F486, and contacts head-to-head with the side chain of F498 [22,39,42,43]. This was very critical for the orientation of helices H11, H11′ and H12. When the side chain of W317 is pushed to *trans*-conformation by the binding of the inverse agonist (Fig. 10B), this residue comes in direct steric clash with the side chain of F498 at H11'. Such steric clash shifts the H11 up about 5.0 Å (Fig. 10C and D), and makes the helical structures of H11' and H12

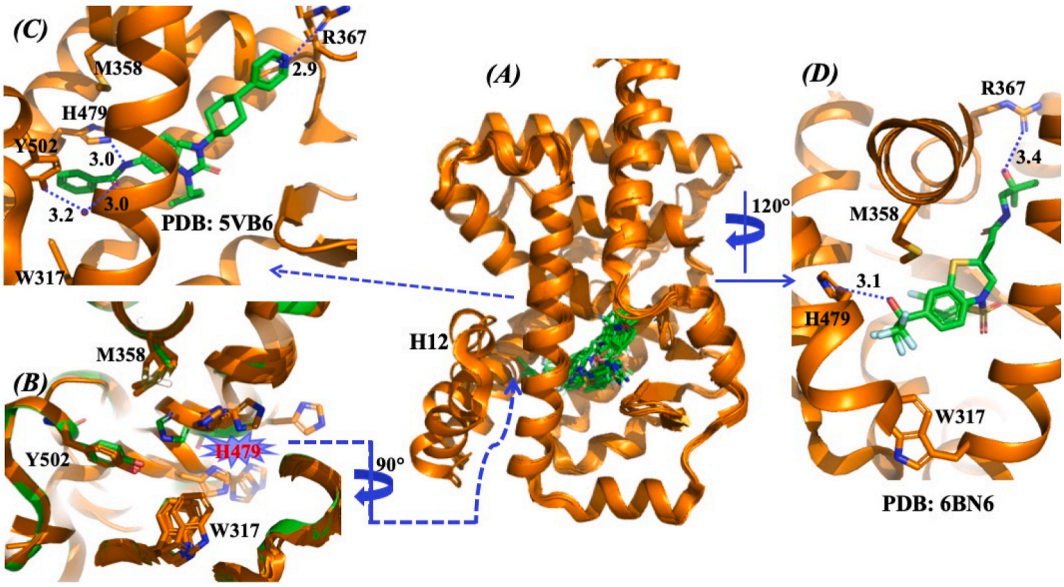

Fig. 7. Inverse agonists breaking the H479-Y502-F506 triplet interactions of RORγt. (A) X-ray binding structures of RORγt LBD with inverse agonists, the PDB codes are 5VB5, 5VB6, 5UFR, 5UHI, 6A22, 6BN6, 6NAD, and 6Q2W. These inverse agonists are shown in green sticks in the binding pocket. The helix H11 is shifted obviously, and the H12 is mostly distorted. (B) Comparison at the local structures of the H479-Y502-F506 triplet, M358 and W317 of these x-ray structures, with that of the same receptor binding with an agonist (PDB code 5YP6, green ribbon). The side chain of H479 is pushed away from the bottom of the binding pocket by the bound inverse agonists, although the M358 and W317 could maintain at almost the same positions. (C) Atomic interactions between the RORγt LBD and the inverse agonist, derived from the x-ray structure (PDB code 5VB6), of which the inverse agonist could gain a hydrogen bond with the H479 side chain. Dashed lines represent the hydrogen bonds with labeled distances (Å). (D) Typical example of the x-ray structure of RORγt LBD bound with an inverse agonist (PDB code 6BN6). The details of inter-molecular interactions include the hydrogen bonding of the inverse agonist with the H479 side chain with distances labeled in Å.

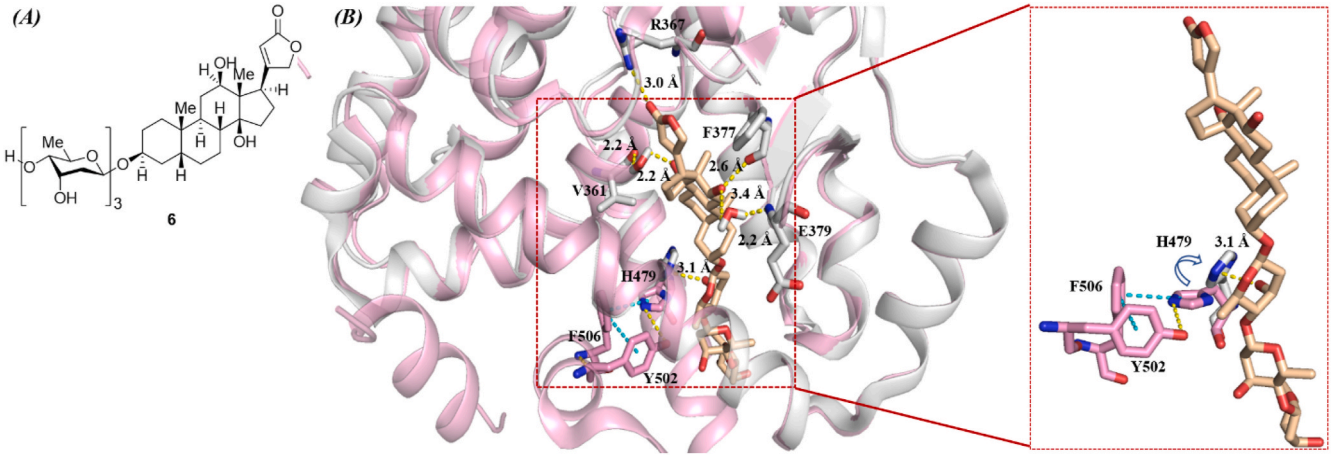

Fig. 8. Structure of compound **6** and its cocrystal structures with RORγt-LBD. (A) Structure of inverse agonist **6**. (B) Superposition of the RORγt LBDs in complex with **6** (PDB code 3B0W, compound 6 in wheat stick and protein in gray cartoon) and protein in the agonistic state (PDB code 3L0L, in pink cartoon). Hydrogen bonds are indicated by yellow dashed lines and π-π stacking is indicated by blue dashed lines.

severely disrupted or totally uncoiled (Fig. 10C and D) [38,77,79,82,84,85].

Among these discovered inverse agonists, the pyridin-substituted benzamide compound as revealed in PDB 6CN6 is able to shift ~110° in the conformation of the side chain of W317, and also maintains a hydrogen bond with the side chain of H479 in RORγt LBD, showing great potency (IC_{50} = 5.0 nM by TR-FRET cofactor recruitment assay) with RORγt receptor(Fig. 11A) [83]. A recently identified inverse agonist with thiazole skeleton was able to exceptionally tune the conformation of both the side chain of M358 and the side chain of W317 (Fig. 11B), showing high level affinity (K_d = 0.48 nM by ThermoFluor assay) with RORγt receptor [84]. One more typical example is a recently designed N-aryl imidazole compound as a class of potent RORγt inverse agonist (Fig. 11C) [85]. This class of compounds embed very deep inside the binding pocket, tune the side chain of W317 to the *trans*-conformation, and maintained a hydrogen bond with the side chain of H479 in RORγt LBD. Based on this mechanism, compound revealed in PDB 6Q7H shows inverse agonistic activity with a IC_{50} of 31 nM in a TR-FRET assay. All these three classes of compounds are evaluated to be potent, selective and bioavailable RORγt inverse agonists. In our recent study [21], we explored the mode of action of our newly discovered N-sulfonamide tetrahydroquinoline derivatives by performing ~100 ns MD simulations on the molecular-modeled RORγt LBD binding structures. Our MD results described dynamic process of how the binding of an inverse agonist could change the conformation of the side chain of W317, perturb the hydrophobic cluster around W317 (Figs. 3D and 10B), and how the helical structures of H11, H11′ and H12 got distorted and collapsed. We found that residue W317 did act as the inducer to destabilize the H11'

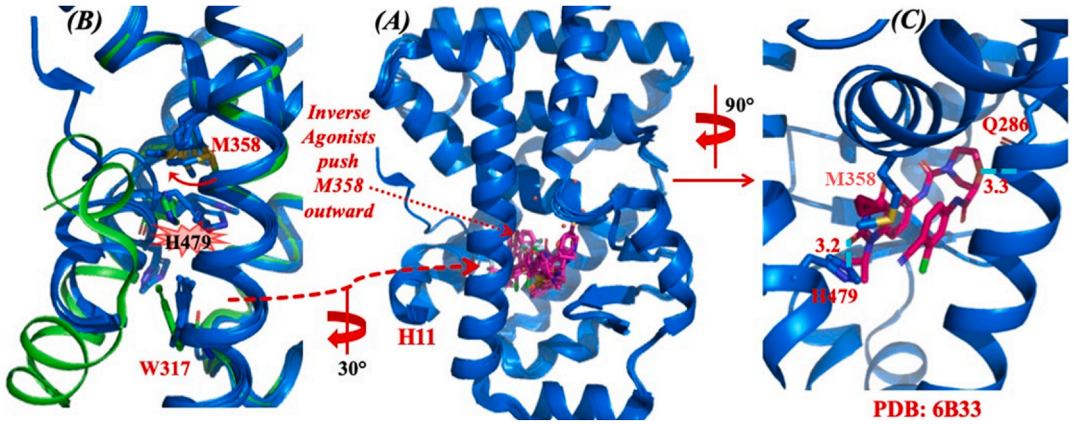

Fig. 9. RORγt inverse agonism by perturbing the M358 side chain. (A) X-ray structures of RORγt LBD binding with inverse agonists, retrieved from PDB codes as 4ZJW, 5ETH, 5IXK, 5NTQ, 5ZA1, and 6B33. These inverse agonists are shown in pink sticks in the binding pocket. The helices H11′ and H12 are obviously uncoiled. (B) These X-ray structures are compared with that of the same receptor binding with an agonist (PDB code 5YP6, green ribbon), at the local structures around residues W317, M358 and H479. The M358 side chain is pushed outward to *trans*-conformation. (C) Atomic interactions for the RORγt LBD and the inverse agonist at the binding pocket, this is derived from the x-ray structure (PDB code 6B33), of which the inverse agonist could maintain a hydrogen bond with the H479 side chain. Dashed lines represent the hydrogen bonds with labeled distances (Å).

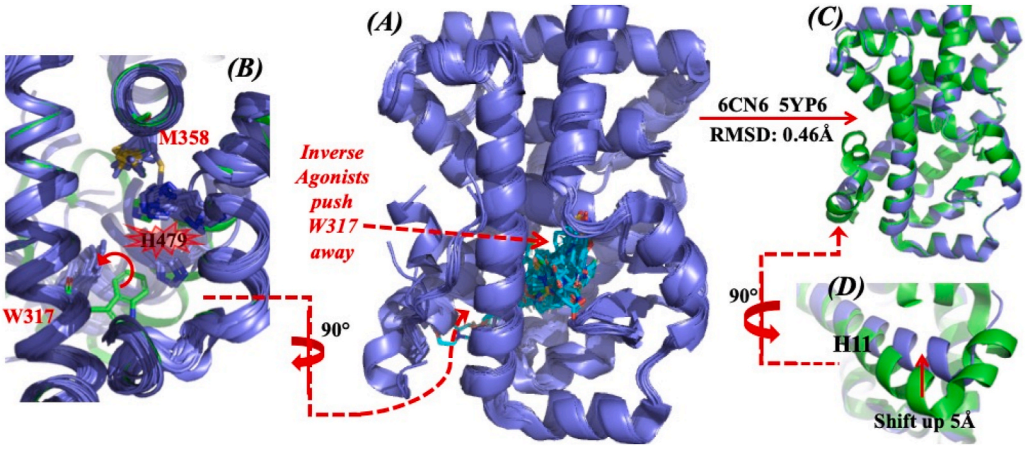

Fig. 10. RORγt inverse agonism by interfering the W317 side chain. (A) X-ray structures of RORγt LBD binding with inverse agonists from typical x-ray structures (PDB code: 3B0W, 4QM0, 4WQP, 4ZJR, 4ZOM, 5APK, 5AYG, 5M96, 5NTK, 5NTP, 5W4R, 5X8Q, 6BR2, 6BR3, 6CN5, 6CN6, 6CVH, 6IVX, 6J1L, 6NWT, 6Q6M, 6Q6O, 6Q7A, and 6Q7H). These inverse agonists are colored in cyan sticks inside the binding pocket. The helices H11′ and H12 are obviously uncoiled. (B) These X-ray structures are compared with that of the same receptor binding with an agonist (PDB code 5YP6, green ribbon), at the local structures around residues W317, M358 and H479. The W317 side chain is pushed outward to *trans*-conformation as the red arrow indicated. The H479 is pulled much in close the bottom of the binding pocket. (C) A typical RORγt LBD bound with an inverse agonist (PDB code: 6CN6, shown in purple ribbon), which lost the structure of H11′ and H12, is superimposed to the agonist-bound receptor (shown as green ribbon) with the geometric RMSD of 0.46 Å. (D) Local view of the helix H11 of the superimposed structures.

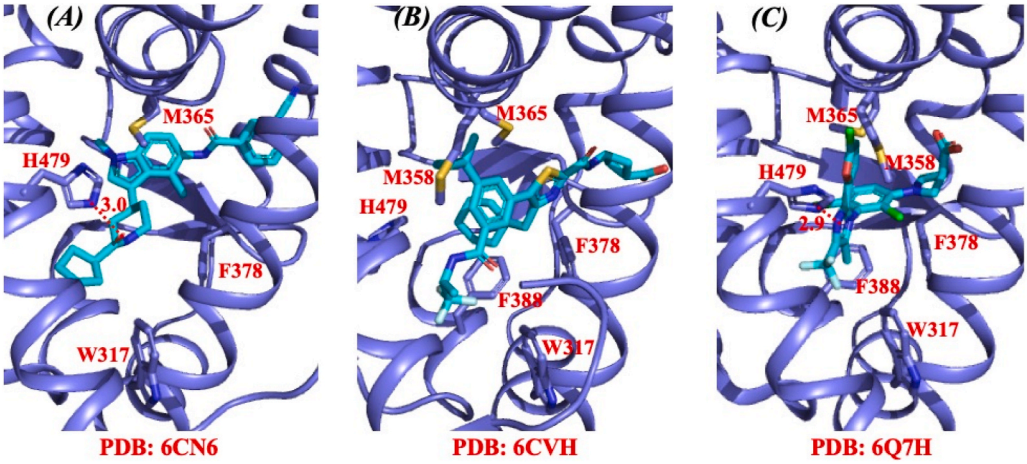

Fig. 11. Representative examples of inverse agonists tuning the conformation of W317 side chain to block the activity of RORγt-dependent gene transcription. Viewing after clock-wise 90° rotation relative to the position as shown in Fig. 10A small molecules are shown in cyan-colored stick style, and dashed lines for hydrogen bonds with labeled distances (Å). (A) The benzamide compound pushed W317 away but kept hydrogen bonding with H479 side chain. (B) The thiazole compound pushed away side chains of both W317 and M358. (C) The smaller imidazole compound binds similarly as that of the benzamide compound, also hydrogen bonded with H479.

and H12, and deactivate RORγt receptor. These MD insights have reasonably explained the observed different conformations of W317 in the reported x-ray structures as discussed above, and demonstrated their inverse agonism through the tuning the side chain of W317 in RORγt.

4. Mode of action for inverse agonists at allosteric site

While great efforts were spent on the design of inverse agonists binding at the orthosteric binding pocket at RORγt LBD, a new type of inverse agonists were discovered to bind at the allosteric site (Figs. 2B and 12) [55–60,87], which occupies the site originally for the coactivator in the apo-state and active RORγt receptor (Figs. 2A and 3A). This site is formed by the helices H4, H5, H11 and H12. The bound inverse agonists interact directly with H12, resulting in unfolding of helix 11′ and orientation of H12 to directly block cofactor peptide binding (Fig. 12A, B and 12D). As this conceptually new binding site is not overlapped with the orthosteric site (Fig. 12D), the obvious advantage of such inverse agonism is that the inverse agonists could more effectively inhibit the RORγt-dependent gene transcription. Such inhibition is also independent on the concentration of natural ligands of RORγt like cholesterol or 25-HC, as such type of inverse agonists do not compete with these previous found agonists in the orthosteric binding pocket [11,37,39,40,44,45,87]. On the contrary, the binding at these two sites is structurally cooperative as revealed in a recent co-crystallization and MD simulations study [87]. It demonstrate that the binding of an agonist at the orthosteric site helps to stabilize the allosteric site, and thus enhances the affinity of the bound inverse agonist at the allosteric site.

As depicted in Figs. 12C and 13, these inverse agonists could fit very well inside this small allosteric binding site, form hydrophobic interactions with side chains of W317, I328, M358, L501, Y502, L505, and F506, while the H479 side chain is pushed down to the bottom of the orthosteric pocket of RORγt LBD [55,57,59]. The polar groups of these inverse agonists could form hydrogen bonds with the backbone –NH group of residues of A497 and F498, the side chain of Q329, and strong hydrophilic interactions with Q484. Among them the compound **MRL-871** as revealed in PDB 4YPQ, which acts as the first allosteric inhibitor identified, have IC_{50} values of 7 nM in a cofactor peptide recruitment assay [55]. Then Glenmark Pharmaceuticals used the **MRL-871** core as the basis for an in silico scaffold hopping screen and contributed to the development of a series of structures based on thienopyrazole scaffold. The compound as revealed in PDB 6TLM shows the highest potency with IC_{50} of 425 ± 61 nM in a TR-FRET coactivator recruitment assay. In our MD simulations [22] on the PDB 5C4O system [55], we found that the H11′ acts as a long arch connecting H11 and H12, and H12 could survive well as a regular helix during the whole simulation time of 100 ns. We also found that the most flexible components in the 5C4O system are the helix H2 and the neighboring β-sheets at the entrance area of the orthosteric binding pocket of the receptor [22].

5. Conclusion and perspective

RORγt plays an important role in multiple inflammatory and immunological pathways, the desire to discover small molecules targeting the RORγt has been intensely pursued in the past decades.

Based on the published x-ray structures, we explored the binding details of RORγt with a series of small molecules. For sure, the MOA insights from these structures have been driving the novel design and optimization of hundreds of highly potent agonists and inverse agonists. The results from computational studies using the tool as molecular dynamics simulations also provided further understanding of the binding features of RORγt with ligands.

Among the discovered hundreds of highly potent ligands, some of which have become excellent clinical drugs such as pyrazinone derivative BI 730357 [28]. However, it is really very challenging to transform the potent lead compounds through multiple pharmaceutical tests of both *in vitro* and *in vivo*, into clinically useful agents. Currently, drug discovery and development targeting RORγt needs new crues and directions. For this purpose, we think that the MOA insights together with the artificial intelligence (machine learning and deep learning) aided drug design [35] will definitely lend a helping hand.

Associated content

The Supporting Information is available free of charge on the ACS Publications website. Also provided are the properties and druglikeness

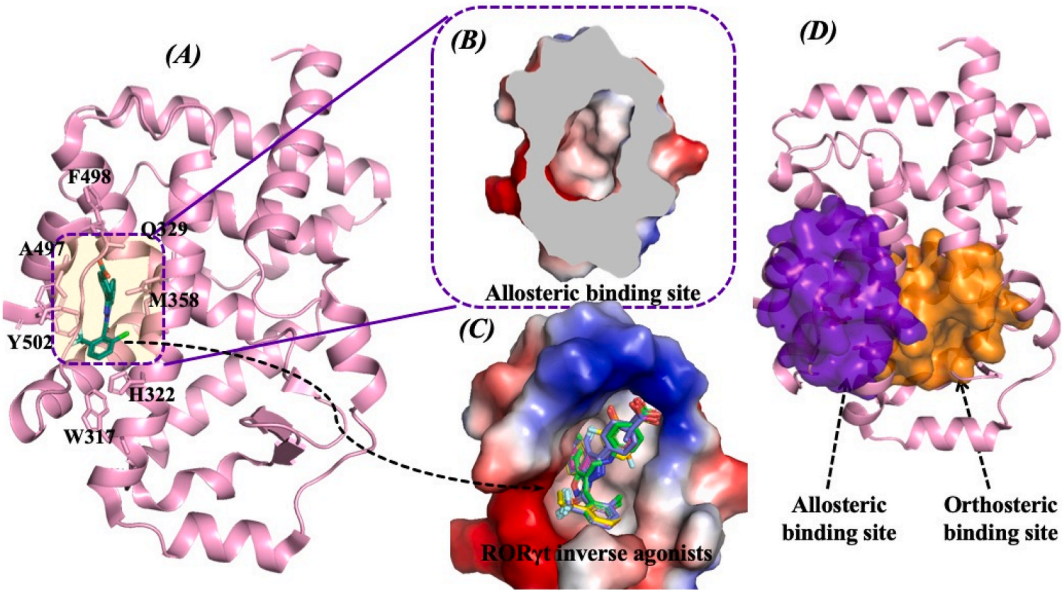

Fig. 12. Representation for allosteric binding site at the RORγt LBD and its binding with inverse agonists. (A) RORγt LBD is shown in colored ribbon, with important residues for inverse agonist binding, rendered from x-ray structure (PDB code 6SAL). (B) The binding site shown as molecular surface and sliced through the top entrance. (C) Reported inverse agonists bound inside the allosteric site, the binding site is shown as molecular surface and colored in electrostatic potentials with red for negative and blue for positive. The small molecules are superimposed from x-ray structures (PDB codes: 4YPQ, 5C4O, 5C4S, 5C4T, 5C4U, 5LWP, 6SAL, 6TLM, 6UCG, 7NEC, 7NP5 and 7NP6). (D) Relative positioning of the allosteric binding site to the orthosteric binding pocket, shown in differently colored molecule surface.

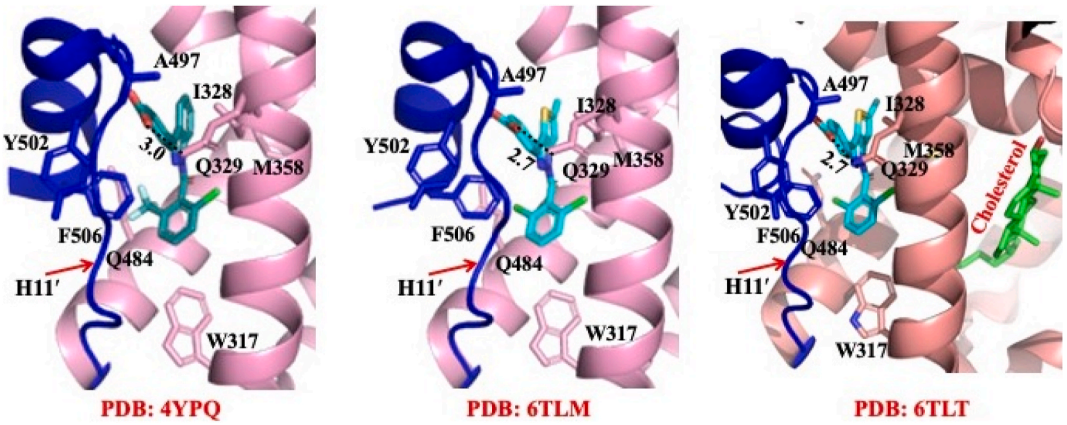

Fig. 13. Typical examples of inverse agonists binding at the allosteric site to stop the activity of RORγt-dependent gene transcription. Viewing similarly as that of Fig. 12A. Uncoiled helix 11 and helix 12 are colored in blue ribbon. The dashed lines indicate hydrogen bonds with labeled distances (Å). (A) The binding structure derived from the PDB code 4YPQ. (B) The complex structure from the PDB 6TLM as another example of the inverse agonist binding at the allosteric site. (C) The complex structure from the PDB 6TLT as an example of the cooperative binding of the inverse agonist at the allosteric site, and a natural agonist like cholesterol at the orthosteric site (similar bindings in other PDBs as 6T4G, 6T4I, 6T4J, 6T4K, 6T4T, 6T4U, 6T4W, 6T4X, 6T4Y, 6T50, 6TLQ).

data of the RORγt-compounds including Tables S1–S17.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2022.115039.

Abbreviations used

LBD	ligand-binding domain
MOA	modes of action
ML	machine learning
DL	deep learning
NRs	Nuclear receptors
RORγt	retinoic acid receptor-related orphan receptor γt
RORE	retinoid-related orphan receptor response element
Th	T-helper
RA	rheumatoid arthritis
MS	multiple sclerosis
A/B	amino-terminal domain
DBD	DNA-binding domain
H12	helix 12
SRC2	steroid receptor coactivator 2
25-HC	25-hydroxycholesterol
RMSD	root-mean square deviation
PD	pharmacodynamic
PK	pharmacokinetic
SAD	single ascending dose
SAR	structure-activity relationship;
DNN	deep neural networks
gTPP	generic Target Product Profile.

References

[1] T.P. Burris, S.A. Busby, P.R. Griffin, Targeting orphan nuclear receptors for treatment of metabolic diseases and autoimmunity, Chem. Biol. 19 (2012) 51–59.
[2] J.S. Lee, D.J. Cua, The emerging landscape of RORγt biology, Immunity 40 (2014) 451–452.
[3] Ivanov II, B.S. McKenzie, L. Zhou, C.E. Tadokoro, A. Lepelley, J.J. Lafaille, D. J. Cua, D.R. Littman, The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells, Cell 126 (2006) 1121–1133.
[4] F. Isono, S. Fujita-Sato, S. Ito, Inhibiting RORγt/Th17 axis for autoimmune disorders, Drug Discov. Today 19 (2014) 1205–1211.
[5] S. Rutz, C. Eidenschenk, J.R. Kiefer, W. Ouyang, Post-translational regulation of RORγt-A therapeutic target for the modulation of interleukin-17-mediated responses in autoimmune diseases, Cytokine Growth Factor Rev. 30 (2016) 1–17.
[6] M. Matsuyama, Y. Ishii, H. Sakurai, S. Ano, Y. Morishima, K. Yoh, S. Takahashi, K. Ogawa, N. Hizawa, Overexpression of RORγt enhances pulmonary inflammation after infection with Mycobacterium Avium, PLoS One 11 (2016), e0147064.
[7] A.M. Jetten, Y. Takeda, A. Slominski, H.S. Kang, Retinoic acid-related Orphan Receptor γ (RORγ): connecting sterol metabolism to regulation of the immune system and autoimmune disease, Curr. Opin. Toxicol. 8 (2018) 66–80.
[8] R. Qiu, Y. Wang, Retinoic acid receptor-related orphan receptor γt (RORγt) agonists as potential small molecule therapeutics for cancer immunotherapy, J. Med. Chem. 61 (2018) 5794–5804.
[9] C. Gege, T. Schlüter, T. Hoffmann, Identification of the first inverse agonist of retinoid-related orphan receptor (ROR) with dual selectivity for RORβ and RORγt, Bioorg. Med. Chem. Lett 24 (2014) 5265–5267.
[10] Y. Wang, W. Cai, G. Zhang, T. Yang, Q. Liu, Y. Cheng, L. Zhou, Y. Ma, Z. Cheng, S. Lu, Y.G. Zhao, W. Zhang, Z. Xiang, S. Wang, L. Yang, Q. Wu, L.A. Orband-Miller, Y. Xu, J. Zhang, R. Gao, M. Huxdorf, J.N. Xiang, Z. Zhong, J.D. Elliott, S. Leung, X. Lin, Discovery of novel N-(5-(arylcarbonyl)thiazol-2-yl)amides and N-(5-(arylcarbonyl)thiophen-2-yl)amides as potent RORγt inhibitors, Bioorg. Med. Chem. 22 (2014) 692–702.
[11] Y. Wang, T. Yang, Q. Liu, Y. Ma, L. Yang, L. Zhou, Z. Xiang, Z. Cheng, S. Lu, L. A. Orband-Miller, W. Zhang, Q. Wu, K. Zhang, Y. Li, J.N. Xiang, J.D. Elliott, S. Leung, F. Ren, X. Lin, Discovery of N-(4-aryl-5-aryloxy-thiazol-2-yl)-amides as potent RORγt inverse agonists, Bioorg. Med. Chem. 23 (2015) 5293–5302.
[12] Y. Wang, W. Cai, Y. Cheng, T. Yang, Q. Liu, G. Zhang, Q. Meng, F. Han, Y. Huang, L. Zhou, Z. Xiang, Y.G. Zhao, Y. Xu, Z. Cheng, S. Lu, Q. Wu, J.N. Xiang, J.D. Elliott, S. Leung, F. Ren, X. Lin, Discovery of biaryl amides as potent, orally bioavailable, and CNS penetrant RORγt inhibitors, ACS Med. Chem. Lett. 6 (2015) 787–792.
[13] A.F. Abdel-Magid, RORc modulators for the treatment of autoimmune diseases, ACS Med. Chem. Lett. 6 (2015) 958–960.
[14] F.R. Santori, P. Huang, S.A. van de Pavert, E.F. Douglass Jr., D.J. Leaver, B. A. Haubrich, R. Keber, G. Lorbek, T. Konijn, B.N. Rosales, D. Rozman, S. Horvat, A. Rahier, R.E. Mebius, F. Rastinejad, W.D. Nes, D.R. Littman, Identification of natural RORγ ligands that regulate the development of lymphoid cells, Cell Metabol. 21 (2015) 286–298.
[15] Y. Huang, M. Yu, N. Sun, T. Tang, F. Yu, X. Song, Q. Xie, W. Fu, L. Shao, Y. Wang, Discovery of carbazole carboxamides as novel RORγt inverse agonists, Eur. J. Med. Chem. 148 (2018) 465–476.

[16] J. Wang, J.X. Zou, X. Xue, D. Cai, Y. Zhang, Z. Duan, Q. Xiang, J.C. Yang, M. C. Louie, A.D. Borowsky, A.C. Gao, C.P. Evans, K.S. Lam, J. Xu, H.J. Kung, R. M. Evans, Y. Xu, H.W. Chen, ROR-γ drives androgen receptor expression and represents a therapeutic target in castration-resistant prostate cancer, Nat. Med. 22 (2016) 488–496.
[17] Y. Zhang, X. Wu, X. Xue, C. Li, J. Wang, R. Wang, C. Zhang, C. Wang, Y. Shi, L. Zou, Q. Li, Z. Huang, X. Hao, K. Loomes, D. Wu, H.W. Chen, J. Xu, Y. Xu, Discovery and characterization of XY101, a potent, selective, and orally bioavailable RORγ inverse agonist for treatment of castration-resistant prostate cancer, J. Med. Chem. 62 (2019) 4716–4730.
[18] J. Zheng, J. Wang, Q. Wang, H. Zou, H. Wang, Z. Zhang, J. Chen, Q. Wang, P. Wang, Y. Zhao, J. Lu, X. Zhang, S. Xiang, H. Wang, J. Lei, H.W. Chen, P. Liu, Y. Liu, F. Han, J. Wang, Targeting castration-resistant prostate cancer with a novel RORγ antagonist elaiophylin, Acta Pharm. Sin. B 10 (2020) 2313–2322.
[19] X. Zhang, Z. Huang, J. Wang, Z. Ma, J. Yang, E. Corey, C.P. Evans, A.M. Yu, H. W. Chen, Targeting feedforward loops formed by nuclear receptor RORγ and kinase PBK in mCRPC with hyperactive AR signaling, Cancers 13 (2021) 1672.
[20] N.K. Lytle, L.P. Ferguson, N. Rajbhandari, K. Gilroy, R.G. Fox, A. Deshpande, C. M. Schürch, M. Hamilton, N. Robertson, W. Lin, P. Noel, M. Wartenberg, I. Zlobec, M. Eichmann, J.A. Galván, E. Karamitopoulou, T. Gilderman, L.A. Esparza, Y. Shima, P. Spahn, R. French, N.E. Lewis, K.M. Fisch, R. Sasik, S.B. Rosenthal, M. Kritzik, D. Von Hoff, H. Han, T. Ideker, A.J. Deshpande, A.M. Lowy, P.D. Adams, T. Reya, A multiscale map of the stem cell state in pancreatic adenocarcinoma, Cell 177 (2019) 572–586, e522.
[21] N. Sun, C. Yuan, X. Ma, Y. Wang, X. Gu, W. Fu, Molecular mechanism of action of RORγt agonists and inverse agonists: insights from molecular dynamics simulation, Molecules 23 (2018) 3181.
[22] C.M. Yuan, H.H. Chen, N.N. Sun, X.J. Ma, J. Xu, W. Fu, Molecular dynamics simulations on RORγt: insights into its functional agonism and inverse agonism, Acta Pharmacol. Sin. 40 (2019) 1480–1489.
[23] J. Tian, N. Sun, M. Yu, X. Gu, Q. Xie, L. Shao, J. Liu, L. Liu, Y. Wang, Discovery of N-indanyl benzamides as potent RORγt inverse agonists, Eur. J. Med. Chem. 167 (2019) 37–48.
[24] N. Sun, H. Guo, Y. Wang, Retinoic acid receptor-related orphan receptor gamma-t (RORγt) inhibitors in clinical development for the treatment of autoimmune diseases: a patent review (2016-present), Expert Opin. Ther. Pat. 29 (2019) 663–674.
[25] Y. Zhu, N. Sun, M. Yu, H. Guo, Q. Xie, Y. Wang, Discovery of aryl-substituted indole and indoline derivatives as RORγt agonists, Eur. J. Med. Chem. 182 (2019), 111589.
[26] N. Sun, X. Ma, K. Zhou, C. Zhu, Z. Cao, Y. Wang, J. Xu, W. Fu, Discovery of novel N-sulfonamide-tetrahydroquinolines as potent retinoic acid receptor-related orphan receptor γt inverse agonists for the treatment of autoimmune diseases, Eur. J. Med. Chem. 187 (2020), 111984.
[27] F.A. Meijer, G.J.M. Oerlemans, L. Brunsveld, Orthosteric and allosteric dual targeting of the nuclear receptor RORγt with a bitopic ligand, ACS Chem. Biol. 16 (2021) 510–519.
[28] C. Harcken, J. Csengery, M. Turner, L. Wu, S. Liang, R. Sibley, S. Brunette, M. Labadia, K. Hoyt, A. Wayne, T. Wieckowski, G. Davis, M. Panzenbeck, D. Souza, S. Kugler, D. Terenzio, D. Collin, D. Smith, R.M. Fryer, Y.C. Tseng, J.P. Hehn, K. Fletcher, R.O. Hughes, Discovery of a series of pyrazinone RORγ antagonists and identification of the clinical candidate BI 730357, ACS Med. Chem. Lett. 12 (2021) 143–154.
[29] F.A. Meijer, M.C.M. van den Oetelaar, R.G. Doveston, E.N.R. Sampers, L. Brunsveld, Covalent occlusion of the RORγt ligand binding pocket allows unambiguous targeting of an allosteric site, ACS Med. Chem. Lett. 12 (2021) 631–639.
[30] Y. Xia, M. Yu, Y. Zhao, L. Xia, Y. Huang, N. Sun, M. Song, H. Guo, Y. Zhang, D. Zhu, Q. Xie, Y. Wang, Discovery of tetrahydroquinolines and benzomorpholines as novel potent RORγt agonists, Eur. J. Med. Chem. 211 (2021), 113013.
[31] X. Ma, N. Sun, X. Li, W. Fu, Discovery of novel N-sulfonamide-tetrahydroisoquinolines as potent retinoic acid receptor-related orphan receptor γt agonists, Eur. J. Med. Chem. 222 (2021), 113585.
[32] X. Xue, A. De Leon-Tabaldo, R. Luna-Roman, G. Castro, M. Albers, F. Schoetens, S. DePrimo, D. Devineni, T. Wilde, S. Goldberg, T. Hoffmann, A.M. Fourie, R. L. Thurmond, Preclinical and clinical characterization of the RORγt inhibitor JNJ-61803534, Sci. Rep. 11 (2021), 11066.
[33] X. Wu, H. Shen, Y. Zhang, C. Wang, Q. Li, C. Zhang, X. Zhuang, C. Li, Y. Shi, Y. Xing, Q. Xiang, J. Xu, D. Wu, J. Liu, Y. Xu, Discovery and characterization of benzimidazole derivative XY123 as a potent, selective, and orally available RORγ inverse agonist, J. Med. Chem. 64 (2021) 8775–8797.
[34] S. Liu, D. Liu, R. Shen, D. Li, Q. Hu, Y. Yan, J. Sun, F. Zhang, H. Wan, P. Dong, J. Feng, R. Zhang, J. Li, L. Zhang, W. Tao, Discovery of a novel RORγ antagonist with skin-restricted exposure for topical treatment of mild to moderate psoriasis, Sci. Rep. 11 (2021) 9132.
[35] M. Moret, M. Helmstädter, F. Grisoni, G. Schneider, D. Merk, Beam search for automated design and scoring of novel ROR ligands with machine intelligence, Angew Chem. Int. Ed. Engl. 60 (2021) 19477–19482.
[36] L. Jin, D. Martynowski, S. Zheng, T. Wada, W. Xie, Y. Li, Structural basis for hydroxycholesterols as natural ligands of orphan nuclear receptor RORgamma, Mol. Endocrinol. 24 (2010) 923–929.
[37] T. Yang, Q. Liu, Y. Cheng, W. Cai, Y. Ma, L. Yang, Q. Wu, L.A. Orband-Miller, L. Zhou, Z. Xiang, M. Huxdorf, W. Zhang, J. Zhang, J.N. Xiang, S. Leung, Y. Qiu, Z. Zhong, J.D. Elliott, X. Lin, Y. Wang, Discovery of tertiary amine and indole derivatives as potent RORγt inverse agonists, ACS Med. Chem. Lett. 5 (2014) 65–68.
[38] O. René, B.P. Fauber, L. Boenig Gde, B. Burton, C. Eidenschenk, C. Everett, A. Gobbi, S.G. Hymowitz, A.R. Johnson, J.R. Kiefer, M. Liimatta, P. Lockey, M. Norman, W. Ouyang, H.A. Wallweber, H. Wong, Minor structural change to tertiary sulfonamide RORc ligands led to opposite mechanisms of action, ACS Med. Chem. Lett. 6 (2015) 276–281.
[39] R.I. Olsson, Y. Xue, S. von Berg, A. Aagaard, J. McPheat, E.L. Hansson, J. Bernström, P. Hansson, J. Jirholt, H. Grindebacke, A. Leffler, R. Chen, Y. Xiong, H. Ge, T.G. Hansson, F. Narjes, Benzoxazepines achieve potent suppression of IL-17 release in human T-helper 17 (TH 17) cells through an induced-fit binding mode to the nuclear receptor RORγ, ChemMedChem 11 (2016) 207–216.
[40] D.J. Marcotte, Y. Liu, K. Little, J.H. Jones, N.A. Powell, C.P. Wildes, L.F. Silvian, J. V. Chodaparambil, Structural determinant for inducing RORgamma specific inverse agonism triggered by a synthetic benzoxazinone ligand, BMC Struct. Biol. 16 (2016) 7.
[41] D.A. Kummer, M.D. Cummings, M. Abad, J. Barbay, G. Castro, R. Wolin, K. D. Kreutter, U. Maharoof, C. Milligan, R. Nishimura, J. Pierce, C. Schalk-Hihi, J. Spurlino, M. Urbanski, H. Venkatesan, A. Wang, C. Woods, X. Xue, J.P. Edwards, A.M. Fourie, K. Leonard, Identification and structure activity relationships of quinoline tertiary alcohol modulators of RORγt, Bioorg. Med. Chem. Lett 27 (2017) 2047–2057.
[42] J. Kallen, A. Izaac, C. Be, L. Arista, D. Orain, K. Kaupmann, C. Guntermann, K. Hoegenauer, S. Hintermann, Structural states of RORγt: X-ray elucidation of molecular mechanisms and binding interactions for natural and synthetic compounds, ChemMedChem 12 (2017) 1014–1021.
[43] Y. Wang, W. Cai, T. Tang, Q. Liu, T. Yang, L. Yang, Y. Ma, G. Zhang, Y. Huang, X. Song, L.A. Orband-Miller, Q. Wu, L. Zhou, Z. Xiang, J.N. Xiang, S. Leung, L. Shao, X. Lin, M. Lobera, F. Ren, From RORγt agonist to two types of RORγt inverse agonists, ACS Med. Chem. Lett. 9 (2018) 120–124.
[44] T. Yukawa, Y. Nara, M. Kono, A. Sato, T. Oda, T. Takagi, T. Sato, Y. Banno, N. Taya, T. Imada, Z. Shiokawa, N. Negoro, T. Kawamoto, R. Koyama, N. Uchiyama, R. Skene, I. Hoffman, C.H. Chen, B. Sang, G. Snell, R. Katsuyama, S. Yamamoto, J. Shirai, Design, synthesis, and biological evaluation of retinoic acid-related orphan receptor γt (RORγt) agonist structure-based functionality switching approach from in house RORγt inverse agonist to RORγt agonist, J. Med. Chem. 62 (2019) 1167–1179.
[45] T.S. Strutzenberg, R.D. Garcia-Ordonez, S.J. Novick, H. Park, M.R. Chang, C. Doebellin, Y. He, R. Patouret, T.M. Kamenecka, P.R. Griffin, HDX-MS reveals structural determinants for RORγ hyperactivation by synthetic agonists, Elife 8 (2019), e47172.
[46] I.J. Enyedy, N.A. Powell, J. Caravella, K. van Vloten, J. Chao, D. Banerjee, D. Marcotte, L. Silvian, A. McKenzie, V.S. Hong, J.D. Fontenot, Discovery of biaryls as RORγ inverse agonists by using structure-based design, Bioorg. Med. Chem. Lett 26 (2016) 2459–2463.
[47] X. Li, M. Anderson, D. Collin, I. Muegge, J. Wan, D. Brennan, S. Kugler, D. Terenzio, C. Kennedy, S. Lin, M.E. Labadia, B. Cook, R. Hughes, N.A. Farrow, Structural studies unravel the active conformation of apo RORγt nuclear receptor and a common inverse agonism of two diverse classes of RORγt inhibitors, J. Biol. Chem. 292 (2017) 11618–11630.
[48] J.K. Barbay, M.D. Cummings, M. Abad, G. Castro, K.D. Kreutter, D.A. Kummer, U. Maharoof, C. Milligan, R. Nishimura, J. Pierce, C. Schalk-Hihi, J. Spurlino, V. M. Tanis, M. Urbanski, H. Venkatesan, A. Wang, C. Woods, R. Wolin, X. Xue, J. P. Edwards, A.M. Fourie, K. Leonard, 6-Substituted quinolines as RORγt inverse agonists, Bioorg. Med. Chem. Lett 27 (2017) 5277–5283.
[49] F. Narjes, Y. Xue, S. von Berg, J. Malmberg, A. Llinas, R.I. Olsson, J. Jirholt, H. Grindebacke, A. Leffler, N. Hossain, M. Lepistö, L. Thunberg, H. Leek, A. Aagaard, J. McPheat, E.L. Hansson, E. Bäck, S. Tångefjord, R. Chen, Y. Xiong, G. Hongbin, T.G. Hansson, Potent and orally bioavailable inverse agonists of RORγt resulting from structure-based design, J. Med. Chem. 61 (2018) 7796–7813.
[50] S. von Berg, Y. Xue, M. Collins, A. Llinas, R.I. Olsson, T. Halvarsson, M. Lindskog, J. Malmberg, J. Jirholt, N. Krutrök, M. Ramnegård, M. Bränström, A. Lundqvist, M. Lepistö, A. Aagaard, J. McPheat, E.L. Hansson, R. Chen, Y. Xiong, T.G. Hansson, F. Narjes, Discovery of potent and orally bioavailable inverse agonists of the retinoic acid receptor-related orphan receptor C2, ACS Med. Chem. Lett. 10 (2019) 972–977.
[51] Z. Lu, J.J. Duan, H. Xiao, J. Neels, D.R. Wu, C.A. Weigelt, J.S. Sack, J. Khan, M. Ruzanov, Y. An, M. Yarde, A. Karmakar, S. Vishwakrishnan, V. Baratam, H. Shankarappa, S. Vanteru, V. Babu, M. Basha, A. Kumar Gupta, S. Kumaravel, A. Mathur, Q. Zhao, L.M. Salter-Cid, P.H. Carter, T.G. Murali Dhar, Identification of potent, selective and orally bioavailable phenyl ((R)-3-phenylpyrrolidin-3-yl) sulfone analogues as RORγt inverse agonists, Bioorg. Med. Chem. Lett 29 (2019) 2265–2269.
[52] D. Marcoux, J.J. Duan, Q. Shi, R.J. Cherney, A.S. Srivastava, L. Cornelius, D. G. Batt, Q. Liu, M. Beaudoin-Bertrand, C.A. Weigelt, P. Khandelwal, S. Vishwakrishnan, K. Selvakumar, A. Karmakar, A.K. Gupta, M. Basha, S. Ramlingam, N. Manjunath, S. Vanteru, S. Karmakar, N. Maddala, M. Vetrichelvan, A. Gupta, R.A. Rampulla, A. Mathur, S. Yip, P. Li, D.R. Wu, J. Khan, M. Ruzanov, J.S. Sack, J. Wang, M. Yarde, M.E. Cvijic, S. Li, D.J. Shuster, V. Borowski, J.H. Xie, K.W. McIntyre, M.T. Obermeier, A. Fura, K. Stefanski, G. Cornelius, J. Hynes Jr., J.A. Tino, J.E. Macor, L. Salter-Cid, R. Denton, Q. Zhao, P.H. Carter, T.G.M. Dhar, Rationally designed, conformationally constrained inverse agonists of RORγt-identification of a potent, selective series with biologic-like in vivo efficacy, J. Med. Chem. 62 (2019) 9931–9946.
[53] R. Nakajima, H. Oono, S. Sugiyama, Y. Matsueda, T. Ida, S. Kakuda, J. Hirata, A. Baba, A. Makino, R. Matsuyama, R.D. White, R. Wurz, Y. Shin, X. Min, A. Guzman-Perez, Z. Wang, A. Symons, S.K. Singh, S.R. Mothe, S. Belyakov, A. Chakrabarti, S. Shuto, Discovery of [1,2,4]Triazolo[1,5-a]pyridine derivatives

as potent and orally bioavailable RORγt inverse agonists, ACS Med. Chem. Lett. 11 (2020) 528–534.
[54] I. Muegge, D. Collin, B. Cook, M. Hill-Drzewi, J. Horan, S. Kugler, M. Labadia, X. Li, L. Smith, Y. Zhang, Discovery of 1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide analogs as new RORC modulators, Bioorg. Med. Chem. Lett 25 (2015) 1892–1895.
[55] M. Scheepstra, S. Leysen, G.C. van Almen, J.R. Miller, J. Piesvaux, V. Kutilek, H. van Eenennaam, H. Zhang, K. Barr, S. Nagpal, S.M. Soisson, M. Kornienko, K. Wiley, N. Elsen, S. Sharma, C.C. Correll, B.W. Trotter, M. van der Stelt, A. Oubrie, C. Ottmann, G. Parthasarathy, L. Brunsveld, Identification of an allosteric binding site for RORγt inhibition, Nat. Commun. 6 (2015) 8833.
[56] G. Ouvry, C. Bouix-Peter, F. Ciesielski, L. Chantalat, O. Christin, C. Comino, D. Duvert, C. Feret, C.S. Harris, L. Lamy, A.P. Luzy, B. Musicki, D. Orfila, J. Pascau, V. Parnet, A. Perrin, R. Pierre, G. Polge, C. Raffin, Y. Rival, N. Taquet, E. Thoreau, L.F. Hennequin, Discovery of phenoxyindazoles and phenylthioindazoles as RORγ inverse agonists, Bioorg. Med. Chem. Lett 26 (2016) 5802–5808.
[57] F.A. Meijer, R.G. Doveston, R. de Vries, G.M. Vos, A.A.A. Vos, S. Leysen, M. Scheepstra, C. Ottmann, L.G. Milroy, L. Brunsveld, Ligand-based design of allosteric retinoic acid receptor-related orphan receptor γt (RORγt) inverse agonists, J. Med. Chem. 63 (2020) 241–259.
[58] R. de Vries, R.G. Doveston, F.A. Meijer, L. Brunsveld, Elucidation of an allosteric mode of action for a thienopyrazole RORγt inverse agonist, ChemMedChem 15 (2020) 561–565.
[59] H. Zhang, B.T. Lapointe, N. Anthony, R. Azevedo, J. Cals, C.C. Correll, M. Daniels, S. Deshmukh, H. van Eenenaam, H. Ferguson, L.G. Hegde, W.J. Karstens, J. Maclean, J.R. Miller, L.Y. Moy, V. Simov, S. Nagpal, A. Oubrie, R.L. Palte, G. Parthasarathy, N. Sciammetta, M. van der Stelt, J.D. Woodhouse, B.W. Trotter, K. Barr, Discovery of N-(Indazol-3-yl)piperidine-4-carboxylic acids as RORγt allosteric inhibitors for autoimmune diseases, ACS Med. Chem. Lett. 11 (2020) 114–119.
[60] F.A. Meijer, A. Saris, R.G. Doveston, G.J.M. Oerlemans, R. de Vries, B.A. Somsen, A. Unger, B. Klebl, C. Ottmann, P.J. Cossar, L. Brunsveld, Structure-activity relationship studies of trisubstituted isoxazoles as selective allosteric ligands for the retinoic-acid-receptor-related orphan receptor γt, J. Med. Chem. 64 (2021) 9238–9258.
[61] N. Sun, Q. Xie, Y. Dang, Y. Wang, Agonist lock touched and untouched retinoic acid receptor-related orphan receptor-γt (RORγt) inverse agonists: classification based on the molecular mechanisms of action, J. Med. Chem. 64 (2021) 10519–10536.
[62] M. Kono, A. Ochida, T. Oda, T. Imada, Y. Banno, N. Taya, S. Masada, T. Kawamoto, K. Yonemori, Y. Nara, Y. Fukase, T. Yukawa, H. Tokuhara, R. Skene, B.C. Sang, I. D. Hoffman, G.P. Snell, K. Uga, A. Shibata, K. Igaki, Y. Nakamura, H. Nakagawa, N. Tsuchimori, M. Yamasaki, J. Shirai, S. Yamamoto, Discovery of [cis-3-({(5 R)-5-[(7-Fluoro-1,1-dimethyl-2,3-dihydro-1 H-inden-5-yl)carbamoyl]-2-methoxy-7,8-dihydro-1,6-naphthyridin-6(5H)-yl}carbonyl)cyclobutyl] acetic acid (TAK-828F) as a potent, selective, and orally available novel retinoic acid receptor-related orphan receptor γt inverse agonist, J. Med. Chem. 61 (2018) 2973–2988.
[63] R. Nakajima, H. Oono, K. Kumazawa, T. Ida, J. Hirata, R.D. White, X. Min, A. Guzman-Perez, Z. Wang, A. Symons, S.K. Singh, S.R. Mothe, S. Belyakov, A. Chakrabarti, S. Shuto, Discovery of 6-Oxo-4-phenyl-hexanoic acid derivatives as RORγt inverse agonists showing favorable ADME profile, Bioorg. Med. Chem. Lett 36 (2021), 127786.
[64] Q. Liu, D.G. Batt, C.A. Weigelt, S. Yip, D.R. Wu, M. Ruzanov, J.S. Sack, J. Wang, M. Yarde, S. Li, D.J. Shuster, J.H. Xie, T. Sherry, M.T. Obermeier, A. Fura, K. Stefanski, G. Cornelius, P. Khandelwal, J.A. Tino, J.E. Macor, L. Salter-Cid, R. Denton, Q. Zhao, T.G.M. Dhar, Novel tricyclic pyroglutamide derivatives as potent RORγt inverse agonists identified using a virtual screening approach, ACS Med. Chem. Lett. 11 (2020) 2510–2518.
[65] C.W. Lugar, C.A. Clarke, R. Morphy, H. Rudyk, S. Sapmaz, R.E. Stites, G.M. Vaught, K. Furness, H.B. Broughton, G.L. Durst, D.K. Clawson, S.L. Stout, S.Y. Guo, J. D. Durbin, K.R. Stayrook, D.D. Edmondson, K. Kikly, N.E. New, H.A. Bina, M. G. Chambers, P. Shetler, W.Y. Chang, V.C. Chang, R. Barr, W.H. Gough, J.P. Steele, B. Getman, N. Patel, B.M. Mathes, T.I. Richardson, Defining target engagement required for efficacy in vivo at the retinoic acid receptor-related orphan receptor C2 (RORγt), J. Med. Chem. 64 (2021) 5470–5484.
[66] M.G. Yang, M. Beaudoin-Bertrand, Z. Xiao, D. Marcoux, C.A. Weigelt, S. Yip, D. R. Wu, M. Ruzanov, J.S. Sack, J. Wang, M. Yarde, S. Li, D.J. Shuster, J.H. Xie, T. Sherry, M.T. Obermeier, A. Fura, K. Stefanski, G. Cornelius, P. Khandelwal, A. Karmakar, M. Basha, V. Babu, A.K. Gupta, A. Mathur, L. Salter-Cid, R. Denton, Q. Zhao, T.G.M. Dhar, Tricyclic-carbocyclic RORγt inverse agonists-discovery of BMS-986313, J. Med. Chem. 64 (2021) 2714–2724.
[67] Z. Ruan, P.K. Park, D. Wei, A. Purandare, H. Wan, D. O'Malley, S. Stachura, H. Perez, C.L. Cavallaro, C.A. Weigelt, J.S. Sack, M. Ruzanov, J. Khan, M. Gururajan, J.J. Wong, Y. Huang, M. Yarde, Z. Li, C. Chen, H. Sun, V. Borowski, J.H. Xie, M. Anthony, M. Agler, B.E. Fink, L.S. Harikrishnan, Substituted diaryl ether compounds as retinoic acid-related orphan Receptor-γt (RORγt) agonists, Bioorg. Med. Chem. Lett 35 (2021), 127778.
[68] Q. Liu, H.Y. Xiao, D.G. Batt, Z. Xiao, Y. Zhu, M.G. Yang, N. Li, S. Yip, P. Li, D. Sun, D.R. Wu, M. Ruzanov, J.S. Sack, C.A. Weigelt, J. Wang, S. Li, D.J. Shuster, J.H. Xie, Y. Song, T. Sherry, M.T. Obermeier, A. Fura, K. Stefanski, G. Cornelius, S. Chacko, P. Khandelwal, S. Dudhgaonkar, A. Rudra, J. Nagar, V. Murali, A. Govindarajan, R. Denton, Q. Zhao, N.A. Meanwell, R. Borzilleri, T.G.M. Dhar, Azatricyclic inverse agonists of RORγt that demonstrate efficacy in models of rheumatoid arthritis and psoriasis, ACS Med. Chem. Lett. 12 (2021) 827–835.
[69] M. Noguchi, A. Nomura, S. Doi, K. Yamaguchi, K. Hirata, M. Shiozaki, K. Maeda, S. Hirashima, M. Kotoku, T. Yamaguchi, Y. Katsuda, P. Crowe, H. Tao, S. Thacher, T. Adachi, Ternary crystal structure of human RORγ ligand-binding-domain, an inhibitor and corepressor peptide provides a new insight into corepressor interaction, Sci. Rep. 8 (2018), 17374.
[70] H. Gong, D.S. Weinstein, Z. Lu, J.J. Duan, S. Stachura, L. Haque, A. Karmakar, H. Hemagiri, D.K. Raut, A.K. Gupta, J. Khan, D. Camac, J.S. Sack, A. Pudzianowski, D.R. Wu, M. Yarde, D.R. Shen, V. Borowski, J.H. Xie, H. Sun, C. D'Arienzo, M. Dabros, M.A. Galella, F. Wang, C.A. Weigelt, Q. Zhao, W. Foster, J. E. Somerville, L.M. Salter-Cid, J.C. Barrish, P.H. Carter, T.G.M. Dhar, Identification of bicyclic hexafluoroisopropyl alcohol sulfonamides as retinoic acid receptor-related orphan receptor gamma (RORγ/RORc) inverse agonists. Employing structure-based drug design to improve pregnane X receptor (PXR) selectivity, Bioorg. Med. Chem. Lett 28 (2018) 85–93.
[71] V.M. Tanis, H. Venkatesan, M.D. Cummings, M. Albers, J. Kent Barbay, K. Herman, D.A. Kummer, C. Milligan, M.I. Nelen, R. Nishimura, T. Schlueter, B. Scott, J. Spurlino, R. Wolin, C. Woods, X. Xue, J.P. Edwards, A.M. Fourie, K. Leonard, 3-Substituted quinolines as RORγt inverse agonists, Bioorg. Med. Chem. Lett 29 (2019) 1463–1470.
[72] J. Amaudrut, M.A. Argiriadi, M. Barth, E.C. Breinlinger, D. Bressac, P. Broqua, D. J. Calderwood, M. Chatar, K.P. Cusack, S.B. Gauld, S. Jacquet, R.V. Kamath, M. E. Kort, V. Lepais, J.M. Luccarini, P. Masson, C. Montalbetti, L. Mounier, D. Potin, O. Poupardin, S. Rouaud, L. Spitzer, C.D. Wallace, Discovery of novel quinoline sulphonamide derivatives as potent, selective and orally active RORγ inverse agonists, Bioorg. Med. Chem. Lett 29 (2019) 1799–1806.
[73] B.P. Fauber, G. de Leon Boenig, B. Burton, C. Eidenschenk, C. Everett, A. Gobbi, S. G. Hymowitz, A.R. Johnson, M. Liimatta, P. Lockey, M. Norman, W. Ouyang, O. René, H. Wong, Structure-based design of substituted hexafluoroisopropanol-arylsulfonamides as modulators of RORc, Bioorg. Med. Chem. Lett 23 (2013) 6604–6609.
[74] J. Chao, I. Enyedy, K. Van Vloten, D. Marcotte, K. Guertin, R. Hutchings, N. Powell, H. Jones, T. Bohnert, C.C. Peng, L. Silvian, V.S. Hong, K. Little, D. Banerjee, L. Peng, A. Taveras, J.L. Viney, J. Fontenot, Discovery of biaryl carboxylamides as potent RORγ inverse agonists, Bioorg. Med. Chem. Lett 25 (2015) 2991–2997.
[75] Y. Sasaki, M. Odan, S. Yamamoto, S. Kida, A. Ueyama, M. Shimizu, T. Haruna, A. Watanabe, T. Okuno, Discovery of a potent orally bioavailable retinoic acid receptor-related orphan receptor-gamma-t (RORγt) inhibitor, S18-000003, Bioorg. Med. Chem. Lett 28 (2018) 3549–3553.
[76] A. Sato, Y. Fukase, M. Kono, A. Ochida, T. Oda, Y. Sasaki, N. Ishii, Y. Tomata, S. Fukumoto, Y.N. Imai, K. Uga, A. Shibata, M. Yamasaki, H. Nakagawa, M. Shirasaki, R. Skene, I. Hoffman, B.C. Sang, G. Snell, J. Shirai, S. Yamamoto, Design and synthesis of conformationally constrained RORγt inverse agonists, ChemMedChem 14 (2019) 1917–1932.
[77] S. Fujita-Sato, S. Ito, T. Isobe, T. Ohyama, K. Wakabayashi, K. Morishita, O. Ando, F. Isono, Structural basis of digoxin that antagonizes RORgamma t receptor activity and suppresses Th17 cell differentiation and interleukin (IL)-17 production, J. Biol. Chem. 286 (2011) 31409–31417.
[78] B.P. Fauber, O. René, G. de Leon Boenig, B. Burton, Y. Deng, C. Eidenschenk, C. Everett, A. Gobbi, S.G. Hymowitz, A.R. Johnson, H. La, M. Liimatta, P. Lockey, M. Norman, W. Ouyang, W. Wang, H. Wong, Reduction in lipophilicity improved the solubility, plasma-protein binding, and permeability of tertiary sulfonamide RORc inverse agonists, Bioorg. Med. Chem. Lett 24 (2014) 3891–3897.
[79] T. Wang, D. Banerjee, T. Bohnert, J. Chao, I. Enyedy, J. Fontenot, K. Guertin, H. Jones, E.Y. Lin, D. Marcotte, T. Talreja, K. Van Vloten, Discovery of novel pyrazole-containing benzamides as potent RORγ inverse agonists, Bioorg. Med. Chem. Lett 25 (2015) 2985–2990.
[80] K. Hirata, M. Kotoku, N. Seki, T. Maeba, K. Maeda, S. Hirashima, T. Sakai, S. Obika, A. Hori, Y. Hase, T. Yamaguchi, Y. Katsuda, T. Hata, N. Miyagawa, K. Arita, Y. Nomura, K. Asahina, Y. Aratsu, M. Kamada, T. Adachi, M. Noguchi, S. Doi, P. Crowe, E. Bradley, R. Steensma, H. Tao, M. Fenn, R. Babine, X. Li, S. Thacher, H. Hashimoto, M. Shiozaki, SAR exploration guided by LE and Fsp(3): discovery of a selective and orally efficacious RORγ inhibitor, ACS Med. Chem. Lett. 7 (2016) 23–27.
[81] S. Hintermann, C. Guntermann, H. Mattes, D.A. Carcache, J. Wagner, A. Vulpetti, A. Billich, J. Dawson, K. Kaupmann, J. Kallen, R. Stringer, D. Orain, Synthesis and biological evaluation of new Triazolo- and Imidazolopyridine RORγt inverse agonists, ChemMedChem 11 (2016) 2640–2648.
[82] M. Noguchi, A. Nomura, K. Murase, S. Doi, K. Yamaguchi, K. Hirata, M. Shiozaki, S. Hirashima, M. Kotoku, T. Yamaguchi, Y. Katsuda, R. Steensma, X. Li, H. Tao, B. Tse, M. Fenn, R. Babine, E. Bradley, P. Crowe, S. Thacher, T. Adachi, M. Kamada, Ternary complex of human RORγ ligand-binding domain, inverse agonist and SMRT peptide shows a unique mechanism of corepressor recruitment, Gene Cell. 22 (2017) 535–551.
[83] M.E. Schnute, M. Wennerstål, J. Alley, M. Bengtsson, J.R. Blinn, C.W. Bolten, T. Braden, T. Bonn, B. Carlsson, N. Caspers, M. Chen, C. Choi, L.P. Collis, K. Crouse, M. Färnegårdh, K.F. Fennell, S. Fish, A.C. Flick, A. Goos-Nilsson, H. Gullberg, P. K. Harris, S.E. Heasley, M. Hegen, A.E. Hromockyj, X. Hu, B. Husman, T. Janosik, P. Jones, N. Kaila, E. Kallin, B. Kauppi, J.R. Kiefer, J. Knafels, K. Koehler, L. Kruger, R.G. Kurumbail, R.E. Kyne Jr., W. Li, J. Löfstedt, S.A. Long, C.A. Menard, S. Mente, D. Messing, M.J. Meyers, L. Napierata, D. Nöteberg, P. Nuhant, M.J. Pelc, M. J. Prinsen, P. Rhönnstad, E. Backström-Rydin, J. Sandberg, M. Sandström, F. Shah, M. Sjöberg, A. Sundell, A.P. Taylor, A. Thorarensen, J.I. Trujillo, J.D. Trzupek, R. Unwalla, F.F. Vajdos, R.A. Weinberg, D.C. Wood, L. Xing, E. Zamaratski, C. W. Zapf, Y. Zhao, A. Wilhelmsson, G. Berstein, Discovery of 3-Cyano- N-(3-(1-isobutyrylpiperidin-4-yl)-1-methyl-4-(trifluoromethyl)-1 H-pyrrolo[2,3- b]pyridin-5-yl)benzamide: a potent, selective, and orally bioavailable retinoic acid receptor-related orphan receptor C2 inverse agonist, J. Med. Chem. 61 (2018) 10415–10439.

[84] C. Gege, M.D. Cummings, M. Albers, O. Kinzel, G. Kleymann, T. Schlüter, C. Steeneck, M.I. Nelen, C. Milligan, J. Spurlino, X. Xue, K. Leonard, J.P. Edwards, A. Fourie, S.D. Goldberg, T. Hoffmann, Identification and biological evaluation of thiazole-based inverse agonists of RORγt, Bioorg. Med. Chem. Lett 28 (2018) 1446–1455.

[85] K. Hoegenauer, J. Kallen, E. Jiménez-Núñez, R. Strang, P. Ertl, N.G. Cooke, S. Hintermann, M. Voegtle, C. Betschart, D.J.J. McKay, J. Wagner, J. Ottl, C. Beerli, A. Billich, J. Dawson, K. Kaupmann, M. Streiff, N. Gobeau, S. Harlfinger, R. Stringer, C. Guntermann, Structure-based and property-driven optimization of N-aryl imidazoles toward potent and selective oral RORγt inhibitors, J. Med. Chem. 62 (2019) 10816–10832.

[86] M. Kotoku, T. Maeba, S. Fujioka, M. Yokota, N. Seki, K. Ito, Y. Suwa, T. Ikenogami, K. Hirata, Y. Hase, Y. Katsuda, N. Miyagawa, K. Arita, K. Asahina, M. Noguchi, A. Nomura, S. Doi, T. Adachi, P. Crowe, H. Tao, S. Thacher, H. Hashimoto, T. Suzuki, M. Shiozaki, Discovery of second generation RORγ inhibitors composed of an Azole scaffold, J. Med. Chem. 62 (2019) 2837–2842.

[87] R. de Vries, F.A. Meijer, R.G. Doveston, I.A. Leijten-van de Gevel, L. Brunsveld, Cooperativity between the orthosteric and allosteric ligand binding sites of RORγt, Proc. Natl. Acad. Sci. U. S. A. 118 (2021).

[88] J.R. Huh, M.W. Leung, P. Huang, D.A. Ryan, M.R. Krout, R.R. Malapaka, J. Chow, N. Manel, M. Ciofani, S.V. Kim, A. Cuesta, F.R. Santori, J.J. Lafaille, H.E. Xu, D. Y. Gin, F. Rastinejad, D.R. Littman, Digoxin and its derivatives suppress TH17 cell differentiation by antagonizing RORγt activity, Nature 472 (2011) 486–490.