Acta Pharmacologica Sinica (2015) 36: 71–87
 1671-4083/15 $32.00
www.nature.com/aps

Review

ROR nuclear receptors: structures, related diseases, and drug discovery

Yan ZHANG[1], Xiao-yu LUO[1], Dong-hai WU[2, *], Yong XU[1, *]

[1]*Institute of Chemical Biology, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China;* [2]*The Key Laboratory of Regenerative Biology, The Guangdong Provincial Key Laboratory of Stem Cell and Regenerative Medicine, Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China*

Nuclear receptors (NRs) are ligand-regulated transcription factors that regulate metabolism, development and immunity. The NR superfamily is one of the major classes of drug targets for human diseases. Retinoic acid receptor-related orphan receptor (ROR) α, β and γ belong to the NR superfamily, and these receptors are still considered as 'orphan' receptors because the identification of their endogenous ligands has been controversial. Recent studies have demonstrated that these receptors are regulated by synthetic ligands, thus emerge as important drug targets for the treatment of multiple sclerosis, rheumatoid arthritis, psoriasis, *etc*. Studying the structural basis and ligand development of RORs will pave the way for a better understanding of the roles of these receptors in human diseases. Here, we review the structural basis, disease relevance, strategies for ligand identification, and current status of development of therapeutic ligands for RORs.

Keywords: nuclear receptor; retinoic acid receptor-related orphan receptor; autoimmune disease; metabolic disorder; rational drug design

Acta Pharmacologica Sinica (2015) 36: 71–87; doi: 10.1038/aps.2014.120; published online 15 Dec 2014

Introduction

The nuclear receptors (NRs) are a large family of ligand-regulated transcriptional factors and include the receptors for steroid hormones, thyroid hormones, lipophilic vitamins, and cholesterol metabolites[1-3]. NRs are involved in a wide variety of biological processes, such as cell proliferation, differentiation, development, and homeostasis[4, 5]. Dysfunction of NR signaling leads to various diseases such as cancer, diabetes, obesity, and autoimmune disorders. The NR superfamily is one of the primary classes of therapeutic drug targets for human disease. The agonists or antagonists of NRs account for approximately 13% of all FDA-approved drugs[6]. Among the top-selling drugs, these include tamoxifen for estrogen receptors in breast cancer, dexamethasone for the glucocorticoid receptor in inflammatory diseases and rosiglitazone for the peroxisome proliferator-activated receptor-γ (PPARγ) in diabetes.

The NR superfamily is composed of 48 members in humans. The members share high sequence identity and conserved domains (Figure 1). The typical domain structure of NRs comprises four major functional regions (Figure 1A). The A/B region refers to the amino-terminal ligand-independent activation function 1 (AF-1) domain. The C region is the highly conserved DNA-binding domain (DBD) and contains two zinc finger motifs to bind distinct DNA response elements. The relatively short but flexible D (hinge) region links the C region to the E region. The E/F region is the carboxy-terminal ligand-binding domain (LBD) with the ligand-dependent activation domain 2 (AF-2). AF-1 and the hinge region are the most divergent, whereas the DBD and LBD regions are the most conserved in sequence and length across the superfamily. Ligand binding induces a conformational change of the LBD and alters the surface to facilitate the recruitment of cofactor proteins, which transcriptionally regulates the induction or repression of target genes (Figure 2).

Approximately half of the NRs have well-characterized natural ligands, whereas the remaining receptors are classified as orphan NRs because they do not have well-characterized ligands[7]. Orphan NRs are an active area of research partly due to the potential for clinical agent development for various diseases[8]. Recent studies have demonstrated that retinoic acid receptor-related orphan receptors (RORs) have been implicated in several physiological and pathological processes.

* To whom correspondence should be addressed.
E-mail wu_donghai@gibh.ac.cn (Dong-hai WU);
xu_yong@gibh.ac.cn (Yong XU)
Received 2014-08-09 Accepted 2014-10-08

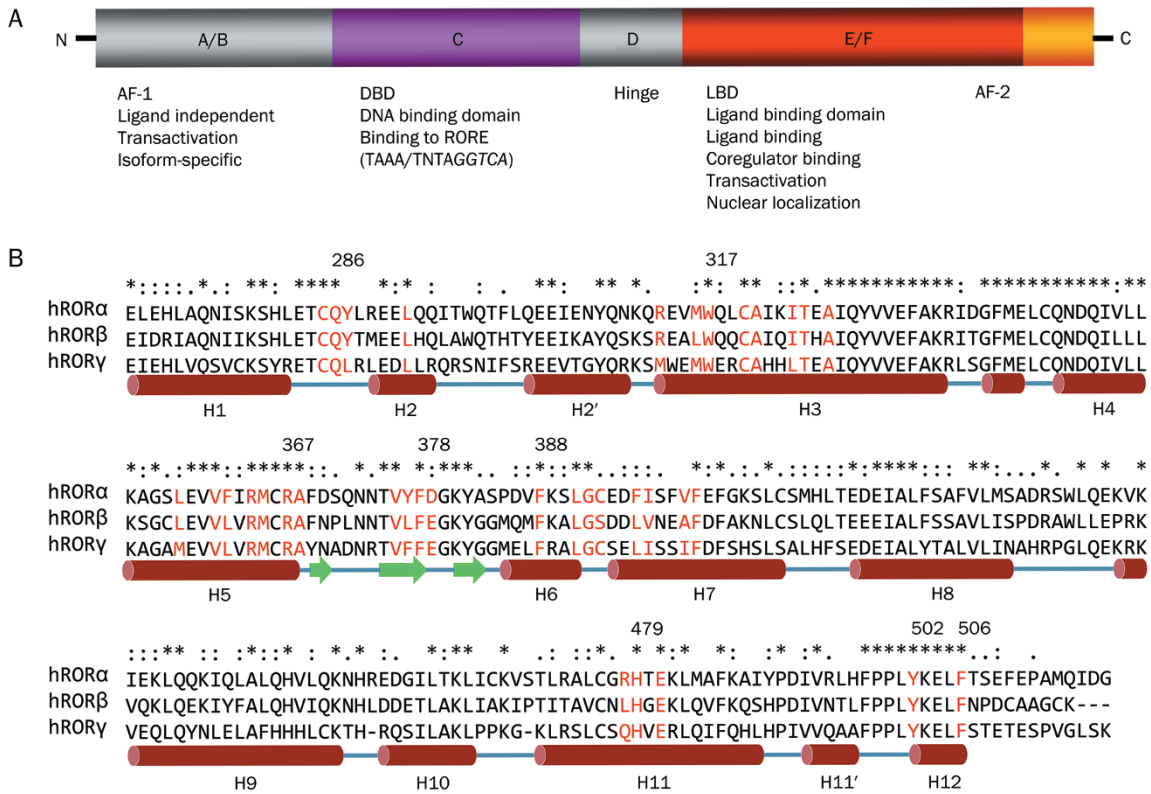


Figure 1. Structural organization of ROR functional domains. (A) Schematic diagram of the domain structure of RORs. Similar to other NRs, RORs display conserved modular domain architecture with a N-terminal ligand-independent activation function 1 (AF-1) domain, followed by a DNA binding domain (DBD), a hinge domain, and a ligand-binding domain with an activation function 2 (AF-2) domain. The DBD binds specific DNA sequences that typically consist of TAAA/TNTA*GGTCA* (termed ROR response element, RORE). (B) Sequence alignment of the ligand binding domain of RORα, RORβ, and RORγ performed using ClustalW. Cartoon presentation of the general architecture of RORs was shown under the corresponding sequences. Identical residues are labeled with an asterisk. Partially conserved residues are labeled with a colon. The residue numbering for RORα, RORβ, and RORγ are E305-G556, E222-K470, and E269-K518, respectively. Residues around the ligand are shown as red letters. Residues important for ligand binding were labeled on top of the sequences.

Therefore, RORs have emerged as important drug targets for the treatment of various diseases, such as multiple sclerosis, rheumatoid arthritis, and psoriasis.

Here, we review the structural basis of the ligand regulation mechanism and related diseases, and the strategies to identify potent and specific ROR modulators. The current status of ROR ligand development from both the literature and patents are also described with their therapeutic potentials.

RORs and ROR-related diseases

The ROR subfamily of transcription factors consists of RORα (NR1F1), RORβ (NR1F2) and RORγ (NR1F3) and has been identified in several mammalian species that exhibit tissue-specific expression of these transcription factors[9, 10]. Each ROR gene generates several receptor isoforms that differ in their amino terminus in humans and rodents because of alternative promoter usage and splicing[11]. The first member of the ROR subfamily of NRs (RORα) was identified in the 1990s based on sequence similarities to the retinoic acid receptor (RAR) and the retinoid X receptor (RXR), which yielded the name 'retinoic acid receptor-related orphan receptor alpha'[12]. RORβ and RORγ were subsequently identified[13, 14]. RORα, RORβ, and RORγ display distinct patterns of tissue expression. RORα is widely expressed in liver, skeletal muscle, skin, lung, adipose tissue, kidney, thymus, and brain[15, 16]. RORβ exhibits a more restricted neuronal-specific expression pattern in the brain, retina, and pineal gland[17, 18]. RORγ is highly expressed in thymus (the thymus-specific isoform is referred to as RORγt), muscle, testis, pancreas, prostate, heart, and liver[10, 19]. The RORs are somewhat unusual in that they recognize and bind as monomers to specific DNA sequences (typically consisting of TAAA/TNTA*GGTCA*), termed ROR response elements (ROREs), as opposed to the majority of other NRs, which bind as dimers[4, 20]. When bound to this response element within the promoter of a target gene, RORs constitutively recruit coactivators, which lead to the transcriptional activation of their target genes. By contrast, another group of orphan NRs, the REV-ERBs, repress transcription by recognizing the same response elements and functionally antagonize the action of the RORs in many cases[21–23]. Recent advances have established that selective inhibition of RORs is a promising therapeutic approach for the treatment of autoim-

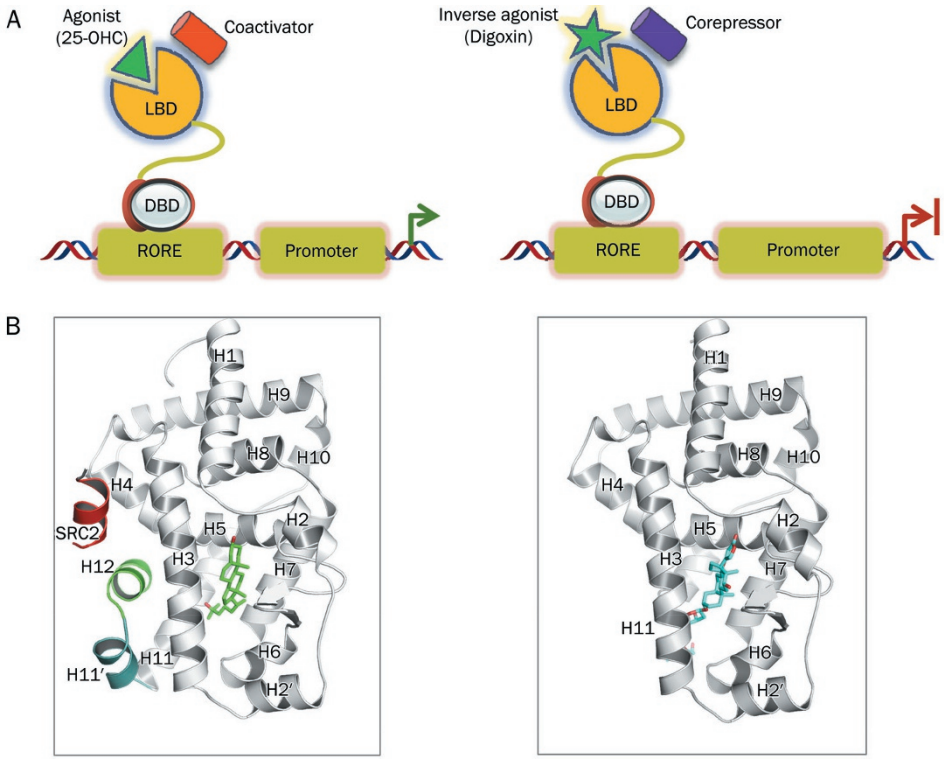


Figure 2. Structural model of ROR agonism and antagonism. (A) RORγ agonists, such as 25-hydroxycholesterol, drive recruitment of transcriptional coactivators, which leads to the modulation and promotion of target gene transcription. Inverse agonists of RORγ, such as digoxin, disrupt recruitment of the transcriptional coactivator and repress target gene expression. (B) Agonist binding induces a conformational change and facilitates binding of the LXXLL motif of coactivators, such as SRC2. Antagonists, such as digoxin, induce a conformational change of helix 12 and circumvent the coactivator recruitment. The coactivator protein and helix 12 are colored in red and green, respectively. The agonist (left, 3L0L.pdb) and inverse agonist (right, 3B0W.pdb) are shown as sticks.

mune diseases, metabolic disorders and some cancers[24–30].

RORs and autoimmune diseases

RORα and RORγt (an isoform of RORγ) are considered to be the master regulators of the development of T helper 17 cells (Th17 cells), which have an essential role in the development of many autoimmune disorders, including multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease and psoriasis[31, 32]. Both RORα and RORγt are required for the full differentiation of naïve $CD4^+$ T cells into interleukin 17 (IL-17)-producing Th17 cells[33–36]. Th17 cells produce numerous cytokines, including interleukin-17 (IL-17), that are known to enhance inflammatory processes. IL-17A expression is directly regulated by RORs through their interaction with ROREs in the IL-17 promoter[33]. The discovery of Th17 cells as critical mediators of autoimmune disorders provides a unique opportunity to develop focused therapeutics that act by inhibiting the function of these cells. The genetic ablation of RORγ alone, or in combination with RORα in mice, led to impaired Th17 cell differentiation and protected the mice from the development of experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis[34]. These data suggest that the targeted inhibition of RORα and RORγ with specific synthetic ligands could potentially provide a means for reducing autoimmune pathology. Both RORα and RORγ, specifically RORγt, have gained significant attention over the past few years because of their essential role in the development of Th17 cells. Therefore, pharmacological repression of RORγ might represent an attractive starting point for the development of a novel therapy for treating inflammatory diseases. The current treatments for known Th17-mediated autoimmune diseases use immunosuppressants with significant side effects. Targeting RORs represents a significant advantage over the current therapies because they specifically target the one arm of the immune system that mediates disease instead of the immune system as a whole.

RORs and metabolic disorder

Both RORα and RORγ play important roles in glucose and lipid metabolism, which is exemplified by the phenotypes of mice with mutations in RORα or RORγ[37, 38]. The staggerer mouse ($RORα^{sg/sg}$) is a natural mutant strain of mouse lacking functional RORα; these animals have lower total plasma cholesterol levels compared with wild-type mice[39]. Loss of RORα in *staggerer* mice results in mice that are resistant to weight gain and hepatic steatosis when placed on a high-fat diet[38]. Suppression of RORα activity may also lead to a decrease in the elevated hepatic glucose output; therefore, RORα inverse

agonists may hold utility in the treatment of metabolic disorders, such as type 2 diabetes[40, 41].

RORγ$^{-/-}$ mice display normal cholesterol and triglyceride levels but slightly reduced blood glucose levels compared with their wild-type counterparts[37]. In double knockout mice, a similar reduction in cholesterol, triglyceride, and blood glucose levels was observed compared with a single knockout. These findings suggest that RORα and RORγ inverse agonists may hold therapeutic potential for the treatment of metabolic syndrome and associated diseases.

Beyond autoimmunity and metabolic diseases, the RORs also offer the potential for the development of drugs that target a range of disorders, such as asthma and cancer[42–44].

Structural basis of RORs

A typical NR LBD exhibits similar structural features with a three-layered fold of approximately 12 alpha-helices and 2–3 β-strands. A hydrophobic ligand binding pocket resides within the bottom portion of the LBD (Figure 2B). The helix 12 (also called AF-2) can adopt multiple conformations depending on the different bound ligands (agonist, inverse agonist or antagonist). Therefore, the LBD can interact with a coactivator or a corepressor to activate or repress gene transcription in the nucleus. Upon the binding of an agonist, the helix 12 along with another region of the LBD forms a hydrophobic groove for the binding of a coactivator (such as steroid receptor coactivator, SRC, Figure 2B). The interaction of the LBD with the coactivator will change upon the binding of an inverse agonist (if the NR has constitutive transcriptional activity). Specifically, helix 12 will change its conformation and position relative to the LBD core and therefore no longer interact with either a coactivator or a corepressor (Figure 2B).

The first co-crystal structure of the ROR subfamily was that of RORβ bound with stearic acid (Table 1, **1**), a fortuitous ligand[45]. All-*trans* retinoic acid (ATRA, **2**) and the synthetic retinoid ALTA 1550 (**3**) were subsequently identified as putative functional ligands and co-crystallized with RORβ[46]. These ligands function as partial antagonists and inhibit RORβ transcriptional activity. Cholesterol (**4**) and cholesterol sulfate (**5**) were subsequently identified by co-crystallization within the ligand binding pocket of RORα in the agonist-bound state, as suggested by a bound coactivator peptide[47, 48]. The first batch of crystal structures of RORγ bound to its agonists 20α-hydroxycholesterol, 22*R*-hydroxycholesterol and 25-hydroxycholesterol (**6**, **7**, **8**) were determined by Jin *et al* in 2010[49]. These structures revealed that these ligands bind to RORγ in an active conformation with helix 12 positioned for coactivator (SRC2-2) recruitment. In 2011, digoxin (**10**) was identified as an inverse agonist and the crystal structure of the RORγ LBD in complex with digoxin was determined by Fujita-Sato *et al*[50]. To date, at least 3 more complex structures have been solved for the synthetic RORγ agonist (**30**) and inverse agonists (**11**, **38**). This structural information illustrated the mechanism of action for agonists or inverse agonists. It is very promising that the structure-guided optimization of ligands can facilitate the development of highly selective and potent ROR modulators.

The crystal structures of RORγ LBD bound with compounds **6**, **7**, and **8** demonstrated that these types of agonists occupy the entire hydrophobic pocket (Figure 3A). They are oriented with the hydroxyl tail toward helix 11 and the A ring toward helixes 1 and 2. The 3β-hydroxyl group of **8** forms a direct hydrogen bond interaction with Gln286 and two water-mediated hydrogen bonds with residues Arg367 and Arg364. The 25-hydroxyl group made one direct hydrogen bond with His479 (helix 11) and one water-mediated hydrogen bond with Try502 (helix 12), thereby stabilizing the π-π interaction network composed of His479, Tyr502 and Phe506 (helix 12). This π-π interaction network, along with Gln487 (helix 11) and Ser507 (helix 12), favors coactivator (SRC2 in this structure) recruitment.

The RORγ LBD crystal structure with bound digoxin demonstrated that digoxin forms extensive hydrophobic and hydrophilic interactions with RORγ (Figure 3B). Digoxin forms 4 direct hydrogen bonds with Arg367, Phe377, His479 (helix 11) and Leu391 (helix 6) and **4** water-mediated hydrogen bonds with Glu379 and Val361 (helix 5). Digoxin disrupts the polar interaction observed in the **8**-bound RORγ LBD, involving His479, Tyr502, and Phe506, which could be important to stabilize the active agonist conformation. Digoxin binding also induces a side chain conformational change in Trp317 (helix 3), which could cause the large movement of helix 12. It is clear that digitoxose Y and Z protrudes between helixes 3 and 11, and prevents the positioning of helix 12 in the active conformation, as observed in the RORγ LBD complexes with **8**.

The benzenesulfonamide compound T0901317 (**11**) was initially identified as a potent agonist of the liver X receptor[51]. When a team from the Scripps Research Institute in Florida performed a screen to profile the activity of a collection of well-characterized NR ligands against all human NRs, they identified T0901317 as a potent inverse agonist of RORα and RORγ. Fauber *et al* from Genentech, Inc obtained the complex crystal structure of T0901317 and RORγ. This structure provided a detailed molecular insight into why T0901317 functioned as an inverse agonist of RORγ but an agonist of FXR, LXR, and PXR. There are several unique structural features in this complex structure in comparison with the structures of other complexes (Figure 3C). T0901317, because of its small size, does not occupy the polar pocket formed by Try281, Gln286, Arg364, Arg367, and Phe378. The phenylsulfonamide group of T0901317 forms a unique π–π stacking interaction with residues Phe378 and Phe388. This interaction was not observed in the structures of LXR and PXR in complex with T9091317, or RORγ in complex with other hydroxycholesterol and digoxin derivatives. The hexafluoroisopropanol hydroxyl group forms a strong hydrogen bond interaction with the His421 residue in LXRα, which results in stabilization of helixes 11 and 12 and the recruitment of the coactivator peptide. However, there are no obvious hydrogen bonds from this hydroxyl group to the protein. The His479 residue undergoes a conformational change because one of the trifluo-

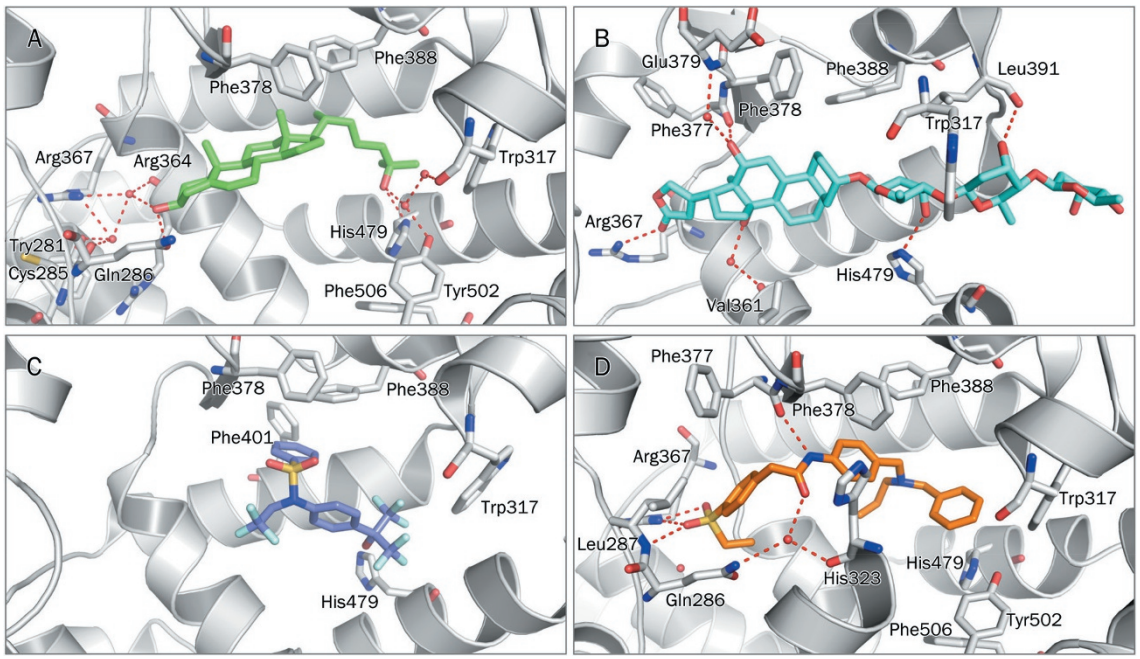


Figure 3. Comparison of the binding of 25-hydroxycholesterol (A), digoxin (B), T0901317 (C), and GSK-2 (D) in the RORγ ligand-binding pocket. Hydrogen bonds are depicted as dashed lines (red). (A) 25-OHC (green) formed direct hydrogen bonds with Gln286 and His479, and water-mediated hydrogen bonds with Arg364, Arg367, and Tyr502. (B) Digoxin (cyan) formed direct hydrogen bonds with Arg367, Phe377, His479, and Leu391, and water-mediated hydrogen bonds with Val361 and Glu379. (C) The phenylsulfonamide group of T0901317 (purple) forms a π−π stacking interaction with Phe378 and Phe388. The ligand and His479 disrupt the interaction network originally formed with Trp317, Tyr502, and Phe506. (D) GSK-2 (orange) formed direct hydrogen bonds with Leu287, Arg367, and Phe377. The amide carbonyl of GSK-2 formed a water-mediated hydrogen bond with Gln286 and His323. The aniline ring of GSK-2 also formed a π−π stacking interaction with Phe378 and Phe388.

romethyl groups occupies its original position. In this case, the ligand and His479 destabilize the interaction network that was originally formed with Trp317, Try502, and Phe506, and disrupt coactivator recruitment. This effect may also explain why T0901317 behaved as an inverse agonist of RORγ.

A team from GlaxoSmithKline developed a series of tertiary amine derivatives as RORγ inverse agonists[52, 53]. However, they only determined the structure of RORγ with agonist **30** (Figure 3D). From this structure, one can clearly determine that this series of ligands form several direct or water-mediated hydrogen bonds. The sulfone moiety forms hydrogen bonds with Arg367 and Leu287. The amide forms one direct hydrogen bond with Phe377 and water-mediated hydrogen bonds with Glu286 and His323. The phenyl group interacts with Trp317 and His479 and therefore stabilizes the network with Try502 and Phe506 in the active conformation. However, the 4-CF_3-phenyl group will disrupt this interaction, and helix 12 cannot maintain its active conformation[52]. This finding may explain why a close derivative with the 4-CF_3-phenyl group behaved as an inverse agonist of RORγ, whereas **30** was an agonist. There is another published crystal structure by Genentech (4QM0.pdb)[54].

All of these crystal structures of the RORγ LBD in complex with its ligands provide the basis for rational drug design to obtain more potent and specific RORγ modulators.

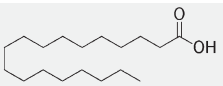
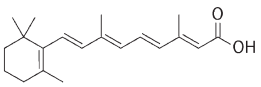
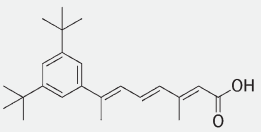
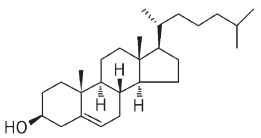
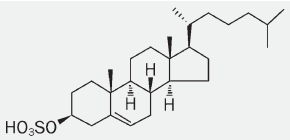
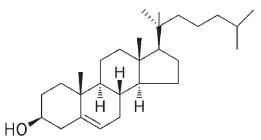
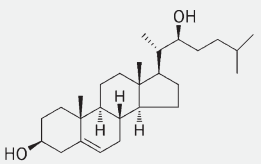
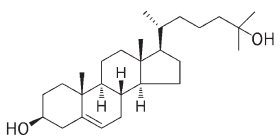
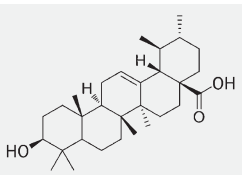
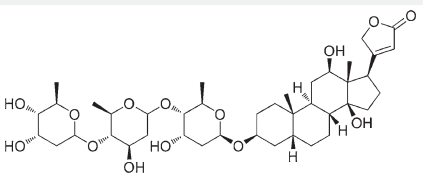
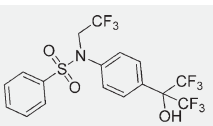
Strategies for the discovery of NR ligands

The identification of potential drug candidates for NRs represents a promising therapeutic approach to various diseases. Several drug development strategies have been developed to identify compounds that bind to NR LBD. Some approaches are rapid, accurate and easy to be developed in a high-throughput screen format to measure the NR-ligand interaction. These strategies are described below.

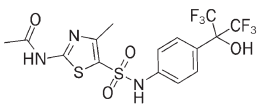
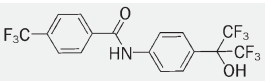
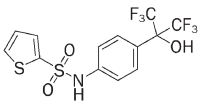
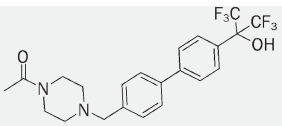
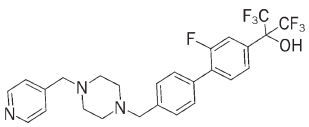
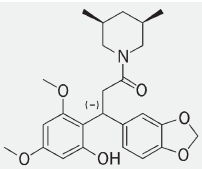
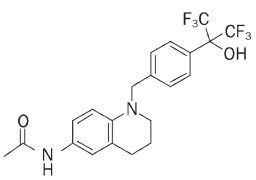
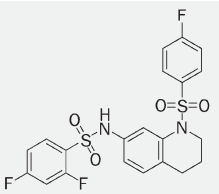
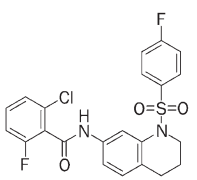
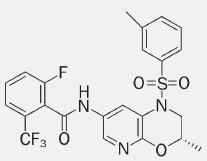
AlphaScreen assay

AlphaScreen, a bead-based Amplified Luminescent Proximity Homogeneous Assay, was first described in 1994 by Ullman based on the principle of luminescent oxygen channeling[55, 56]. The donor and acceptor beads are brought into close proximity when a molecular interaction of binding partners immobilized to these beads occurs (Figure 4A). The excitation of the assay mixture with a high intensity laser at 680 nm initiates a luminescence/fluorescence cascade in the acceptor beads and leads to a highly amplified signal with light output at 520–620 nm. When the acceptor and donor beads are not in proximity, only a very low background signal is generated. The major advantages of AlphaScreen include the high sensitivity, large signal/background ratio, and low reaction volumes (25–40 μL). AlphaScreen has become one of the best methods for the high-throughput screening for the detection of recep-

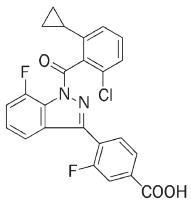
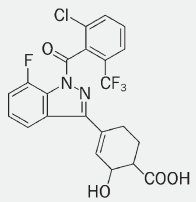
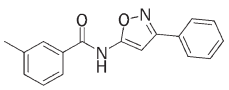
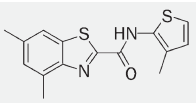
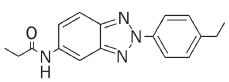
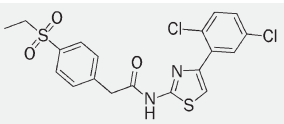
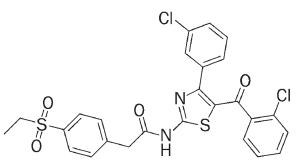
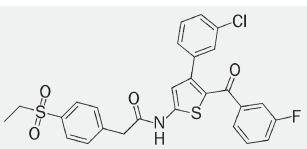
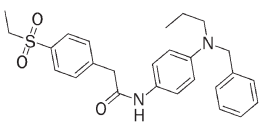
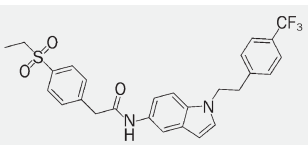
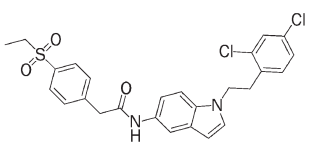
Table 1. Natural and synthetic ligands of RORs.

No	Name	Structure	Subtype activity	PDB ID	Refs
1	Stearic acid		RORβ antagonist	1K4W.pdb (SCR1-2) 1.90 Å	[45]
2	All-*trans* retinoic acid		RORβ antagonist K_d=280 nmol/L (E) K_i=280 nmol/L (E) IC_{50}=0.15 nmol/L (C)	1N4H.pdb (SCR1-2) 2.10 Å	[46]
3	ALTA 1550		RORβ antagonist K_i=160 nmol/L (E) IC_{50}=0.039 nmol/L (C)	1NQ7.pdb (SCR1-2) 1.50 Å	[46]
4	Cholesterol		RORα agonist EC_{50}=200 nmol/L (F)	1N83.pdb 1.63 Å	[48]
5	Cholesterol sulfate		RORα agonist	1S0X.pdb (SCR1-2) 2.20 Å	[47]
6	20α-Hydroxy cholesterol		RORγ agonist EC_{50}=20–40 nmol/L (F)	3KYT.pdb (SCR2-2) 2.35 Å	[49]
7	22(*R*)-Hydroxy cholesterol		RORγ agonist EC_{50}=20–40 nmol/L (F)	3L0J.pdb (SCR2-2) 2.40 Å	[49]
8	25-Hydroxy cholesterol		RORγ agonist EC_{50}=20–40 nmol/L (F)	3L0L.pdb (SCR2-2) 1.74 Å	[49]
9	Ursolic acid		RORγ inverse agonist IC_{50}=680 nmol/L (A)		[86]
10	Digoxin		RORγ inverse agonist IC_{50}=1.98 μmol/L (C) IC_{50}=4.1 μmol/L (D)	3B0W.pdb 2.20 Å	[30, 50]
11	T0901317		RORα/γ inverse agonist RORα IC_{50}=2.0 μmol/L (C) RORγ IC_{50}=1.7 μmol/L (C) RORα K_i=132 nmol/L (E) RORγ K_i=51 nmol/L (E)	4NB6.pdb 2.85 Å	[87, 103]

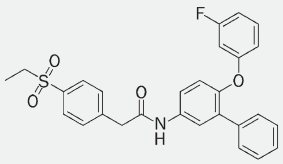
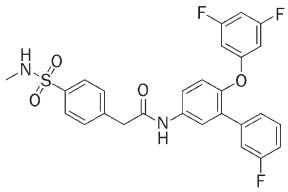
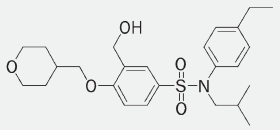
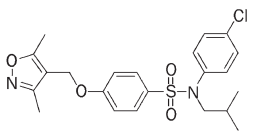
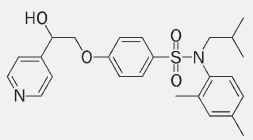
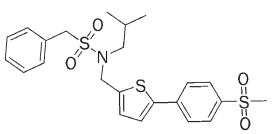
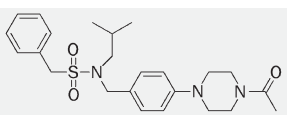
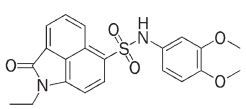
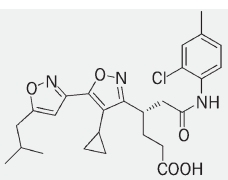
(To be continued)

No	Name	Structure	Subtype activity	PDB ID	Refs
12	SR1001		RORα/γ inverse agonist RORα K_i=172 nmol/L (E) RORγ K_i=111 nmol/L (E) RORγ IC_{50}=117 nmol/L (F)		[29]
13	SR1078		RORα/γ inverse agonist IC_{50}=1–3 μmol/L		[88]
14	SR3335		RORα inverse agonist IC_{50}=480 nmol/L (C) K_i=220 nmol/L (E)		[41]
15	SR1555		RORγ inverse agonist IC_{50}=1.5 μmol/L (C) IC_{50}=1.0 μmol/L (E)		[90]
16	SR2211		RORγ inverse agonist IC_{50}=320 nmol/L (C) K_i=105 nmol/L (E)		[89]
17	ML209		RORγ inverse agonist IC_{50}=500 nmol/L (C) IC_{50}=51 nmol/L (D)		[91, 92]
18	N-(1-(4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)benzyl)-1,2,3,4-tetrahydroquinolin-6-yl)acetamide		RORγ inverse agonist EC_{50}<30 μmol/L (A) IC_{50}<10 μmol/L (B)		[93]
19	2,4-difluoro-N-(1-((4-fluorophenyl)sulfonyl)-1,2,3,4-tetrahydroquinolin-7-yl) benzenesulfonamide		RORγ inverse agonist IC_{50}<1 μmol/L (C)		
20	2-Chloro-6-fluoro-N-(1-((4-fluorophenyl) sulfonyl)-1,2,3,4-tetrahydroquinolin-7-yl)benzamide		RORγ inverse agonist IC_{50}<15 μmol/L (C) IC_{50}<15 μmol/L (A)		[94]
21	(S)-2-fluoro-N-(3-methyl-1-(m-tolyl-sulfonyl)-2,3-dihydro-1H-pyrido[2,3-b] [1,4]oxazin-7-yl)-6-(trifluoromethyl) benzamide		RORγ inverse agonist EC_{50}=6 nmol/L (A)		[95]

(To be continued)

No	Name	Structure	Subtype activity	PDB ID	Refs
22	4-(1-(2-Chloro-6-cyclopropylbenzoyl)-7-fluoro-1*H*-indazol-3-yl)-3-fluorobenzoic acid		RORγ inverse agonist IC_{50}=2 nmol/L (A)		[96]
23	4-(1-(2-Chloro-6-(trifluoromethyl) benzoyl)-7-fluoro-1*H*-indazol-3-yl)-2-hydroxycyclohex-3-enecarboxylic acid		RORγ inverse agonist IC_{50}=2 nmol/L (A)		[96]
24	GSK-1a		RORγ agonist EC_{50}=~100 nmol/L (G)		[97]
25	GSK-1b		RORγ agonist EC_{50}=~100 nmol/L (G)		[97]
26	GSK-1c		RORγ agonist EC_{50}=~100 nmol/L (G)		[97]
27	GSK-6a		RORγ inverse agonist pIC_{50}=6.0 (A)		[53]
28	GSK-8h		RORγ inverse agonist pIC_{50}=7.8 (A) pIC_{50}=6.7 (B)		[53]
29	GSK-9g		RORγ inverse agonist pIC_{50}=7.8 (A) pIC_{50}=7.9 (B)		[53]
30	GSK-2		RORγ agonist EC_{50}=20 nmol/L (A)	4NIE.pdb (SCR2-2) 2.01 Å	[52]
31	GSK-13		RORγ inverse agonist IC_{50}=5 nmol/L (A) IC_{50}=31 nmol/L (B)		[52]
32	GSK-21		RORγ inverse agonist IC_{50}=6 nmol/L (A) IC_{50}=9 nmol/L (B)		[52]

(To be continued)

No	Name	Structure	Subtype activity	PDB ID	Refs
33	2-(4-(Ethylsulfonyl)phenyl)-N-(6-(3-fluorophenoxy)-[1,1′-biphenyl]-3-yl) acetamide		RORγ inverse agonist IC_{50}<50 nmol/L (A)		[98]
34	N-(6-(3,5-difluorophenoxy)-3′-fluoro-[1,1′-biphenyl]-3-yl)-2-(4-(N-methyl-sulfamoyl)phenyl)acetamide		RORγ inverse agonist IC_{50}<50 nmol/L (A)		[98]
35	N-(4-Ethylphenyl)-3-(hydroxymethyl)-N-isobutyl-4-((tetrahydro-2*H*-pyran-4-yl) methoxy)benzenesulfonamide		RORγ inverse agonist pIC_{50}=7.7 (A) pIC_{50}=7.5 (B)		[100]
36	N-(4-chlorophenyl)-4-((3,5-dimethyl-isoxazol-4-yl)methoxy)-N-isobutyl-benzenesulfonamide		RORγ inverse agonist pIC_{50}>7.8 (A) pIC_{50}>6.0 (B)		[100]
37	N-(2,4-dimethylphenyl)-4-(2-hydroxy-2-(pyridin-4-yl)ethoxy)-N-isobutyl-benzenesulfonamide		RORγ inverse agonist pIC_{50}>7.8 (A) pIC_{50}>6.0 (B)		[99]
38	N-isobutyl-N-((5-(4-(methylsulfonyl) phenyl)thiophen-2-yl)methyl)-1-phenylmethanesulfonamide		RORγ inverse agonist IC_{50}=130 nmol/L (E)	4QM0.pdb	[54, 101]
39	N-(4-(4-acetylpiperazin-1-yl)benzyl)-N-isobutyl-1-phenylmethanesulfonamide		RORγ inverse agonist EC_{50}=120 nmol/L (C) EC_{50}=57 nmol/L (A)		[54]
40	N-(3,4-dimethoxyphenyl)-1-ethyl-2-oxo-1,2-dihydrobenzo[cd]indole-6-sulfonamide		RORγ inverse agonist IC_{50}=50 nmol/L (C)		[62]
41	JTE-151		RORγ antagonist EC_{50}<3 μmol/L (C)		[102]

(A) TR-FRET (co-activator binding assays); (B) Th17 cell differentiation assay; (C) GAL4-DBD NR-LBD luciferase reporter assay; (D) Fluorescence polarization displacement assay; (E) Radioligand binding assay; (F) AlphaScreen assay; (G) IL-17 reporter assay.

tor-ligand interactions, such as ligand-induced NR-coregulator interactions[57]. Another advantage of AlphaScreen is that it can distinguish between an agonist and an antagonist using different coactivator or corepressor peptides.

An increasingly greater number of natural or synthesized NR ligands have been identified by AlphaScreen[49, 58–60]. Using

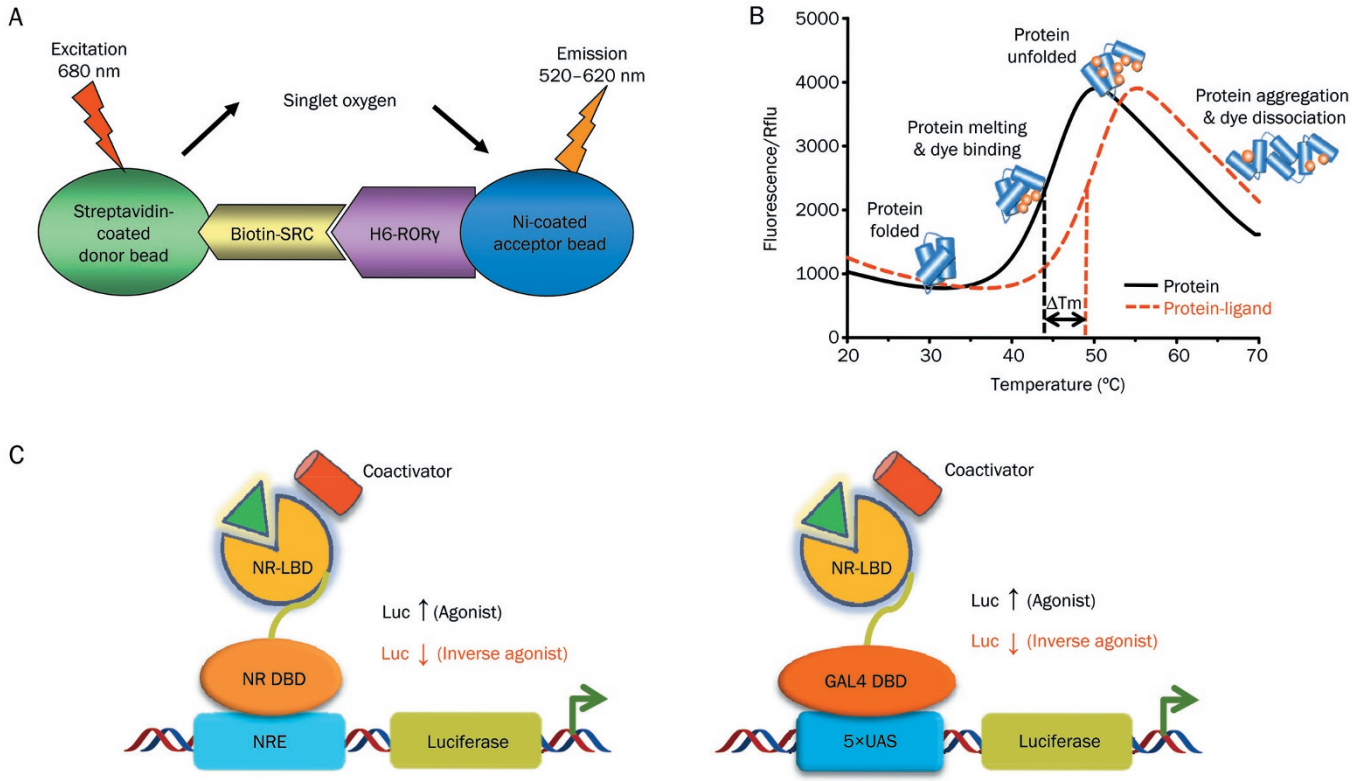


Figure 4. Representative drug discovery strategies. (A) Schematic representation of the AlphaScreen assay (Amplified Luminescent Proximity Homogenous Screen Assay Screen). H6-RORγ is immobilized on Ni-chelating acceptor beads and the biotinylated coactivator (Biotin-SRC) on streptavidin-coated donor beads. Donor beads contain a photosensitizer that, upon activation at 680 nm, converts ambient oxygen to singlet oxygen. If the acceptor beads are brought into close proximity of the donor beads by a RORγ-coactivator interaction, energy is transferred from the singlet oxygen to the thioxene derivatives in the acceptor beads, which results in light emission at 520–620 nm. Addition of a RORγ inverse agonist represses the signal of acceptor bead-immobilized H6-RORγ and donor-bead-biotin-coactivator. (B) Schematic representation of fluorescence intensity versus temperature of the melting protein in the presence of SYPRO orange. Ligand binding to a target protein can stabilize a protein's native state reflected in the increased melting temperature (T_m) of the bound protein. Monitoring of the ΔT_m of apo and ligand-bound proteins can be used to determine the ligand binding affinity. (C) Cell-based reporter assays. [left] Ligand binds to the NR LBD and the NR DBD binds to the nuclear receptor response element upstream of the reporter gene to activate transcription. [right] Upon ligand binding, the GAL4-NR-LBD binds to the GAL4 UAS to activate transcription.

Ni-chelating acceptor beads and streptavidin-coated donor bead pairs from PerkinElmer, a his-tag fusion NR protein and biotinylated coregulator peptides, we also found some NR modulators. These ligands include a PPARγ agonist[61], an orphan receptor RORγ antagonist[62], and natural compounds as agonists for the orphan receptors TR4 and COUP-TFII[63, 64].

DSF

Differential scanning fluorimetry (DSF), also known as the protein thermal shift assay (TSA), is an increasingly popular method to identify specific ligands or nonspecific protein stabilizing conditions, such as buffers, salts, pH and small molecule ligands[65–67]. TSA monitors the thermal unfolding of proteins in the presence of an environmentally sensitive fluorescent dye, such as SYPRO Orange, and measures the melting temperature (T_m) as a readout (Figure 4B). The SYPRO Orange dye is highly fluorescent in a non-polar environment (hydrophobic core residues exposed to the dye), whereas the fluorescence is quenched in aqueous surroundings. The melting temperature is the midpoint of transition from native to unfolded state and provides information on the thermal stability of a protein in apo or holo form.

DSF is a rapid and inexpensive screening method to identify ligands that bind and stabilize proteins. It has been demonstrated that the stabilizing effect of a ligand upon binding is proportional to the concentration and affinity of the ligand[68–70]. Compounds with similar physicochemical properties can be ranked based on their relative ΔT_m values[71]. DSF is compatible with a standard real-time PCR instrument and can be performed in 96-well format using 10 μL reaction volumes and with a small amount of protein required. DSF has received substantial attention and has been widely applied for the development of drug candidates in recent years[70, 72, 73].

DeSantis and Sekiguchi *et al* successfully identified ERα and PXR ligands using DSF as a high-throughput assay[74, 75]. Our group developed a high-throughput assay for RORγ ligand identification using DSF. The positive control digoxin exhibited a 10 °C temperature shift compared with DMSO (T_m=66 °C

for digoxin and T_m=56 °C for DMSO). A series of benzenesulfonamide-containing compounds (**19**) were identified from DSF screening of our in-house chemical library and could stabilize RORγ by 5–7 °C (unpublished data).

Cell-based reporter assay

A cell-based reporter assay may provide valuable information regarding the functional activity, potency and selectivity of a ligand that interacts with NRs. Transient and stable transfections are two types of cell-based systems for evaluating NR transactivation, and the former is the most common. The NR LBD fused with a GAL4-DBD is cotransfected with a reporter construct that contains GAL4 upstream activation sequences (UAS) of a reporter gene such as luciferase (Figure 4C). The regulation of transactivation of the NR by binding of a ligand may be measured by the expression level of the reporter gene. For native promoter reporter assays, the cells are cotransfected with plasmids that encode a full-length NR and their cognate luciferase reporters such as RORE-luc (Figure 4C). There are many modified versions of gene reporter assay systems for the measurement of receptor activation or repression. These systems are easily used to rapidly determine compound selectivity and potency in NRs[76–79]. Similar to the AlphaScreen assay, the cell-based transactivation assay can distinguish between an agonist and an antagonist.

Virtual screening approaches

Virtual screening (VS) is a knowledge-based approach which can be divided into structure-based and ligand-based methods. For structure-based virtual screening, the most frequently used method is molecular docking when the 3D structure of the target is available. The ligand-based methods have also been widely used because it is unnecessary to know 3D information for the targets. There have been several well-established methods demonstrated in the literature, such as 3D-QSAR, ligand-based pharmacophore searching, scaffold-hopping, virtual library design, and shape-based screening. Readers are encouraged to refer to the excellent reviews by Jiang *et al* for various computational drug design methods[80, 81]. Virtual screening has been widely used for the discovery of NR ligands[62, 82–85].

Beyond the strategies previously described, fluorescence resonance energy transfer (FRET) or time-resolved FRET has also been used as a high-throughput tool for the discovery of NR modulators[52, 86]. Furthermore, differential hydrogen/deuterium exchange mass spectrometry (H/D-Ex) has been used to monitor the conformational change from the apo-form to ligand-bound form of a NR LBD[29, 41].

Discovery of ROR modulators

The RORs were initially identified as orphan receptors, and it was not clear that these receptors were regulated by small molecule ligands. The co-crystal structure of the LBD of RORα bound to cholesterol indicated that various cholesterol derivatives, such as 7-oxygenated sterols, may act as physiological ligands to influence ROR activity. Given the specific tissue distribution of each ROR isoform and their potential role in pathophysiological conditions, they have been linked to autoimmune diseases and metabolic disorders, as previously described. The discovery and development of natural and synthetic ligands, including agonists, antagonists, and inverse agonists that modulate the activity of these receptors is in high demand.

Ursolic acid (UA, **9**), a natural carboxylic acid ubiquitously present in plants, was identified as a strong and selective inhibitor for RORγt function[86]. UA was identified from a small chemical library with more than 2000 known bioactive compounds. UA inhibited IL-17 production not only in developing Th17 cells but also in mature Th17 cells. Mice that received UA treatment were resistant to EAE, which indicates UA can be used to develop a treatment for Th17-mediated diseases. UA was demonstrated to inhibit the binding of RORγ-LBD to coactivator peptide SRC-1 with an IC_{50} of 0.68 μmol/L. Consistent with the results of retroviral overexpression and reporter gene assays, UA did not inhibit the binding of RORα-LBD to the coactivator peptide, which suggests that UA is a RORγt-specific antagonist. As a natural triterpene scaffold molecule, ubiquitously present in plants and human diets, UA is relatively non-toxic and is well tolerated both orally and topically in humans and rodents. These characteristics suggest that UA has a great advantage in the development of more selective RORγ modulators.

Digoxin (**10**), a well-known cardiac glycoside used clinically in the treatment for various heart conditions, was identified as a specific inhibitor for RORγ in 2011[30, 50]. Huh *et al* performed a chemical screen with 4812 compounds and determined that digoxin inhibited RORγ transcriptional activity in a *Drosophila* S2 cell luciferase reporter assay with an IC_{50} value of 1.98 μmol/L. Digoxin inhibition of RORγ was specific, with no activity against RORα, DAF-12, AR, or LXRα. Digoxin can directly bind to RORγ-LBD and displace 25-hydroxycholesterol (**8**) with an IC_{50} of 4.1 μmol/L. Circular dichroism analysis also demonstrated that digoxin increased the thermal stability of RORγ-LBD. Digoxin inhibited murine Th17 cell differentiation without affecting the differentiation of other T cell lineages, including Th1, Th2, and regulatory T cells. Digoxin was effective in delaying the onset and reducing the severity of symptoms in a mouse model of multiple sclerosis (EAE). Digoxin is unlikely to be used therapeutically for inflammatory and autoimmune diseases because of its toxicity. However, it may still serve as a good template for the development of a safer RORγ antagonist.

In 2010, the Griffin and Burris laboratories at Scripps Florida demonstrated that the benzenesulfonamide LXR agonist T0901317 also functions as a potent inverse agonist of RORα and RORγ via suppression of their basal transcriptional activity[87]. Griffin *et al* demonstrated, for the first time, that a synthetic ligand could bind directly to and modulate the transcriptional activity of RORα and RORγ with high affinity (K_i=132 and 51 nmol/L for RORα and RORγ, respectively). It is interesting that T0901317 inhibited the constitutive transactivation activity of both GAL4-RORα and GAL4-

RORγ with little or no activity on GAL4-RORβ. In control cells transfected with GAL4-VP16 and the UAS reporter, no repression of GAL4-VP16 transactivation of the luciferase gene was observed, which suggests that the repression induced by T0901317 is not a result of nonspecific luciferase effects or cellular toxicity. Treatment of cells that expressed GAL4-LXRα, GAL4-RORα, or GAL4-RORγ with increasing concentrations of T0901317 demonstrated an excellent dose response, with an estimated EC_{50} of 0.25 μmol/L (LXRα) and estimated IC_{50} values of 2.0 μmol/L (RORα) and 1.7 μmol/L (RORγ). Medicinal chemistry efforts focused on the T0901317 scaffold led to the development of a series of non-sterol, synthetic ROR modulators.

In a 2011 patent, Scripps Research Institute worked on the T0901317 scaffold to explore SAR in search of selective RORγ ligands. They determined that SR1001 (**12**) targets both RORα and RORγ and that both receptors are required for the development of Th17 cell-mediated autoimmune diseases[29, 33, 34]. SR1078 (**13**) was the first identified agonist of RORα and RORγ[88]. SR3335 (**14**) was the first potent RORα-specific inverse agonist[41]. Additional work on the SR1001 scaffold, directed at the design of RORγ-selective inverse agonists, led to the identification of two RORγ-specific ligands, SR1555 (**15**) and SR2211 (**16**)[89, 90].

SR1001 (**12**) has a hexafluoroisopropanol-substituted phenyl group with an N-linked sulfonamide in the *para* position (Figure 5A). There are some major structural differences between SR1001 and T0901317. A more elaborate substituted thiazole is attached to the sulfonamide sulfur in SR1001; however, a phenyl ring is attached to this portion in T0901317. The trifluoroethyl group on the sulfonamide nitrogen in T0901317 was simplified to an N-H in SR1001. SR1001 repressed both GAL4–RORα and GAL4–RORγ transcriptional activities in a dose-dependent manner, but demonstrated no effect on LXRα activity. The specificity of SR1001 was also assessed in a panel that comprised all 48 human NRs in a cell-based cotransfection assay, and no activity was observed on receptors other than RORα or RORγ. The direct binding of SR1001 to RORα and RORγ was examined using competitive radioligand binding assays. SR1001 dose-dependently displaced [^{3}H]25-hydroxycholesterol binding to RORα and RORγ (K_i=172 and 111 nmol/L, respectively). SR1001 reduced the interaction of a co-activator TRAP220 NR box 2 peptide with RORγ in a dose-dependent manner (IC_{50}=117 nmol/L).

SR1078 (**13**) contained the same hexafluoroisopropanol carboxylic acid isostere as T0901317 but used a secondary amide, rather than a tertiary sulfonamide, to link the two phenyl rings. In an AlphaScreen assay, increasing doses of SR1078 resulted in a dose-dependent reduction in the ability of RORγ to recruit the TRAP220 coactivator NR box. In a cell-based GAL4-NRLBD cotransfection assay, SR1078 significantly inhibited the constitutive transactivation activity of RORα and RORγ but had no effect on the activity of FXR, LXRα, or LXRβ. These data clearly demonstrate that SR1078 selectively targeted RORα and RORγ and no longer functioned as a LXR/FXR agonist.

Additional modifications of the T0901317 and SR1078 scaffolds led to the discovery of SR3335 (**14**), the first potent RORα-specific inverse agonist. SR3335 was initially identified by its ability to inhibit the constitutive activity of RORα in a GAL4-RORα-LBD cotransfection assay. In a biochemical radioligand binding assay that used [^{3}H]25-hydroxycholesterol as a label, it was clear that unlabeled SR3335 dose-dependently competed for binding to the RORα LBD (K_i=220 nmol/L). SR3335 did not compete well for binding when the RORγ LBD was utilized. In a cell-based cotransfection assay, SR3335 significantly inhibited the constitutive transactivation activity of RORα (IC_{50}=480 nmol/L) (partial inverse agonist activity) but had no effect on the activity of LXRα or RORγ.

Using a modular chemistry approach, modifications to the SR1001 scaffold were performed to develop SAR to diminish RORα activity while maintaining selectivity over LXR. The compounds were profiled using a screening approach based on radioligand binding in a Scintillation Proximity Assay (SPA) format. SR2211 (**16**) displays exquisite selectivity for RORγ over RORα in both biochemical and cell-based assays, with a K_i value of 105 nmol/L at RORγ and no detectable binding to RORα. To assess the functional transcriptional activity of SR2211, cell-based assays using GAL4-NR-LBD cotransfection assays (LBDs of RORα, RORγ, LXRα, FXR, and VP-16) were performed. SR2211 treatment did not have an impact on the transcriptional activity of RORα, whereas the inhibition of RORγ activity was observed with an IC_{50} of 320 nmol/L.

A screen of SR1001 derivatives in a GAL4-NR co-transfection assay demonstrated that SR1555 (**15**) was devoid of LXR, FXR, and RORα activity but that it repressed the activity at RORγ in a dose-dependent manner (IC_{50}≈1.5 μmol/L). SR1555 was only able to displace [^{3}H]T0901317 from the ligand binding domain (LBD) of RORγ (IC_{50}=1.0 μmol/L) and not RORα, which confirmed that SR1555 was indeed specific for RORγ.

To identify selective RORγ antagonists, Scripps Research Institute performed a quantitative high-throughput screen of 310 000 compounds using a cell-based RORγ gene reporter assay to detect RORγ transcriptional inhibitors. The initial hit displayed an IC_{50} value of 3.3 μmol/L in the RORγ assay with no activity in control assays, including the RORα assay. The initial hit also inhibited Th17 cell differentiation, which indicated that this was a viable scaffold for the development of SAR studies. These efforts led to the discovery of ML209 (SR9805, **17**), which is a RORγ inhibitor with an IC_{50} value of 0.5 μmol/L[91, 92]. Notably, in a panel of 20 NRs, only weak activity was reported for ERRα, LXRα, and thyroid hormone receptor α and β (TRα and TRβ). In accordance with the cell-based RORγ gene reporter assay, ML209 was 12-fold more potent (IC_{50}=51 nmol/L) compared with its enantiomer (IC_{50}=605 nmol/L) in the competition assays[91, 92].

In 2012, a team from Innovimmune disclosed a series of related indoline and tetrahydroquinoline modulators of RORγ. The SAR for these compounds appeared to be tight, with only subtle structural variations giving rise to RORγ agonist or inverse agonist activity, as assessed by a coactivator peptide recruitment assay. Compound **18** described in these patents

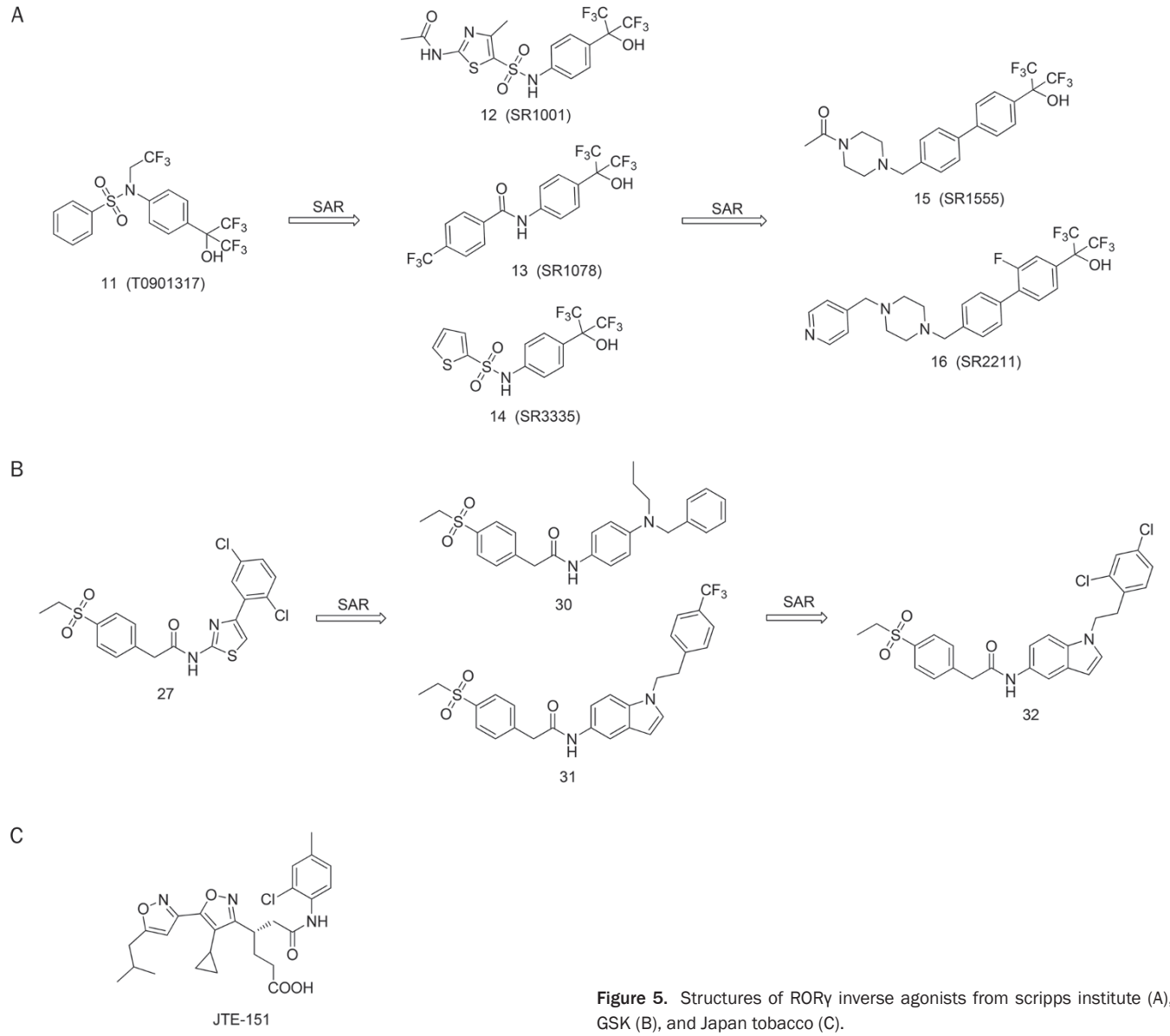


Figure 5. Structures of RORγ inverse agonists from scripps institute (A), GSK (B), and Japan tobacco (C).

bears some resemblance to T0901317. All compounds displayed a modest inhibition of IL-17A and IL-17F production in a human PBMC assay (EC_{50}<10 μmol/L). The compounds were identified as potent RORγ inverse agonists in a TR-FRET assay (EC_{50}<30 μmol/L). It will be interesting to determine whether this recently disclosed set of compounds from Innovimmune are selective for RORγ over LXR[93].

N-Acylated tetrahydroquinolines, indazoles, benzoxazine arylsulfonamides and related analogues have recently been reported in patents as potential inverse agonists of RORγ. Nonetheless, there are several noticeable trends throughout these series. First, the N-1 amine of the tetrahydroquinoline, indazole, and benzoxazine rings is preferably sulfonylated. It is not clear what the nature of this effect is, other than to force the rings out of co-planarity, but this type of substitution dominates the majority of the examples. Compound **20** from Lycera's patent contained a 2,6-disubstituted benzamide at the 7-position of the tetrahydroquinoline core[94]. These compounds are claimed as RORγ inhibitors for reducing IL-17 and treating immune and inflammatory disorders. The compounds were tested for their ability to inhibit RORγ activity in cells using a GAL4-driven reporter gene assay in HEK293 cells. The compounds were also tested for binding in a RORγ-LBD TR-FRET assay. No clear data are provided other than whether the IC_{50} values are less than or greater than 15 μmol/L, which made it impossible to decipher any SAR. To further explore SAR, compound **21** was found to exhibit an EC_{50} of 6 nmol/L in a SRC1-2 coactivator peptide recruitment assay[95].

Merck disclosed a series of indazoles and azaindazoles as inverse agonists of RORγ. A series of sulfonylated indazoles were described in a European patent application from Merck. Two of the most potent compounds, **22** and **23**[96], exhibited IC_{50} values of 2 nmol/L. This series is similar to that from

Lycera, in which the 2,6-bishalogenated benzoyl substitution pattern appears on the indazole nitrogen atom in this case. Although the claims indicate the core can be an indole, all examples described are indazoles. This series also appears to tolerate sulfonyl substitution on the indazole nitrogen, as several potent analogs of this type are also described. Indazole ring substitution, as well as aza-analogs, is tolerated.

Our group also identified N-acylated tetrahydroquinolines, indazoles, benzoxazine arylsulfonamides and related analogues in the TSA screening. Compound **19** was demonstrated to have an IC_{50} value of less than 1.0 μmol/L using a GAL4-driven reporter gene assay in HEK293 cells (unpublished data).

GSK has conducted substantial research on the discovery of RORγ modulators. A series of aromatic amide derivatives were identified as agonists of RORγ using a TR-FRET assay. Compounds **24**, **25**, and **26** were evaluated for SRC1 recruitment. These compounds were also demonstrated to stimulate RORγ-dependent IL-17 expression in a luciferase reporter assay with an EC_{50} value of ~100 nmol/L. These compounds were demonstrated to directly interact with RORγ using a thermal stability shift assay measured by circular dichroism[97]. Compounds **24**, **25**, and **26** stabilized RORγ by 0.49, 2.26, and 2.90 °C, respectively.

In 2012, high-throughput screening of the GSK in-house compound collection using a FRET assay resulted in the identification of thiazole amide compound **27** as a RORγ inhibitor with a pIC_{50} of 6.0. The binding of compound 27 to the RORγt LBD was confirmed with a thermal shift of 7.1 °C in a thermal shift assay and a pK_i of 6.4 in a radioligand binding assay. In the subsequent evaluation in a cell-based assay, compound **27** inhibited Th17 cell differentiation with 49% of maximum inhibition at 10 μmol/L. In all patent applications, the 2-(4-(ethylsulfonyl)phenyl)acetamide group of **27** was fixed, and the SAR study was explored on the right-hand side with the thiazole amide as a core structure. This moiety appears to be critical for activity. The central core of compound **27** is always thiazole, or thiophene. SAR studies of compound **27** led to the identification of thiazole ketone amide compound **28** and thiophene ketone amide compound **29** with high binding affinities and inhibitory activities for Th17 cell differentiation. For example, compound **28** exhibited a FRET pIC_{50} of 7.8 and a Th17 pIC_{50} of 6.7. Compound **29** exhibited excellent inhibitory activity on a Th17 cell differentiation assay with a pIC_{50} of 7.9, and in a RORγ FRET assay with a pIC_{50} of 7.8. The reason why thiazole ketone amides exhibited lower Th17 potency compared with RORγ FRET potency is unclear and cannot be explained by membrane permeability because both thiazole ketone amides and thiophene ketone amides have reasonably good membrane permeability[53].

SAR exploration on the right-hand side (RHS) of compound **27** (Figure 5B) led to the identification of tertiary amine compound **30** as a potent RORγt agonist with an EC_{50} of 20 nmol/L in a dual FRET assay. Following this assessment, compound **31** was synthesized and confirmed to be a potent RORγt inverse agonist with an IC_{50} of 5 nmol/L (FRET, max%=104). Linker exploration and rational design led to a series of indole-based analogues as more potent RORγt inverse agonists. Compound **32** was discovered as a potent RORγt lead with an IC_{50} less than 10 nmol/L in a FRET assay[53].

In a separate but somewhat related patent, Glenmark disclosed a series of biaryl and heterobiaryl amide analogues as modulators of RORγ. In patent applications, the 4-substituted ethyl sulfone phenacetyl amide is constant. These compounds were strikingly similar to the compounds described in 2013 by the team at GSK. Compounds **33** and **34** from Glenmark were described and tested in a TR-FRET assay and found to have IC_{50} values <50 nmol/L[98].

Another patent disclosure from GSK described a series of N-aryl benzenesulfonamides as RORγ inverse agonists. Compounds **35**, **36**, and **37**[99, 100] from the patent were potent RORγ inverse agonists with pIC_{50} values >7.8 in the SRC1-2 coactivator peptide recruitment assay. These compounds also inhibited IL-17 production in a human PBMC assay (pIC_{50}>6.0). Two follow-up patents on the same series were disclosed and exemplified subtle changes on the periphery of the compounds. Compound **35** was reported as a racemate, and its separate enantiomers were also described in the same patent. Compound **35** (the racemate) was a potent inhibitor with a pIC_{50}=7.7 in a TR-FRET assay and a pIC_{50}=7.5 in a human PBMC assay.

Genentech recently described a series of sulfonamides as modulators of RORγ. The compounds were assessed for their ability to bind to the RORγ LDB by displacing [^{3}H]25-hydroxycholesterol in a radioligand binding assay. These compounds were strikingly similar compared with the compounds described earlier by the team at GSK. Compound **38** was demonstrated to be a potent inhibitor with an IC_{50} of 130 nmol/L. Compound **39** demonstrated inhibition activity of RORγ LBD recruitment of a peptide derived from the SRC1 co-activator protein (EC_{50}=57 nmol/L), which was shown in GAL4 cellular constructs to have an EC_{50} value of 120 nmol/L[54, 101].

Our group recently discovered a new series of sulfonamide RORγ antagonists using a structure-based virtual screening approach in conjunction with medicinal chemistry optimization and biological evaluation. The derivatives were synthesized or purchased, and assessed with the AlphaScreen assay and luciferase reporter gene assays. The derivatives demonstrated remarkably improved activity. The most potent was compound **40**, which showed an IC_{50} value of 50 nmol/L in a GAL4-driven luciferase reporter assay[62].

One patent from Japan Tobacco disclosed a series of isoxazole- or triazole-based compounds as RORγ inverse agonists. Some of the reported compounds in the patent exhibited EC_{50} values less than 3.0 μmol/L in a GAL4-driven luciferase reporter assay[102]. Japan Tobacco recently announced a phase I clinical trial of a RORγ inverse agonist, JTE-151 (Figure 5C). This trial will provide a detailed description of the long-term *in vivo* safety profiles of this RORγ inverse agonist.

Conclusions

Orphan NRs are potential drug targets, and their functions can be regulated by potent and specific agonists or inverse agonists. The identification of the natural ligands for the retinoic acid receptor-related orphan receptors remains controversial, although a range of oxysterols can potently bind to and modulate the function of RORγ. Since the benzenesulfonamide LXR agonist T0901317 was identified as an inverse agonist of both RORα and RORγ, significant progress have been made regarding the identification of novel ligands for the RORs. It is obvious that the determination of more complex structures of RORs and their ligands can facilitate the structure-based design of potent and selective ROR modulators. Further optimization for improved drug-like properties of these compounds will have a high potential for the treatment of metabolic and autoimmune diseases.

Acknowledgements

We gratefully acknowledge financial support from the National Natural Science Foundation of China (Grant 81373325), the National Key Basic Research Program of China (973 Program, Grant 2013CB910601), the "100 Talents Project" of CAS, and the Guangzhou Bureau of Science and Information Technology, China, (Grants 2012Y2-00051, 2013Y2-00048),"Interdisciplinary Cooperation Team" Program for Science and Technology Innovation of the CAS.

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