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Review paper

Small molecule inhibitors of RORγt for Th17 regulation in inflammatory and autoimmune diseases

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ABSTRACT

As a ligand-dependent transcription factor, retinoid-associated orphan receptor γt (RORγt) that controls T helper (Th) 17 cell differentiation and interleukin (IL)-17 expression plays a critical role in the progression of several inflammatory and autoimmune conditions. An emerging novel approach to the therapy of these diseases thus involves controlling the transcriptional capacity of RORγt to decrease Th17 cell development and IL-17 production. Several RORγt inhibitors including both antagonists and inverse agonists have been discovered to regulate the transcriptional activity of RORγt by binding to orthosteric- or allosteric-binding sites in the ligand-binding domain. Some of small-molecule inhibitors have entered clinical evaluations. Therefore, in current review, the role of RORγt in Th17 regulation and Th17-related inflammatory and autoimmune diseases was highlighted. Notably, the recently developed RORγt inhibitors were summarized, with an emphasis on their optimization from lead compounds, efficacy, toxicity, mechanisms of action, and clinical trials. The limitations of current development in this area were also discussed to facilitate future research.

1. Introduction

A wide family of transcription factors known as nuclear receptors (NRs) controls gene expression in a ligand-dependent way [1]. The sequence similarity of many NRs has led to their identification; however, these receptors have no identified natural ligands and are termed orphan NRs. Retinoid-associated orphan receptors (RORs) belong to the NR superfamily. The general structural features of RORs are a variable N-terminal domain, a central highly conserved DNA-binding domain (DBD), a hinge, and a C-terminal ligand-binding domain (LBD) [2]. Recognizing and binding to receptors and ligands is the responsibility of LBD. In the RORs subfamily, there are three members: RORα [3], RORβ [4], and RORγ [5], encoded by *RORA*, *RORB*, and *RORC*, respectively. All of these receptors are transcription factors that are ligand dependent. RORγt as one of isoforms of RORγ is a dominant transcription factor which is both sufficient and necessary for induction of the transcription of interleukin (IL)-17 and the production of the closely related cytokines in naive $CD4^+$ T helper (Th) cells from both mouse and human [6]. RORγt cooperates with cytokines of

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IL-6, IL-23, IL-21 and transforming growth factor β (TGF-β) and transcription factors of signal transducer and activator of transcription 3 (STAT3), interferon regulatory factor 4 (IRF4), eomesodermin (Eomes), runt-associated transcription factor 1 (Runx1) and c-Rel to induce differentiation of primitive $CD4^+$ T cells to Th17 cells and secretion of IL-17 [7]. It was found that Th17 cells were decreased in RORγt-deficient mouse, which attenuated the occurrence of inflammatory and autoimmune diseases, such as multiple sclerosis (MS), psoriasis, rheumatoid arthritis (RA), systemic lupus erythematosus, and inflammatory bowel disease (IBD) [6,8,9]. Therefore, it has been highlighted that regulating RORγt activity and Th17 maturation is a promising strategy for relieving these diseases.

IL-17 antibodies have been developed and authorized for handling psoriasis which have shown good clinical efficacy. However, inhibiting IL-17 solely is not enough to increase clinical efficacies in certain conditions, including RA and Crohn's disease (CD) [10]. Some small molecules indirectly targeting IL-17 have thus been introduced into the field of vision. Small-molecule drugs targeting RORγt can interfere with the Th17 development and inhibit the production of multiple cytokines, thus reducing inflammation. RORγt becomes an attractive druggable target for the treatment of Th17-induced diseases.

In the current review, the role of RORγt in Th17 regulation and Th17-related inflammatory and autoimmune diseases was highlighted. The recently developed small-molecule RORγt inhibitors were summarized, with an emphasis on their optimization from lead compounds, efficacy, toxicity, mechanisms of action, and clinical trials. The limitations of current development in this area were also discussed to facilitate future research.

2. The role of Th17 cells in inflammatory and autoimmune diseases

Th1 and Th2 cells were considered as the main pathogenic factors of inflammatory diseases before Th17 cells were discovered. The discovery of Th17 cells provides a novel understanding of importance of Th subsets in occurrence of inflammatory diseases. Subsequently, it is found that pathogenicity of Th17 is dominated by their secreted cytokine IL-17 [11,12]. It has been demonstrated that Th17 is closely associated with conditions of psoriasis, experimental autoimmune encephalomyelitis (EAE), RA, IBD, and so on [13], and high expression of Th17 cytokines such as IL-17, IL-22 and tumor necrosis factor-α (TNF-α) has been determined in these diseases. Inflammatory responses are remarkably induced by the cytokines IL-17A/IL-17F mainly excreted by Th17, which subsequently induces the release of granulocyte colony-stimulating factor and C–X–C motif (CXC) chemokines [11,12].

It was reported that IL-23 in the inflammatory epidermal dendritic cells of psoriasis activated Th17 to produce IL-17, and stimulated keratinocytes to produce many chemokines, such as CXCL1, CXCL2, CXCL3, CXCL5 and CXCL8, which recruited neutrophils to the inflammatory site [14,15]. IL-17A, IL-22 and TNF-α also stimulated the production of C–C motif chemokine ligand 20 (CCL20) in human keratinocytes and maintained the presence of C–C motif chemokine receptor 6 (CCR6) cells in peripheral inflamed tissues [16]. Campbell et al. [17] found that inhibition of CCR6 reduced psoriatic plaques in a murine model of psoriasis.

It is evident that Th17 and IL-17 are pathogenic in arthritis. Lubberts et al. [18] showed that collagen II-immunized mouse with overexpressed IL-17 had more severe joint damage. In the reactivation of antigen-induced arthritis (AIA), inhibiting endogenous IL-17 reduced joint inflammation and bone degradation [19]. Moreover, increased Th17 cells and elevated IL-17 level were determined in RA animals [20]. Th17 cytokines increased the production of CCL20, a ligand for CCR6, and recruited $CCR6^+$ Th17 cells to the inflammatory sites [20]. It is worth noting that CCR6 expression required significant amounts of RORγt or other cytokines including IL-1. In addition, CCL20 was produced through the synergy of IL-1β, TNF-α and IL-17 [21]. Therefore, CCR6 contributes to Th17 activity in autoimmune diseases, particularly in RA.

In EAE mice, the increased production of IL-17A in central nervous system induced the secretion of CCL2, CCL12, CXCL1, CXCL2 and CXCL5 chemokines [22,23]. These chemokines infiltrated lymphocytes into the CNS which were subsequently involved in lesion formation in MS and EAE. Similarly, IL-17 elevated IL-6 in astrocytes and co-regulated CCL20 expression in astrocytes, thereby promoting the recruitment of CCR6-expressing cells into CNS [24]. Lock et al. [25] observed enhanced expression of genes encoding IL-6, IL-17A, and interferon-γ (IFN-γ) in MS lesions. Notably, Kang et al. [26] found that the severity of EAE was reduced when astrocytes were deficient of Act1, a key regulatory protein for IL-17 signaling, indicating that EAE is primarily caused by IL-17 signaling. Additionally, Ivanov et al. [6] detected that Th17 cells in RORγt-deficient mice had significantly reduced cytokine levels and were not susceptible to EAE, suggesting an important contribution of Th17 in EAE.

Th17 and its cytokines are also closely involved in pathogenesis of IBD. It was found that dextran sulfate sodium (DSS) challenge in IL-17A deficient mice only led to mild colitis compared to the wide type mice [27]. Moreover, *IL-17R*$^{-/-}$ mice were protected against murine colitis induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS) [28]. Th17 cytokines enhanced neutrophil chemokine expression and neutrophil influx into inflammatory sites [29]. IBD patients and mice with DSS-induced experimental colitis expressed more of the chemokine CCL20 [29]. The primary activity of CCL20 was to recruit $CCR6^+$ immunocytes, which migrated to the inflamed intestinal mucosa during active ulcerative colitis (UC) [30].

IL-17 is also related to allergic asthma pathogenesis. Allergic sensitization of the airway can cause severe Th17 response. IL-17 recruited neutrophils, resulting in neutrophilia and neutrophil-dependent airway hyperresponsiveness (AHR). Besides, Th17-induced airway inflammatory condition and AHR showed steroid resistance [31,32].

Atherosclerosis is a chronic inflammatory disease of the major arteries. Th17 cells and IL-17 are highly correlated with the onset of atherosclerosis [33,34]. IL-17 induced the release of CXCL1, CXCL2 and CXCL8 chemokines from endothelial cells and vascular smooth muscle cells, which aggravated the inflammation of atherosclerotic plaques [35]. Moreover, IL-17A induced upregulation of pro-inflammatory mediators, including IL-1A and IL-6, which drove the formation of atherosclerotic plaques [36].

In addition, Th17 cells play an important role in the pathogenesis of coronavirus disease 2019 (COVID-19). Patients with COVID-19 exhibited high levels of IL-17 and granulocyte-macrophage colony-stimulating factor (GM-CSF) [37]. It has been shown that lung tissue destruction may be caused by recruitment of neutrophils mediated by Th17 cells [37].

Taken together, Th17 and the related cytokines like IL-17 causing inflammation has a significant impact on the progression of autoimmune diseases or inflammation-related diseases. Many researchers have thus highlighted the benefits of targeting Th17 cell regulation in the treatment of multiple related disorders [38,39].

3. RORγt as a key target for Th17 regulation

3.1. The role of RORγt in Th17 functionality

It is demonstrated that RORγt plays an essential role in modulating Th17 differentiation and several cytokines production. Therefore, RORγt is identified as a key target for Th17 cell modulation to manage Th17-related diseases [39].

3.1.1. Th17 differentiation and RORγt

Initially, $CD4^+$T cells were thought to differentiate into Th1 and Th2 subsets. In contrast to conventional Th1 and Th2 cells, Th cells that produce IL-17 represent a distinct lineage of inflammatory T cells [40]. IL-17A, IL-17F, IL-21, IL-22 and GM-CSF are pro-inflammatory cytokines generated by Th17, a $CD4^+$ T cell subset [41,42], which contribute to Th17-related diseases (Table 1) [6,14–32]. IL-17A and IL-17F, members of the IL-17 family, are highly homologous and interact with the same receptor [43].

Various cytokines control how Th17 cells differentiate (Fig. 1). Primitive T cells are co-induced by IL-6 and TGF-β for differentiation into Th17 [44,45]. IL-6 and TGF-β regulate RORγt expression by integrating different *cis*-regulatory elements. TGF-β binds to conserved noncoding sequence 6 (CNS6) by inducing Sma- and Mad-related (SMAD) proteins; on the other hand, IL-6-induced STAT3 binds to CNS9 to a larger extent and CNS6 to a partial extent [46]. These Th17 cells are generally considered as non-pathogenic, and in some cases show protective effects. Non-pathogenic Th17 cells express more cytokines associated with immunosuppression. In the gut, for example, production of IL-22 is mediated by segmented filamentous bacteria to maintain tissue homeostasis and defend pathogenic microorganisms [47]. Additionally, the expression of IL-17 and IL-10 induced by TGF-β and IL-6 eliminates pathogenic functions in EAE [48]. Therefore, the pathogenic activity of Th17 is not mediated by TGF-β and IL-6. However, high concentration of TGF-β mediates forkhead box protein P3 (Foxp3) expression and inhibits IL23R expression, thus antagonizing RORγt, facilitating Treg development and suppressing Th17 differentiation [49,50]. IL-21 and IL-23 promote or maintain development of Th17, while IL-4 and IFN-γ show a negatively modulatory effect [51]. Additionally, IL-2-induced signal transducer and STAT5 activation reduces Th17 cell differentiation [52].

Nevertheless, Th17 differentiation can proceed into the other direction (Fig. 1), with IL-6, IL-23, IL-1β or IL-6, IL-23 and TGF-β3 promoting the production of pathogenic Th17 cells [53,54]. In Th17 differentiation driven by IL-6, IL-23 and IL-1β, recruiting steroid receptor coactivator 3 (SRC3) to the *Il17a* and *Il1r1* promoter regions by RORγt required p300 acetyltransferase [55]. In addition, Lee et al. [56] found that serum amyloid A proteins replaced TGF-β and, cooperating with IL-6, also induced pathogenic Th17, leading to the development of inflammation-related diseases. Kleinewietfeld et al. [57] found that NaCl drove autoimmune diseases by inducing pathogenic Th17 cells, and subsequently Wu et al. [58] demonstrated that this phenomenon was induced by inducing serum glucocorticoid kinase 1 and promoting tissue inflammation. Chen et al. [59] showed that leukotriene B4 (LTB4) inhibited the differentiation of Treg cells and may promote the generation of Th17 cells, while prostaglandin E2 (PGE2) inhibited the differentiation of both Treg and Th17 cells. Therefore, LTB4 and PGE2 may play an important role in the pathogenesis of RA by regulating the differentiation of Treg and Th17 cells [59]. Compared with non-pathogenic Th17, pathogenic Th17 expresses increased pro-inflammatory cytokines/chemokines (*Cxcl3*, *Ccl4*, *Ccl5*, *Ccl3*, *Csf2*, *Il3*, *Il22*, and *Il17*) as well as several transcription factors [54]. It is worth noting that GM-CSF is secreted by pathogenic Th17, and as a

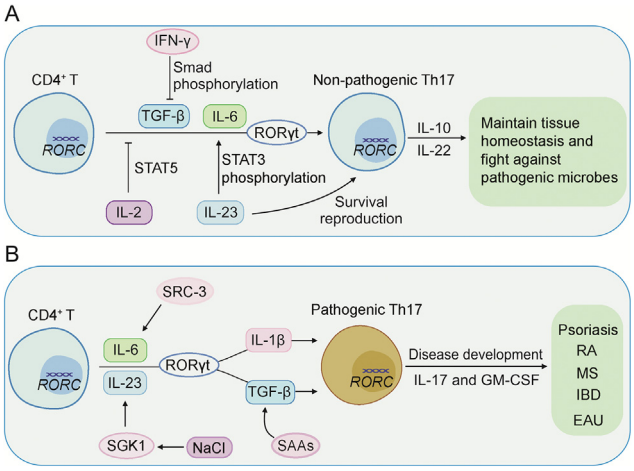


Fig. 1. $CD4^+$ T cells under induction can differentiate into non-pathogenic T helper 17 (Th17) cells or pathogenic Th17 cells. (A) Under the co-induction of interleukin (IL)-6 and transforming growth factor-β (TGF-β), $CD4^+$ T cells differentiate into non-pathogenic Th17 cells, which is also regulated by interferon-γ (IFN-γ), IL-23, IL-2, IL-4, and other cytokines. (B) IL-6, IL-23, IL-1β or IL-6, IL-23, TGF-β induce the production of pathogenic Th17 cells. Pathogenic Th17 cells secrete cytokines such as IL-17 and granulocyte-macrophage colony-stimulating factor (GM-CSF), which drive psoriasis, rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD), and experimental autoimmune uveitis (EAU). RORγt: retinoid-associated orphan receptor γt; SRC3: steroid receptor coactivator 3; SGK1: serum glucocorticoid kinase 1; SAAs: serum amyloid A proteins.

Table 1
Pathogenic mechanisms of T helper (Th) 17 cells and their cytokines in related diseases.

Disease	Related Th17 cytokines	Pathogenic mechanism	Refs.
MS/EAE	IL-17 and IL-23	Th17 cytokines promote the secretion of chemokines CCL2, CCL12, CXCL1, CXCL2, and CXCL5, which recruit lymphocytes into the central nervous system. Th17 cytokines promote the expression of CCL20 and promote the recruitment of CCR6-expressing cells to the central nervous system.	[6,22–26]
Psoriasis	IL-17 and IL-23	Th17 cytokines stimulate keratinocytes produce CXCL2, CXCL3, CXCL5, and CXCL8, which recruit neutrophils to sites of inflammation. Th17 cytokines stimulate the expression of CCL20 in keratinocytes and recruit $CCR6^+$ cells to the inflammatory site of psoriasis.	[14–17]
RA	IL-17; IL-23; IL-21; and GM-CSF	Th17 cytokines stimulate the chemokine CCL20 to recruit $CCR6^+$ lymphocytes to sites of inflammation.	[18–21]
IBD	IL-17; IL-21; and IL-22	Th17 cytokines increase expression of the chemokine CCL20 and promote $CCR6^+$ cells migrating to the inflamed intestinal mucosa.	[27–30]
Asthma	IL-17	Th17 cytokines promote the release of CXC chemokines, which recruit neutrophils to the airways.	[31,32]

MS: multiple sclerosis; EAE: experimental autoimmune encephalomyelitis; IL: interleukin; CCL: C–C motif chemokine ligand; CXCL: C–X–C motif (CXC) chemokine ligand; CCR: C–C motif chemokine receptor; RA: rheumatoid arthritis; GM-CSF: granulocyte-macrophage colony-stimulating factor; IBD: inflammatory bowel disease.

key pathogenic agent of Th17 cells, GM-CSF is sufficient to cause autoimmune diseases. For example, Th cells deficient of GM-CSF could not induce neuroinflammation, while *Il17a*$^{-/-}$ T helper cells secreted GM-CSF and induced EAE [60]. Additionally, GM-CSF secreted by Th17 increased inflammation in an arthritis model [61]. These findings suggest that differentiation of pathogenic Th17 is highly involved in pathogenesis of inflammatory as well as autoimmune diseases.

Notably, non-pathogenic Th17 only accounts for a very small proportion of Th17, which requires both RORγt and IL-10-associated transcriptional factors including c-MAF, Ikzf3, and aryl hydrocarbon receptor for differentiation [62]. However, currently it is unclear that whether the IL-10^{+} non-pathogenic Th17 cells act as intermediate state of Th17 and Treg transition [62]. It is generally acknowledged that under inflammation the pathogenic Th17 is the key driver of inflammation, and the differentiation of pathogenic Th17 through RORγt is essential for initiation of disease conditions [60,63]. Therefore, RORγt presents as a prospective target for Th17-associated disorders.

3.1.2. RORγt and transcription of IL-17 and other cytokines

As a crucial transcriptional factor, RORγt is important for the development of Th17 as well as IL-17 production. RORγt induces Th17 differentiation, thereby promoting IL-17 expression and secretion [6,64]. In Th17, CNS2 can interact with the promoters of *Il17a* and *Il17f* and is sufficient to regulate their selective transcription, while acetyl histone H3, a permissive histone marker in the promoter regions of CNS2 and IL-17, is under the regulation of RORγt [65]. In addition, IL-17 expression driven by RORγt in vitro is compromised by the targeted ablation of CNS2 [65]. Therefore, RORγt is a key regulator for IL-17 transcription (Fig. 2).

The LBD of RORγt participates in recruitment of coactivators and corepressors and regulates expression of RORγt-related genes (Fig. 2). SRC1 is a coactivator of RORγt. The LXXLL motifs of coactivators interact with receptors, and LXXLL peptides which interact with RORγ can effectively antagonize RORγ-induced transcription activation [66]. SRC1 phosphorylation induced by the protein kinase C-θ, a T cell receptor (TCR) signaling protein, degrades Foxp3, which reverses the inhibitory effect of Foxp3 on RORγt transcriptional activity [67]. In addition, RORγ interacts with the regulatory region of Mi-2b and inhibits RORγ transcriptional activity [68].

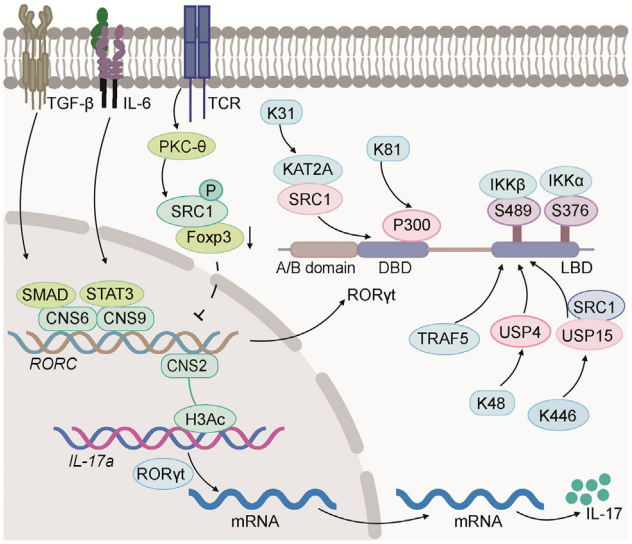


Fig. 2. Retinoid-associated orphan receptor γt (RORγt) mediates interleukin-17 (IL-17) transcription. The post-translational regulatory mechanisms of RORγt include ubiquitination, acetylation, and phosphorylation. TGF-β: transforming growth factor β; TCR: cell receptor; PKC-θ: protein kinase C-θ; SRC: steroid receptor coactivator; Foxp3: forkhead box protein P3; SMAD: Sma- and Mad-related protein; STAT: signal transducer and activator of transcription 3; CNS: conserved noncoding sequence; H3Ac: acetyl histone H3; mRNA: messenger RNA; DBD: DNA-binding domain; IKK: Iκb kinase; LBD: ligand-binding domain; TRAF: tumor necrosis factor receptor-associated factor; USP: ubiquitin-specific protease.

RORγt post-translational regulation is also a key mechanism regulating RORγt-mediated IL-17 transcriptional activation (Fig. 2). Ubiquitination of RORγt-K31 activates histone acetyltransferase (HAT) KAT2A and stables its binding to SRC1, thereby promoting RORγt-mediated differentiation of Th17 cells [69]. HAT P300 interacts with RORγt at its K81 residue and acetylates RORγt to promote RORγt-mediated IL-17 transcriptional activation [70]. Sirtuin 1, a protein deacetylase showing anti-inflammatory function, deacetylates RORγt, increases RORγt activity, enhances Th17 cell development and activity, and increases the progression of EAE in mouse models [71]. Iκb kinase (IKK)α binds and phosphorylates RORγt at S376, which increases the transcription function of RORγt and promotes the Th17 differentiation [72]. In EAE animal models, phosphorylation of RORγt at S489 by IKKβ increases interaction of RORγt and aryl hydrocarbon receptor, promotes RORγt transcriptional activity, and increases disease severity [73]. Additionally, ubiquitin-specific protease (USP) 4-mediated deubiquitination of RORγt promotes RORγt function and IL-17A transcription [74]. USP17-induced deubiquitination of RORγt maintains RORγt protein expression [75]. USP15-induced deubiquitination of RORγt at K446 promotes RORγt-mediated Th17 differentiation via boosting the recruitment of coactivator SRC1 [76]. Tumor necrosis factor receptor-associated factor 5-mediated ubiquitination of RORγt stabilizes RORγt protein level and promotes IL-17A expression [77].

RORγt is also critical for transcription of IL-22 and GM-CSF. Runx1 and RORγt cooperate to increase IL-22 transcription through its distal enhancer of CNS-32 [78]. It is reported that RORγt and aryl hydrocarbon receptor control IL-22 secretion by CD4^{+} T cells activated with IL-21 [79]. Notably, RORγt drives generation of GM-CSF in Th17, and this is required for the effector process in autoimmune condition [60].

3.2. The structure and regulation of RORγt

Three different subtypes of RORs exist: RORα, RORβ, and RORγ, which are encoded by *RORA*, *RORB*, and *RORC*, respectively [39,80]. Different tissues express them differently, and they are involved in different physiological processes (Table 2) [39,80–82]. RORα is found in different tissues, including the liver, skeletal muscles, skin, thymus, lungs, adipose, kidneys, brain, and blood [39,81]. RORβ is only expressed in the central nervous system [39]. The expression of RORγ is similar to RORα, but it's high in the thymus. Two isomers of RORγ are found: RORγ1 and RORγt (RORγ2). RORγ1 is found in liver, fat, skeletal muscles and kidneys, and RORγt is thought to be expressed most commonly by immune cells in the thymus and others [82]. RORγt regulates thymocyte growth, development and survival by recruiting SRCs [83]. RORγt is closely linked to inflammatory and other autoimmune diseases. In this section, the structure of RORγt and its regulation will be summarized.

3.2.1. The structure of the RORγt

RORγt has a typical NR domain, which is comprised of four main functional domains: an A/B domain with activation function 1, a DBD, a hinge, and a C-terminal LBD with ligand-dependent AF2 (Fig. 3) [84]. RORs modulate gene expression through recognizing and binding ROR response elements (ROREs), which are consisted of an AGGTCA core motif and a 5-bp A/T rich sequence [85,86]. The flexible

Table 2
The classification, distribution, and function of retinoid-associated orphan receptor (ROR) family.

Name	Coding gene	Classification	Distribution	Physiological and pathological process	Refs.
RORα	*RORA*	RORα1, RORα2, RORα3, and RORα4	Liver, skeletal muscle, skin, thymus, lung, adipose tissue, kidney, brain, and blood	Liver gluconeogenesis, lipid metabolism, and atherosclerosis	[39,80,81]
RORβ	*RORB*	RORβ1 and RORβ2	Central nervous system	Participate in the processing of sensitive information in the spinal cord, thalamus, and cerebellar cortex	[39,80]
RORγ	*RORC*	RORγ1 and RORγt (RORγ2)	Liver, fat, skeletal muscle, kidney, and thymus	Psoriasis, rheumatoid arthritis, multiple sclerosis, and other autoimmune diseases	[39,80,82]

length of A/B domain helps DBD to recognize and bind specific DNA sequences to regulate gene expression [86]. The DBD is the most conserved region, containing a short motif responsible for DNA binding specificity [87]. The hinge region connects DBD to LBD and often contains DNA minor groove binding residues placed only at the C-terminal of DBD, called C-terminal extensions [88]. LBD includes 12 typical canonical α-helices (H1–H12) and two other helices (H2' and H11') containing ligand binding sites. LBD cooperates with co-activators or co-inhibitors to control transcription [89].

The study of the co-crystal structure of RORγt is helpful for drug design based on structure and ligand in future. Nearly 96 co-crystal structures of RORγt have been reported [90], which includes apo RORγt and RORγt bound to natural agonist, synthetic agonist, partial agonist, and inverse agonist [90]. The crystal structure of RORγt-LBD in apo state mainly adopts active conformation. RORγt utilizes the unique His479–Tyr502–Phe506 triplet to provide significant interaction energy, anchoring H12 in an active conformation and further stabilizing it with a helical packing with a unique H11' element [91]. The binding of endogenous ligand further stabilizes the active conformation of RORγt. The agonist acts similarly to the endogenous ligand in that it forms an extended aromatic cluster with the His479–Tyr502–Phe506 triplet and further stabilizes H12 in the active conformation [91]. The RORγt inverse agonist can disrupt the active conformation of H12 and redirect the RORγt to inactivation by directly disrupting the tightly interacting His479–Tyr502–Phe506 triplet [91].

Small molecule regulators bind to the orthosteric binding site (OBS) or allosteric binding site (ABS) in LBD to control the transcriptional activity of RORγt. The OBS, a pocket generated by H3, H5, H6, H7, H11 and H12, is generally considered to be the target of endogenous ligands [84]. Another new site, ABS, is found near the OBS, which is formed with H3, H4, H5, H11 and H12 [92].

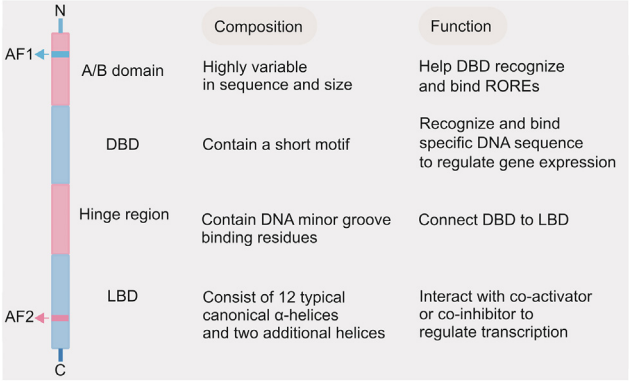


Fig. 3. The structure and function of retinoid-associated orphan receptor γt (RORγt). It includes the A/B domain, DNA-binding domain (DBD), hinge region, and ligand-binding domain (LBD). AF1/2: activation function 1/2; ROREs: ROR response elements [84]. Reprinted from Ref. [84] with permission.

Compared to the OBS, the size of ABS is smaller, and its location is near H12 [84]. Most RORγt regulators bind to OBS, similar to the endogenous ligands. Binding to agonists causes conformational changes that stabilizes the positions of the H12 and AF-2 and leads to recruitment of coactivators. Similarly, binding of antagonists or inverse agonists ligands usually induces H12 redirection or destabilizes (and possibly unbinds) the H12 and AF-2, thereby preventing recruitment of co-activators or enhancing recruitment of co-suppressors, ultimately leading to transcriptional suppression at specific sites [92,93]. Generally, the interaction with allosteric binding sites endows novel small-molecule antagonistic or inverse agonistic ligands of RORγt with high potency and selectivity [92].

3.2.2. *The regulation of RORγt*

RORγt is expressed in particular immunocytes including Th17 cells [6]. The expression of RORγt is modulated by various cytokines and transcriptional factors (Fig. 4).

Cytokines such as IL-6, IL-21, IL-23, and other cytokines have the ability to activate STAT3, thereby promoting the expression of RORγt [42,94–96]. TGF-β is a positive regulator of RORγt expression [97], which is through regulating IRF4 [98,99]. Eomes inhibits RORγt expression, and the expression of Eomes is down-regulated independent on SMAD activated by TGF-β [100]. The c-Rel is activated upon TCR signals and controls RORγt expression by directly

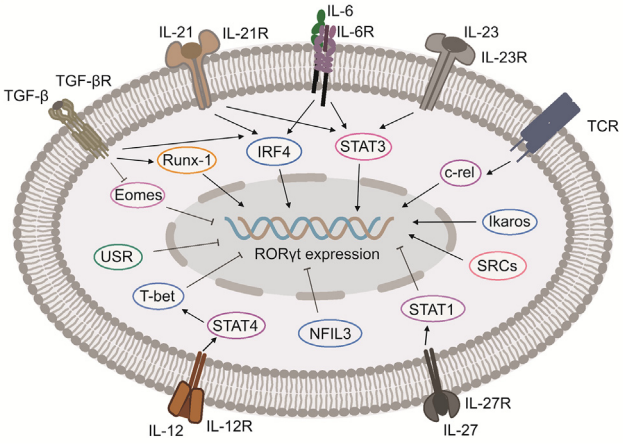


Fig. 4. Retinoid-associated orphan receptor γt (RORγt) as a key regulator for T helper (Th) 17 differentiation is modulated by positive and negative factors, including the cytokines of interleukin (IL)-6, IL-23, IL-21, transforming growth factor-β (TGF-β), IL-12 and IL-27, as well as the transcription factors of signal transducer and activator of transcription 1/4 (STAT1/4), interferon regulatory factor 4/8 (IRF4/8), eomesodermin (Eomes), c-Rel, runt-associated transcription factor 1 (Runx1), Ikaros, steroid receptor coactivators (SRCs), upstream stimulatory factor (USF), T-bet, and nuclear factor interleukin 3 (NFIL3).

interacting with RORγt gene promoter [101]. Runx1 increases RORγt expression via TGF-β signaling pathway [102]. SRCs including the SRC1 (NCoA1), SRC2 (NCoA2), and SRC3 (NCoA3), participate in most NRs transcription activity [103]. RORγt recruits SRCs to regulate thymus cell survival in vivo [83]. In addition, Ikaros [104] and IRF8 [105] also regulate the expression of RORγt.

The negative regulation of RORγt expression is related to multiple factors. IL-12 inhibits RORγt expression via STAT4 and T-bet [106]. IL-27 inhibits the expression of RORγt through STAT1 activation [107]. Both upstream stimulatory factor [108] and nuclear factor interleukin 3 [109] inhibit RORγt expression.

4. Small-molecule RORγt inhibitors for managing inflammatory and autoimmune diseases

Giving the role of RORγt/Th17 axis in inflammatory and autoimmune diseases as well as the understanding of RORγt structure and regulation, inhibition of RORγt/Th17 axis by developing modulators has been identified as a key to treat related diseases. Targeting Th17 cell and its related cytokines have become a research hotspot in the therapy of Th17-related diseases. Notably, IL-17 suppressors are mainly a class of biological agents which directly act on IL-17 ligand or its receptors. In fact, there are several IL-17 inhibitors already on the market. For example, the monoclonal antibodies, secukinumab and ixekizumab, which inhibit the IL-17A ligand, are approved to treat psoriasis, psoriatic arthritis, and ankylosing spondylitis [110,111]; brodalumab blocks IL-17 receptor (IL-17RA) and is approved to treat psoriasis [112]. IL-17 antibodies have the advantages of rapid onset, robust response and good tolerability. However, as with most biological antibodies, upper respiratory tract infections and injection site reactions are the most frequent adverse reactions.

In addition, some small molecules indirectly targeting IL-17 have also been introduced into the field of vision. Compared to monoclonal antibodies that require injection, some small-molecule inhibitors that act on IL-17A/IL-17RA can be administered orally with higher patient compliance [113]. Several inhibitors for Janus kinase 1/2, tyrosine kinase 2, and phosphodiesterase 4 have been developed for treatment of various autoimmune and inflammatory diseases [114–117], all of which exhibit an inhibitory effect on IL-17 production. Notably, small-molecule drugs targeting RORγt can interfere with Th17 differentiation and inhibit the synthesis of multiple cytokines including IL-17, thus reducing inflammation. Two types of small-molecule inhibitors have been developed for targeting RORγt: inverse agonists and antagonists, both of which modulate RORγt function and Th17-mediated inflammation. Thus, RORγt becomes a promising therapeutic target to the therapy of Th17-induced diseases.

4.1. Psoriasis

Psoriasis is a common skin condition with chronic inflammation, and its pathogenesis has not been completely understood. According to numerous studies, Th17 cells contribute to the progression of psoriasis, which invade the skin, release IL-17, and then trigger the production of CXC chemokines by keratinocytes [118–120]. RORγt controls the growth, upkeep, and operation of cells that produce IL-17. Thus, RORγt is a promising target to the therapy of psoriasis [121]. The major regulatory mechanism of targeting RORγt is suppression of *Il17* gene transcription and then reduction of IL-17 production. Secondly, it affects the recruitment of cofactors through suppressing the recruitment of co-activators PGC1α and NCoA1 or increasing co-inhibitors NCoR1 and NCoR2 (Fig. 5). Several RORγt antagonists and inverse agonists have been developed to treat psoriasis.

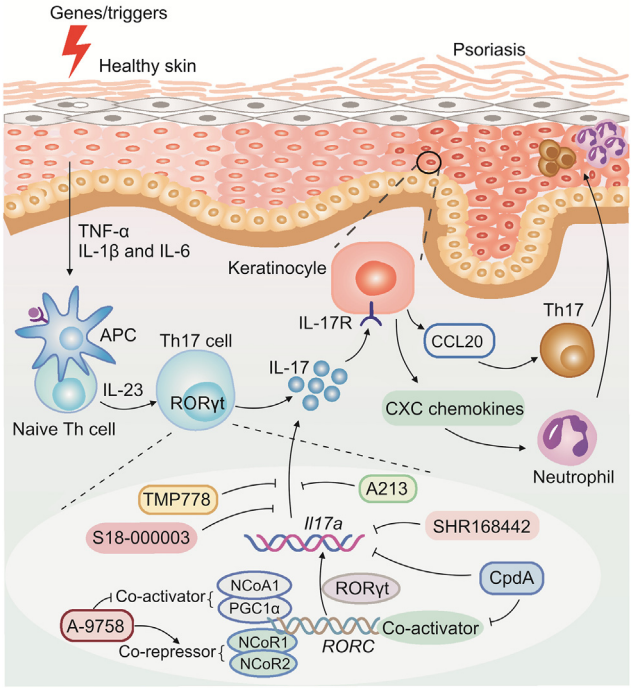


Fig. 5. Mechanisms of retinoid-associated orphan receptor γt (RORγt) inhibitors in improving psoriasis. Compounds like TMP778, S18–000003, A213, SHR168442, and CpdA inhibited the transcription of *interleukin (Il) 17* gene, and finally it inhibited the production of IL-17. A-9758 inhibited the recruitment of coactivators of NCoA1 and PGC1α and induced co-repressors of NCoR1 and NCoR2 to inhibit RORγt-mediated IL-17 transcription. CpdA could also inhibit recruitment of coactivators. TNF-α: tumor necrosis factor-α; APC: antigen-presenting cell; Th: T helper; CCL: chemokine C–C ligand; CXC: C–X–C motif; CCR: chemokine C–C receptor.

Sasaki et al. [122] identified methyl ester derivative **1** through high-throughput screening and hit-to-lead optimization (Fig. 6). A series of new RORγt modulators were obtained by structure-activity relationship (SAR) research of the lead compound **1**. Compound **4** (S18-000003) as an ethylsulfonylbenzyl derivative was found to be a selective RORγt antagonist (Fig. 6) [122]. S18-000003 showed half maximal inhibitory concentration (IC_{50}) value of 29 nM in cell-based human RORγt-GAL4 promoter reporter assay and inhibited human Th17 differentiation with IC_{50} of 13 nM [122]. Further study showed that S18-000003 had an oral bioavailability of 54.5%, and oral S18-000003 inhibited IL-17 production in IL23-challenged mice [122]. Moreover, topical application of S18-000003 (0.1%–8%) in a 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-caused psoriasis model showed significant therapeutic effect dose-dependently with little thymus side effects [123].

Similarly, the selective antagonist A213 (IC_{50} for human Th17 differentiation, 4 nM) inhibited IL-17-secreting cells, and oral application of A213 (30–300 mg/kg) reduced skin inflammation in two mouse psoriatic models: the IL-23-induced model and the K5.Stat3C transgenic mice [124]. In addition, Liu et al. [125] performed structural modification on GSK805 and obtained compound **6** with excellent efficacy in vitro with IC_{50} value of 62 nM. A selective RORγ inverse agonist SHR168442 was found by SAR optimization (Fig. 7) [125]. SHR168442 inhibited *Il17* gene transcription with IC_{50} of 15 nM and decreased IL-17 cytokine production with IC_{50} of 20 nM [125]. In an animal model of imiquimod (IMQ)-mediated psoriasis-like inflammation, 4% and 8% SHR168442 displayed a remarkable decrease in erythematous scores in a dose-dependent manner, showing good skin limiting exposure.

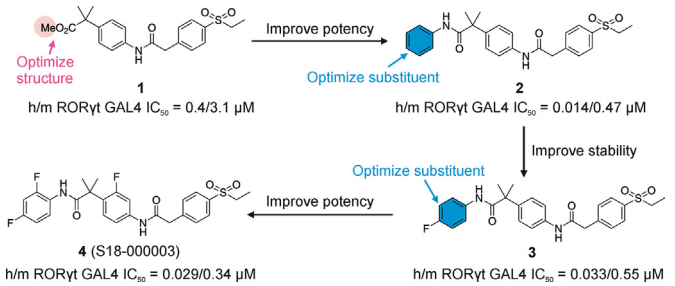


Fig. 6. Design strategy used from lead compound **1** to retinoid-associated orphan receptor γt (RORγt) inhibitor **4** (S18-0000003) showed high potency through a series of optimizations [122]. h/m RORγt GAL4: luciferase reporter assay for human/mouse RORγt; IC_{50}: half maximal inhibitory concentration. Reprinted from Ref. [122] with permission.

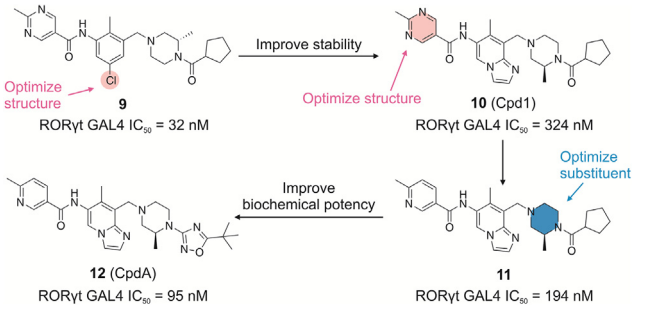


Fig. 8. The high potency retinoid-associated orphan receptor γ (RORγ) inhibitor **12** (CpdA) was optimized based on compound **9** [126]. IC_{50}: half maximal inhibitory concentration; RORγt GAL4: luciferase reporter assay for RORγt. Reprinted from Ref. [126] with permission.

Subsequently, in an animal model of IL-23-mediated psoriasis-like inflammation, skin thickness was improved by 97% after topical application of 8% SHR168442 compared to controls [125].

In addition, Hintermann et al. [126] found compound **9** to be a highly effective RORγt inverse agonist. However, its solubility and stability in human liver microsomes was low. Therefore, based on compound **9**, derivatives **10** and **11** were synthesized to increase the stability in the liver microsome assays [126]. Further SAR study revealed that **12** (CpdA) had higher biochemical potency (Fig. 8) [126]. It was found that CpdA had a good pharmacological effect, which inhibited the expression of RORγt-LBD via inhibiting the binding of RORE site at the promoter of the RORγt target gene, or by interfering with the cofactors of RORγt-LBD [127]. The IC_{50} value of RORγt-GAL4 promoter reporter gene was 95 nM [127]. CpdA selectively inhibited IL-17A expression in naive and memory $CD4^+$T cells (IC_{50} = 15 and 27 nM, respectively) [127]. CpdA reduced pro-inflammatory human keratinocytes and skin responses, and therefore had enormous opportunity in the treatment of psoriasis [127].

The inverse agonist TMP778 inhibited mouse Th17 differentiation with IC_{50} of 0.1 μM and regulated IL-17A production with IC_{50} of 0.1 μM, and the expression of Th17 signaling genes (*Il17a* and *Il17f*) was decreased in TMP778 treated mouse cells [128]. Genome-wide transcriptional profiling study showed that TMP778 had little effect on the expression of genes unrelated to the transcriptional characteristics of Th17, indicating that TMP778 had a high selectivity [129]. In vivo, subcutaneous injection of TMP778 reduced IMQ-induced psoriatic dermatitis in mice [129].

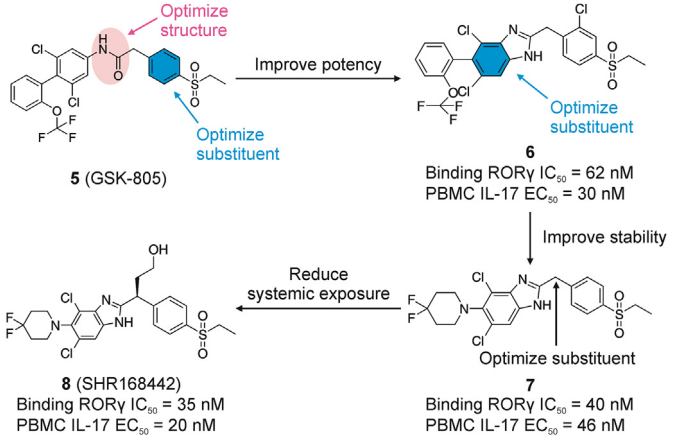


Fig. 7. Optimization of highly active and selective retinoid-associated orphan receptor γ (RORγ) inhibitor **8** (SHR168442) based on compound **5** (GSK-805) [125]. EC_{50}: concentration for 50% of maximal effect; IC_{50}: half maximal inhibitory concentration; PBMC: peripheral blood mononuclear cells. Reprinted from Ref. [125] with permission.

In addition to antagonists, inverse agonists of RORγt are also effective against psoriatic skin inflammation. BMS-986251, an RORγt inverse agonist, showed significant efficacy when taken orally (2–20 mg/kg) in acanthosis mouse and IMQ-induced model, and displayed dose-dependent blocking of secretion of IL-17F [130]. However, Haggerty et al. [131] found that BMS-986251 at a dose of 5 or 25 mg/kg resulted in the occurrence of thymus lymphoma in a 6-month rasH2-Tg mouse carcinogenicity study. Subsequently, Yang et al. [132] conducted SAR study on tricyclic-carbocyclic analogues and found that BMS-986313 was an RORγt inverse agonist with a structure different from that of BMS-986251. BMS-986313 showed concentration for 50% of maximal effect (EC_{50}) value of 3.6 nM in RORγt-GAL4 promoter reporter assay and EC_{50} value of 50 nM in IL-17-stimulated human whole blood (hWB) assay. It also showed strong efficacy at doses of 5–20 and 3–15 mg/kg in the IMQ-mediated skin lesion model and the IL-23-mediated acanthosis model [132]. Whether BMS-986313 possesses thymus toxicity similar to BMS-986251 is currently unknown.

In addition, the inverse agonist A-9758 inhibited the recruitment of co-activators PGC1α and NCoA1 with IC_{50} value of 49 and 110 nM, respectively, and increased the recruitment of co-suppressors NCoR1 and NCoR2 with EC_{50} of 60 and 43 nM, respectively [133]. A-9758 suppressed IL-17A secretion in human $CD4^+$ T cells and mouse Th17 with IC_{50} of 100 and 38 nM, respectively [133]. IL-23-induced psoriatic dermatitis was significantly alleviated by oral administration of A-9758 (1, 10, and 100 mg/kg) in vivo [133].

4.2. RA

Psoriatic arthritis shares many features with RA and destructive arthritis. A considerable elevation in IL-17-producing Th17 cell was reported in patients with RA, as well as elevated levels of IL-17, which synerges with TNF-α and IL-6 to aggravate inflammation [10]. It is showed that inhibition of RORγt could alleviate arthritis symptoms.

Digoxin is an inhibitor of RORγt transcription (IC_{50} for RORγ, 1.98 nM), which does not affect RORα activity [134]. However, high concentrations of digoxin were cytotoxic. 20,22-dihydrodigoxin-21,23-diol (Dig(dhd)) and digoxin-21-salicylidene (Dig(sal)) are non-cytotoxic derivatives (Fig. 9A) [134]. Dig(dhd) and Dig(sal) specifically inhibited the production of human IL-17 [134]. Digoxin inhibited Th17 differentiation and reduced IL-17 through binding to RORγt LBD [135]. Digoxin inhibited collagen-induced arthritis (CIA) and may be also used to treat other Th17-related diseases [136]. The antagonistic effect of digoxin may be due to its binding between helices H3 and H11 in

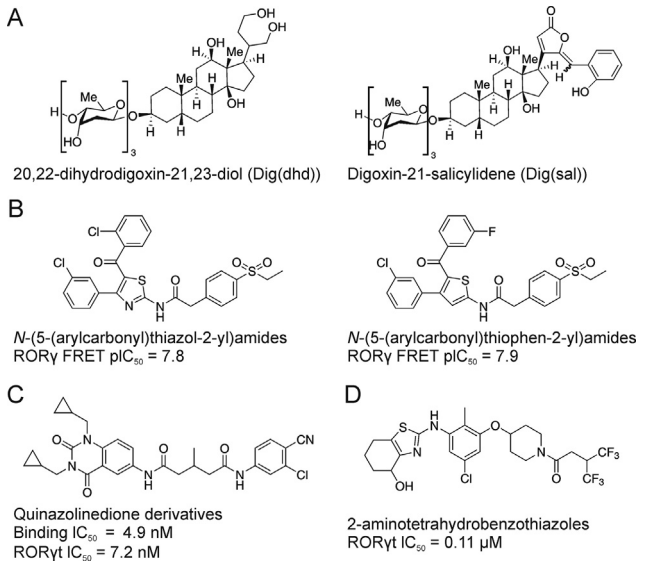


Fig. 9. Chemical structure of (A) digoxin derivatives [134], (B) *N*-(5-(arylcarbonyl) thiazol-2-yl)amides and *N*-(5-(arylcarbonyl)thiophen-2-yl)amides [148,149], (C) quinazolinedione derivatives [147], and (D) 2-aminotetrahydrobenzothiazoles [165]. FRET: fluorescence resonance energy transfer assay; IC_{50}: half maximal inhibitory concentration; pIC_{50}: −log (IC_{50}). Reprinted from Refs. [134,147–149,165] with permission.

the ligand-binding pocket of RORγt-LBD, resulting in a blockage of helical H12 localization in the active conformation, thus spatially impeding the formation of LBD-coactivators [135]. In addition, intraperitoneal injection of FC99 (100 mg/kg), a discovered benzenediamine derivative, inhibited zymosan-induced mouse arthritis and alleviated RA-like symptoms, which was associated with the reduction of RORγt expression in Th17 cells [137]. Imidazopyridine Cpd1, as a selective inverse agonist of RORγt, suppressed the generation of IL-17A with IC_{50} value of 60 nM [138]. Cpd1 bound to RORγt-LBD in place of the coactivating peptide, and attenuated the transcription of RORγt target gene. Further study showed that oral administration of the compound (15 or 45 mg/kg) alleviated the pathological symptoms of AIA in rats [138].

JNJ-61803534, an effective RORγ inverse agonist, effectively inhibited RORγt driven transcription. The compound inhibited IL-17A level dose-dependently in human and mouse with IC_{50} value of 230 nM and 172 nM, respectively [139]. In vivo, oral administration of JNJ-61803534 (3–100 mg/kg) reduced inflammation in models of CIA and IMQ-induced skin inflammation [139]. In clinical trial, the compound was well tolerated at a dose of 200 mg, with a plasma half-life of 164–170 h [139]. JNJ-54271074, an effective RORγt inverse agonist with high selectivity, suppressed RORγt-mediated transcription with IC_{50} of 9 nM, reduced co-activator recruitment, and increased interaction with co-repressor proteins [140]. Oral administration of JNJ-54271074 (10, 30 or 60 mg/kg) dose-dependently inhibited joint inflammation in CIA mice [140]. Moreover, SR2211 was an RORγ inverse agonist that inhibited primary T cell differentiation to Th17 and reduced IL-17 production, showing therapeutic effect in a CIA model and dose-dependent (8–20 mg/kg) [141].

4.3. MS/EAE

Th17 cells driven by IL-23 are widely acknowledged as pathogenic in animal models of human MS and EAE [142]. IL-23 and RORγt are critical for the expression of GM-CSF in Th17 cells. The expression of GM-CSF can lead to the occurrence of EAE. However, Th17 cells with the absence of *CSF2* encoding GM-CSF cannot induce EAE [60,143]. In MS, Th17/Treg cells are biased toward Th17 cells in terms of transcription factors, and Th17 and Treg cell development requires RORγt, RORα, and Foxp3 [144]. Therefore, MS pathogenesis is influenced by the imbalance of Th17/Treg cell-related responses. EAE is a disease model mediated mainly by specific sensitized $CD4^+$ T cells, which is an ideal animal model of MS. EAE has the same clinical, biochemical, immunological, and pathological characteristics with human MS. Therefore, MS pathogenesis and treatment have been extensively studied using this model.

Ursolic acid (UA) inhibited RORγt-LBD binding to the LXXLL-containing peptide in SRC1 and effectively inhibited RORγt function with IC_{50} of 0.68 μM, thereby inhibiting Th17 differentiation and IL-17 expression. UA administration could improve the disease symptoms of myelin oligodendrocyte glycoprotein-induced EAE in mice [145]. SR1001 bound to RORγt-LBD, induced conformational changes within the LBD, such as repositioning of H12, inhibited coactivators binding, and increased the recruitment of co-repressors, thereby inhibiting RORγt transcriptional activity [146]. In EAE, treatment with SR1001 (25 mg/kg) effectively hindered the progression of EAE and alleviated the disease symptoms of EAE [146]. In addition, the quinazolinedione derivatives [147], *N*-(5-(arylcarbonyl)thiazol-2-yl)amides and *N*-(5-(arylcarbonyl)thiophen-2-yl)amides (Figs. 9B and C) [147–149] were RORγt inhibitors and displayed in vivo effect in EAE models.

Kono et al. [150] optimized and synthesized a number of tetrahydropyridine derivatives. Firstly, the lipophilicity of tetrahydroisoquinoline **13** was decreased through substitution of the trimethylsilyl residue and structure-based drug design (SBDD)-guided scaffold exchange, and compounds **14** and **15** were obtained, which had low lipophilicity. Subsequently, optimization of the carboxylate tether revealed that compound **16** (TAK-828F) was a strong and specific oral inverse agonist of RORγt (Fig. 10) [150]. The regulator suppressed the recruitment of SRC1 with IC_{50} of 59 nM and blocked the transcription of RORγt with IC_{50} of 9.5 nM [151]. In mouse model of EAE, the administration of TAK-828F (0.3–3 mg/kg) decreased the symptoms of EAE dose-dependently, and further study suggested that it reduced the Th17 cell-mediated inflammatory response via decreasing IL-17A production [152].

In addition, as a selective RORγt inverse agonist, TMP778 significantly suppressed Th17 and reduced generation of IL-17. Subcutaneous administration of TMP778 slowed onset of the disease and significantly improved the progression of EAE [153]. In order to reduce the activity of RORα, in the SAR study of SR1001, Kumar et al. [154] found that SR2211, a selective RORγ inverse agonist, inhibited RORγ transcriptional activity with IC_{50} of 320 nM, thereby inhibiting IL-17 production and protecting mice from the development of EAE.

4.4. IBD

IBD is a specific chronic inflammatory condition in intestine that includes UC and CD. Although CD and UC are traditionally linked to Th1 or Th2 responses, it is now established that cytokines generated by Th17 are crucial for the pathophysiology of intestinal inflammation that affects patients with IBD [155]. The IL-17 level was obviously increased in IBD patients, and the expression of *IL23R* and *RORC* in $CD4^+$ cells was significantly upregulated [156,157]. Leppkes et al. [158] found that transfer of ROR-deficient T cells into the colon did not result in IL-17 secretion and initiation of colitis in RAG1/mice. Th17 is critical in the development of IBD, and RORγt presents as a new treatment target for IBD.

RORγt inverse agonist TAK-828F could improve the Th17/Treg imbalance by inhibiting Th17 differentiation and IL-17 generation

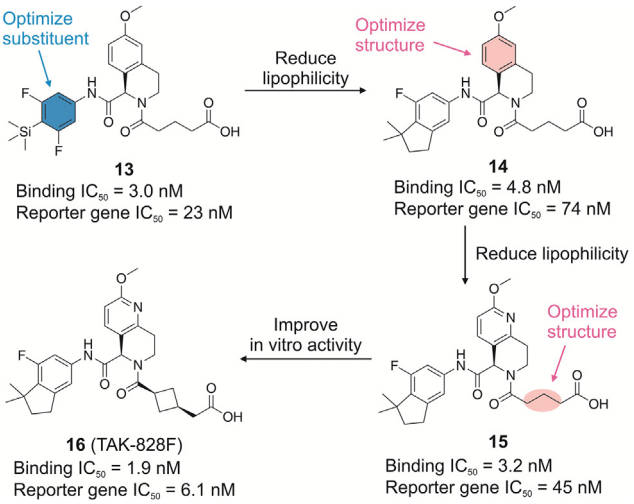


Fig. 10. Optimal design strategy from compound **13** to retinoid-associated orphan receptor γt (RORγt) inhibitor **16** (TAK-828F) [150]. IC_{50}: half maximal inhibitory concentration. Reprinted from Ref. [150] with permission.

in colon (IC_{50} in mouse and human whole blood assay, 720 nM and 120 nM, respectively) [159–161]. Further study showed that the inhibitor was orally effective at 1 or 3 mg/kg in colitis model [159–161]. RORγt inverse agonist GSK805 selectively inhibited Th17 differentiation and cytokine IL-17 secretion in vivo, and treatment of GSK805 (10 mg/kg, p.o.) suppressed the inflammatory response in mouse models (*IL-10*$^{-/-}$ mice and *Rag1*$^{-/-}$ mice) [162]. The RORγt inhibitor VPR-254 reduced IL-17 expression with an IC_{50} of 0.60 μM in vitro. VPR-254 was used to improve colitis symptoms in DSS-induced (15/25 mg/kg, i.p.), TNBS-induced (25 mg/kg, i.p.), and anti-CD40 antibody-induced (50 mg/kg, p.o.) IBD mouse models [163]. RORγt inverse agonist BI119 controlled the expression of the genes (*RORC, Il17a,* and *Il17f*) and their proteins [164]. In $CD4^+$ $CD45RB^{high}$ T cell transfer model, oral administration of BI119 (100 mg/kg) significantly improved the symptoms of enteritis [164]. In addition, 2-aminotetrahydrobenzothiazoles simultaneously inhibited RORγt/DHODH with an IC_{50} of 0.110 μM and alleviated acute colitis symptoms mediated by DSS in mice (Fig. 9D) [165].

4.5. Experimental autoimmune uveitis (EAU)

EAU is an animal model of severe intraocular inflammatory eye disease that is induced by Th1 and Th17 cells. Previous study demonstrated that TMP778 is a selective RORγt inhibitor. TMP778 significantly inhibited EAU development by reducing the secretion of IL-17 and by decreasing the number of lymphocytes expressing these cytokines [166]. Further study showed that TMP778 injected subcutaneously in the abdomen at 20 mg/kg significantly inhibited development of EAU [166]. Tan et al. [167] screened 1.3 million compounds and found that both CQMU151 and CQMU152 were non-steroid-type RORγt inverse agonists that inhibited Th17 cell differentiation. In vivo intraperitoneal injection of CQMU151 or CQMU152 at 25 mg/kg reduced the number of Th17 cells and the secretion of IL-17 in the retina of mice, thereby reducing severity of EAU [167].

4.6. Asthma

A chronic airway inflammatory disorder with specific feature of reversible blockage of the airways, AHR, and inflammation is termed allergic asthma. The role of IL-17 in asthma was further confirmed in a mouse model, where allergic sensitization of the airway induced a strong Th17 response, and elevation of IL-17 increased airway neutrophils and AHR resistance to steroid [31,32]. RORγt overexpression was associated with AHR with neutrophil airway inflammation in mice. RORγt is one of the main factors determining airway inflammation and steroid sensitivity phenotype in asthma [168]. Thus, blocking Th17 signaling may be effective in treating asthma that is resistant to steroids.

The RORγt inhibitor BIX119 specifically blocked IL-17 with IC_{50} of 120 nM and improved AHR and neutrophilia [169]. VTP-938, as a selective inverse agonist of RORγt, inhibited RORγ with IC_{50} value of 0.9 nM [170]. VTP-938 prevented activated RORγ-LBD from interacting with LXXLL and stimulated its interaction with the corepressor NCOR1 [170]. Additionally, VTP-938 inhibited activation of the IL-17 promoter in human. Oral administration of VTP-938 (30 mg/kg) inhibited allergen-induced IL-17 secretion and neutrophil inflammation in asthmatic animal models, and significantly reduced AHR [170].

5. Clinical application of RORγt inhibitors

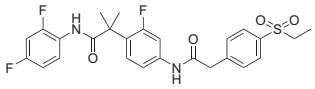
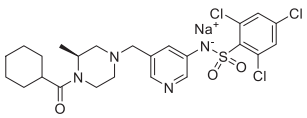
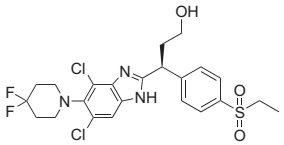
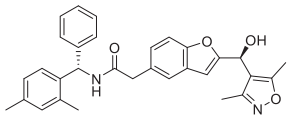
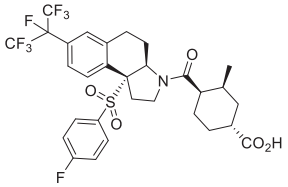
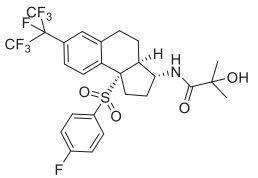
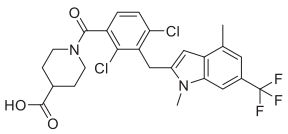
As summarized in Table 3 [122–125,128–130,132–141,145,146,150–154,159–164,166,167,169,170], numerous RORγt inhibitors (antagonists and inverse agonists) are actively being researched for the therapy of probable inflammatory disorders due to significance of RORγt in autoimmune and inflammatory diseases. Some of the reported RORγt inhibitors, such as JTE-451 (Retezorogant), BI730357 (Bevurogant), ABBV-157 (Cedirogant), RTA-1701 and AUR101, have also entered clinical trial evaluation (Table 4) [121,139,171–186]. However, it is irritating because of various safety concerns or a lack of effectiveness. Some drugs that were already undergoing clinical trials, including GSK2981278, PF-06763809, JNJ-61803534, VTP-43742 (Vimirogant), TAK-828F, and AZD0284, were halted or put on hold to allow for more research.

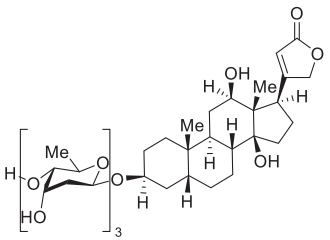
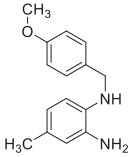
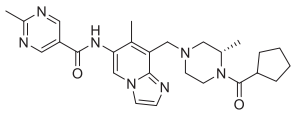
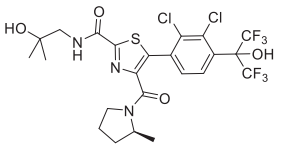
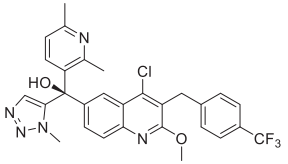
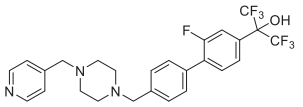
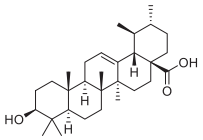
JTE-451, an RORγ antagonist developed by Japan Tobacco, was assessed in a 16-week phase II study in 152 patients for plaque psoriasis. The main clinical trial results were that oral administration of 100 and 200 mg of JTE-451 reduced psoriasis area and severity index (PASI) by 75% in 33.3% and 42.0% of subjects, respectively [172]. Its development as an oral drug was terminated in February 2021 and is now in phase I clinical trial for topical application [121].

Pharmacokinetic studies of BI730357, an RORγ antagonist, were completed in healthy volunteers in a phase I clinical trial [173]. A phase II trial based on its safety, tolerability, and efficacy was subsequently completed in 274 patients with plaque psoriasis. Oral administration of 25, 50, 100, and 200 mg of BI730357 reduced PASI by 75% in 5%, 7.7%, 10.3%, and 30% of subjects, respectively [174]. A medication called ABBV-157 was also being developed to treat persistent plaque psoriasis. A phase I clinical trial was completed in 65 healthy volunteers with plaque psoriasis [175]. Phase II trials based on the safety and efficacy of ABBV-157 were terminated in 2022 [176]. Phase I clinical trials of RTA-1701 have been completed, with safety, tolerability, pharmacokinetics, and pharmacodynamics evaluated based on RTA-1701 in 90 healthy volunteers [177]. AUR101, an RORγ inhibitor for moderate to severe psoriasis, was tested in a phase II study based on efficacy and safety studies in 90 patients with chronic psoriasis [178]. However, currently no results were posted for the studies of ABBV-157, RTA-1701, and AUR101.

GSK2981278 is an RORγ inverse agonist being developed for the therapy of topical plaque psoriasis. The safety, tolerability, and clinical efficacy of topical GSK2981278 ointment was evaluated in

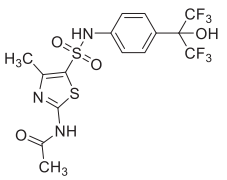
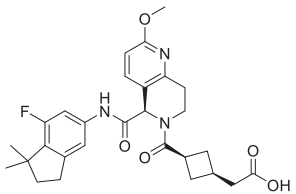
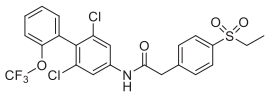
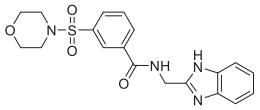
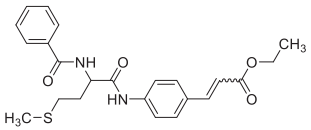
Table 3
The developed retinoid-associated orphan receptor (RORγt) small molecule inhibitors (antagonists and inverse agonists).

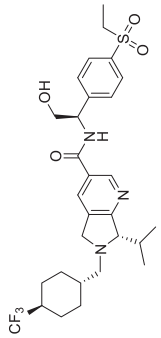
Name	Structure	Type	Characteristics and mechanisms		Refs.
			In vitro or in silico	In vivo	
S18-000003		Antagonist; ethylsulfonylbenzyl derivative	Competitive RORγt binding assay: <0.03 μM; h/m RORγt GAL4: IC_{50} = 29/340 nM (selective over RORα, RORβ, and LXRα/β); h/m Th17: IC_{50} = 13/200 nM; binding to RORγt-LBD	**Models:** IL-23-treated psoriasis mouse model; TPA-induced psoriasis K14.Stat3C transgenic mouse model **Finding:** (oral 30 or 100 mg/kg; topical 0.1%–8%) inhibiting Th17 differentiation and IL-17 production; oral bioavailable (54.5%); low risk of thymus side-effects	[122,123]
A213		Antagonist	Competitive binding assay: IC_{50} = 89 nM; mTh17: IC_{50} = 4 nM; hRORγt pSG5: >100 nM (selective over RORα, RORβ, and PPARγ) binding with RORγt LBD at H12	**Models:** IL-23-induced psoriasis model and K5.Stat3C transgenic mice **Finding:** (oral 30–300 mg/kg) inhibiting IL-17-producing cells	[124]
SHR168442		Inverse agonist	RORγ: IC_{50} = 15 ± 9 nM (selective over RORα)	**Models:** IMQ-induced and IL-23-induced psoriasis-like skin inflammation mouse models **Finding:** (8% SHR168442) inhibiting the production of IL-17	[125]
TMP778		Inverse agonist; benzofuran derivative	h/m IL-17: IC_{50} = 5/100 nM; mTh17: IC_{50} = 100 nM; FRET assay: IC_{50} = 7 nM (selective over RORα and RORβ)	**Models:** IMQ-induced cutaneous inflammation; EAE; EAU **Finding:** (5 or 20 mg/kg, s.c.) inhibiting Th17 differentiation and IL-17 production; inhibiting RORγt whole genome transcriptional profile	[128,129,166]
BMS-986251		Inverse agonist; carboxylic acid derivative	RORγt GAL4 EC_{50} = 12 nM (selective over PXR, LXRα, and LXRβ); hWB IL-17: EC_{50} = 24 nM	**Models:** IMQ-induced skin inflammation model **Finding:** (oral 20 mg/kg) inhibiting production of IL-17F	[130]
BMS-986313		Inverse agonist; tricyclic-carbocyclic analogues	RORγt GAL4: EC_{50} = 3.6 ± 2 nM (selective over RORα, RORβ, LXRα/β, and PXR); hWB IL-17: EC_{50} = 50 ± 13 nM; binding to RORγt-LBD	**Models:** IMQ-induced skin lesion model and the IL-23-induced acanthosis model **Finding:** (6 or 15 mg/kg) inhibition IL-17A and IL-17F mRNAs	[132]
A-9758		Inverse agonist	Radioligand competition binding assay: IC_{50} = 27 nM; h/m IL-17: IC_{50} = 100/38 nM	**Models:** IL-23-induced psoriatic dermatitis **Finding:** (oral 100 mg/kg) inhibiting the gene signature associated with IL-23 exposure; (60 mg/kg) inhibiting IL-17A production	[133]

Digoxin		Inverse agonist; cardiac glycosides	RORγ: IC_{50} = 1.98 μM (selective over RORα, DHR3, LXRα, and VP16) binding to RORγt-LBD	**Models:** collagen-induced arthritis **Finding:** (2 or 5 mg/kg, i.p.) reducing the proportion of Th17	[134–136]
FC99		Benzenediamine derivative	Inhibition of RORγt expression	**Models:** zymosan-induced arthritis **Finding:** (100 mg/kg, i.p.) inhibiting IL-17 production and reducing the proportion of Th17	[137]
Cpd1		Inverse agonist; imidazopyridines	RORγt: IC_{50} = 60 nM (selective over RORα and RORβ); hIL-17A: IC_{50} = 56 nM; hWB IL-17A: IC_{50} = 134 nM; binding to RORγt-LBD	**Models:** antigen-induced rat arthritis model **Finding:** (oral 15 or 45 mg/kg) inhibiting IL-17A production	[138]
JNJ-61803534		Inverse agonist	RORγt: IC_{50} = 9.6 ± 6 nM (selective over RORα and RORβ); h/m IL-17A: IC_{50} = 230/172 nM	**Models:** collagen-induced arthritis; IMQ-induced skin inflammation **Finding:** (oral 100 mg/kg) inhibiting IL-17A production	[139]
JNJ-54271074		Inverse agonist	RORγt: IC_{50} = 9 nM (selective over RORα, RORβ); hIL-17A: IC_{50} = 17 nM	**Models:** collagen-induced arthritis **Finding:** (60 mg/kg) inhibiting IL-17A-producing cells	[140]
SR2211		Inverse agonist	RORγ GAL4: IC_{50} = 320 nM (selective over RORα, LXR, and FXR)	**Models:** EAE; collagen-induced arthritis **Finding:** (20 mg/kg, i.p.) inhibiting Th17 differentiation and IL-17 production	[141,154]
Ursolic acid		Inverse agonist; carboxylic acid	RORγt: IC_{50} = 0.68 ± 0.1 μM (selective over RORα); Th17: IC_{50} = 0.56 ± 0.1 μM	**Models:** MOG-induced EAE **Finding:** inhibiting IL-17 production	[145]

(*continued on next page*)

Table 3 (*continued*)

Name	Structure	Type	Characteristics and mechanisms		Refs.
			In vitro or in silico	In vivo	
SR1001		Inverse agonist	RORγ: IC_{50} = 117 nM (selective over RORβ, LXRα); binding to RORγt-LBD	**Models:** EAE **Finding:** (25 mg/kg, i.p.) inhibiting IL-17 production	[146]
TAK-828F		Inverse agonist; tetrahydronaphthyridine derivative	h/m RORγt: IC_{50} = 6.1/9.5 nM (selective over RORα and RORβ); hIL-17: IC_{50} = 19 nM; h/mWB IL-17: IC_{50} = 430/720 nM	**Models:** EAE; IBD **Finding:** (oral 1 or 3 mg/kg) inhibiting Th17 differentiation and IL-17A gene expression	[150–153,159–161]
GSK805		Inverse agonist	Inhibition of Th17 cell differentiation and IL-17 production	**Models:** IBD **Finding:** (oral 10 mg/kg) reduced IL-17^+ Th17 cell	[162]
VPR-254	Unknown	Inverse agonist	RORγt: IC_{50} = 0.28 μM PBMC IL-17: IC_{50} = 0.6 μM	**Models:** DSS, TNBS, and anti-CD40 antibody-induced murine models of colitis **Finding:** (15 or 25 mg/kg, i.p.) inhibiting IL-17 production	[163]
BI119	Unknown	Inverse agonist	hRORγ: IC_{50} = 260 nM (selective over RORα and RORβ); binding to RORγt-LBD	**Models:** IBD **Finding:** (oral 100 mg/kg) inhibiting Th17-related genes and proteins	[164]
CQMU151		Inverse agonist	mTh17: EC_{50} = 13.43 μM; binding to RORγt-LBD (selective over RORα)	**Models:** EAU; EAE **Finding:** (25 mg/kg) inhibiting IL-17 production	[167]
CQMU152		Inverse agonist	mTh17: EC_{50} = 22.21 μM; binding to RORγt-LBD (selective over RORα)	**Models:** EAU; EAE **Finding:** (25 mg/kg) inhibiting IL-17 production	[167]
BIX119	Unknown	Inverse agonist	mWB IL-17: IC_{50} = 120 nM	**Models:** mouse house dust mite model **Finding:** (oral) inhibiting IL-17 and IL-22 production	[169]

VTP-938		Inverse agonist; thiazolopyrrolidine derivative	RORγ: IC_{50} = 0.9 nM (selective over RORα and RORβ); binding to RORγt-LBD	**Models:** HDE-induced allergic asthma [170] **Finding:** (oral 30 mg/kg) inhibiting Th17 differentiation and IL-17 production

h/m RORγt GAL4: luciferase reporter assay for human (h)/mouse (m) RORγt; IC_{50}: half maximal inhibitory concentration; Th: T helper; LBD: ligand-binding domain; IL: interleukin; TPA: 12-O-tetradecanoylphorbol-13-acetate; FRET: fluorescence resonance energy transfer; IMQ: imiquimod; EAE: experimental autoimmune encephalomyelitis; EAU: experimental autoimmune uveitis; EC_{50}: concentration for 50% of maximal effect; PXR: pregnane X receptor; LXR: liver X receptor; hWB: human whole blood assay; mRNA: messenger RNA; DHR: drosophila nuclear receptor; VP: viral protein; FXR: farnesoid X receptor; MOG: myelin oligodendrocyte glycoprotein; DSS: dextran sulfate sodium salt; PBMC: human peripheral blood mononuclear cell; TNBS: 2,4,6-trintirobenzenesulfonic acid; mWB: mouse whole blood assay; IBD: inflammatory bowel disease; HDE: house dust extract.

subjects with plaque psoriasis in phase I/II clinical trials. The results showed that topical use of 0.03%, 0.1%, 0.8%, and 4% of GSK2981278 ointment did not improve psoriatic lesions [179]. In a phase I randomized double-blind study of PF-06763809 in patients with plaque psoriasis, the inverse agonist showed acceptable safety parameters. But local use of 2.3%, 0.8%, or 0.23% PF-06763809 had no remarkable alleviation in psoriasis symptoms compared with controls. The negative result may be due to insufficient interaction (less contact or shorter duration) between the compound and *RORC2* in the skin [180]. In addition, physicochemical properties play an important role in topical administration. In general, the maximum flux of a drug through the skin is inversely proportional to the molecular weight of the compound, and the degree of absorption of the compound into the skin is bell-shaped in relation to the lipophilicity of the compound, while the receptor prefers molecules with larger molecular weight and more lipophilicity [181]. Therefore, it's hard to find compounds that can be absorbed through the skin and have good receptor affinity. JNJ-61803534 is a potent RORγt inhibitor, which could effectively improve IMQ-mediated skin inflammation in animal. It had a strong inhibitory effect on IL-23 and IL-17 pathways in preclinical studies and had good safety and efficacy in human subjects [139]. However, the study was terminated due to the toxicity of the compound in rabbit embryos [139]. Vitae Pharmaceuticals completed a phase I study of the RORγt-inverse agonist VTP-43742 [182]. A phase II study in patients with psoriasis also showed a signal of efficacy; however, the trial was subsequently terminated as reversible transaminase elevation was found in several patients [183]. The RORγ antagonist AZD0284 inhibited IL-17 secretion and showed good efficacy in imiquimod induced inflammation model, which successfully entered phase I clinical trials [184,185]. However, a phase I study of AZD0284, evaluating safety and efficacy in psoriasis, was discontinued in 2019 [186].

As outlined above, although many RORγt inhibitors have entered clinical trials, there are still some problems in clinical trials. First, some of them are lack of efficacy. This may be due to underexposure of the target drug (such as less exposure or shorter duration), especially for certain mode of administration such as topical administration. Second are the safety issues. In general, safety concerns are due to the selectivity of compounds to the RORα and RORβ isoforms or other related NRs. Inhibition of RORγt has also been reported to skew TCRα gene rearrangement and limit the diversity of T cell banks, which may also be related to the safety of the inhibitors [187]. Although inhibition of RORγ offers a prospect for the treatment of immune diseases, the risk of thymic aberration has been repeatedly reported. For example, the development of BMS-986251, an RORγt inverse agonist, was terminated as thymic lymphoma was observed [131]. Cpd1 and Cpd2 effectively inhibited Th17 cell development and production of related cytokines; however, they induced thymic aberration [188]. This has been a challenge in developing drugs that target RORγt. Therefore, it is necessary to explore safety issues in ongoing preclinical and clinical studies in depth. Finally, current clinical studies mainly focus on the use of RORγt inhibitors on treatment of psoriasis. The feasibility of the application of RORγt modulators for other conditions warrants more endeavors.

6. Conclusion and future perspective

The RORγt-Th17 axis is widely identified to be a key pathogenic factor in inflammatory and autoimmune diseases. RORγt, as an important transcriptional factor of Th17 cells, can promote Th17 to generate IL-17 and other cytokines, which can cause inflammatory or autoimmune diseases. Therefore, blocking of RORγt has become an ideal strategy for the therapy of these pathological conditions. It has been established by repeated experimental studies that RORγt

Table 4
Discontinued or completed clinical trials of retinoid-associated orphan receptor (RORγt) inhibitors.

Name	Clinical trial phase	Disease	Outcomes	Refs.
JNJ-61803534	Phase I (randomized/double-blind)	Healthy subjects	Terminated: due to rabbit embryo toxicity	[139]
JTE-451 (Retezorogant)	Phase II (randomized/double-blind)	Plaque psoriasis	Completed: efficacy and safety studies in 152 patients Results: oral administration of 100 and 200 mg of JTE-451 reduced psoriasis area and severity index (PASI) by 75% in 33.3% and 42.0% of subjects, respectively.	[121,172]
BI 730357 (Bevurogant)	Phase I (non-randomized)	Healthy subjects	Completed: study of pharmacokinetic Results: unpublished	[171,173,174]
	Phase II (randomized/double-blind)	Plaque psoriasis	Completed: tolerability and efficacy studies Results: oral administration of 25, 50, 100, and 200 mg of BI 730357 reduced PASI by 75% in 5%, 7.7%, 10.3%, and 30% of subjects, respectively.	
ABBV-157 (Cedirogant)	Phase I (randomized/double-blind)	Healthy subjects; chronic plaque psoriasis	Completed: pharmacokinetic, safety, and tolerability studies Results: unpublished	[175,176]
	Phase II (randomized/double-blind)	Psoriasis	Terminated: due to unknown reason	
RTA-1701	Phase I (randomized/double-blind)	Healthy subjects	Completed: safety, tolerability, pharmacokinetics, and pharmacodynamics studies Results: unpublished	[177]
AUR101	Phase II (randomized/double-blind)	Chronic plaque psoriasis	Completed: efficacy and safety studies Results: unpublished	[178]
GSK2981278	Phase I, phase II (randomized/double-blind)	Plaque psoriasis	Completed: study of safety and efficacy Results: psoriasis did not improve after treatment with GSK2981278 (0.03%, 0.1%, 0.8%, and 4%) or drug-loaded ointment, and scores were unchanged in most test areas (≥87%) at day 19.	[179]
PF-06763809	Phase I (randomized/double-blind)	Chronic plaque psoriasis	Completed: safety, tolerability and efficacy studies Results: It was well tolerated and had an acceptable safety profile. Local use of 2.3%, 0.8%, and 0.23% PF-06763809 did not reduce skin infiltration thickness or disease biomarkers.	[180,181]
VTP-43742 (Vimirogant)	Phase I (randomized/double-blind)	Healthy subjects; psoriasis	Completed: safety, tolerability, pharmacokinetics, and pharmacodynamics studies Results: unpublished	[182,183]
	Phase II	Psoriasis	Terminated: due to elevated reverse transaminase	
AZD0284	Phase I (randomized/double-blind)	Healthy subjects; plaque psoriasis	Terminated: under preclinical evaluation.	[184–186]

small-molecule inhibitors are crucial in treating a range of autoimmune illnesses, including psoriasis, RA, MS, IBD, etc.

However, RORγt inhibitors have not yet found with widespread clinical application. Some RORγt inhibitors have been found to have poor efficacy or poor safety in clinical trials, which are forced to stop further studies. In most cases, low potency and low specificity are the main reasons. For instance, in the structural optimization of RORγt inverse agonist, activation of the pregnane X receptor by RORγt inhibitors may lead to self-induction of cytochrome P450 enzyme, resulting in reduced exposure and efficacy of RORγt inhibitors. Moreover, RORγt inhibitors may display potential thymic side effects such as thymic lymphocytosis, thymic lymphoma, and thymic aberration. To obtain drugs with both efficacy and safety, known compounds are sometimes modified structurally to produce derivatives. Therefore, to ensure the smooth passage of RORγt inhibitors into the clinic, subsequent efforts may be focused more on increasing efficacy/selectivity or reducing toxicity.

In addition, most previous studies on RORγt inhibitors have focused on their use in autoimmune diseases, especially in the treatment of psoriasis. However, recently, RORγt inhibitors have been gradually found to be effective in steroid-insensitive allergic asthma and so on. Therefore, it may be a direction of future research investigating the influence of RORγt on IL-17-mediated inflammation and explore the application of RORγt inhibitors in more diseases.

CRediT author statement

Jiuping Zeng and **Mingxing Li:** Writing - Original draft preparation, Reviewing and Editing, Visualization; **Qianyun Zhao** and **Meijuan Chen:** Writing - Original draft preparation, Visualization; **Long Zhao**: Supervision, Writing - Reviewing and editing; **Shuin Wei** and **Huan Yang:** Writing - Original draft preparation; **Yueshui Zhao**, **Anqi Wang**, **Jing Shen**, **Fukuan Du**, **Yu Chen**, **Shuai Deng**, **Fang Wang**, and **Zhuo Zhang:** Visualization, Writing - Reviewing and Editing; **Zhi Li**, **Tiangang Wang**, and **Shengpeng Wang:** Visualization; **Zhangang Xiao**: Conceptualization, Writing - Reviewing and Editing; **Xu Wu**: Conceptualization, Writing - Reviewing and Editing, Visualization, Funding acquisition.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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