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Review

Targeting Orphan Nuclear Receptors for Treatment of Metabolic Diseases and Autoimmunity

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The nuclear receptor (NR) superfamily is composed of 48 members in humans and includes receptors for steroid hormones, thyroid hormone, various lipids and oxysterols. This superfamily has been a rich source of drug targets for myriad diseases including inflammation, cancer, and metabolic disorders. Approximately half of the superfamily have well characterized natural ligands whereas the remaining receptors are considered orphan receptors and remain a focus of a number of investigators assessing their ability to be regulated by ligands. Here, we review recent discoveries that yield important insight into the druggability of three orphan nuclear receptors: the retinoic acid receptor-like orphan receptors (RORs), peroxisome proliferator-activated receptor γ (PPARγ), and liver receptor homolog-1 (LRH-1).

Introduction

The nuclear receptor (NR) superfamily is composed of 48 members in humans and includes receptors for steroid hormones, thyroid hormone, various lipids, and oxysterols. NRs function as ligand-dependent transcription factors and are share a modular domain structure (Figure 1) (Mangelsdorf et al., 1995). A general characteristic of members of the superfamily is a highly conserved DNA binding domain (DBD) also called a C region. Amino-terminal to the C region is the A/B region and is highly variable among superfamily members. This domain, depending on the receptor, may contain ligand-independent transcriptional activation activity. The second most conserved region is carboxy-terminal to the DBD is the ligand binding domain (LBD) or E region. This domain is responsible for recognition and binding of the receptor's ligand as well as ligand-dependent transcriptional activity. A relatively short region connects the C region to the E region (region D) and is known as the hinge domain. Some receptors also contain a region carboxy-terminal to the ligand binding domain known as the F region.

Approximately half of the superfamily have well characterized natural ligands whereas the remaining receptors are considered orphan receptors and remain a focus of a number of investigators assessing their ability to be regulated by ligands (Kliewer et al., 1999; Mangelsdorf and Evans, 1995). The vast majority of receptors that have identified natural ligands are also validated targets for clinical drugs. This superfamily has been a rich source of drug targets for myriad diseases including inflammation, cancer, and metabolic disorders. Thus, there remains significant interest in identification of ligands that regulate orphan members of the NR superfamily due to their potential for utilization as potential drugs to treat human disease.

Over the past 2 years, there have been significant breakthroughs in the identification of novel ligands for several orphan NRs and in this review we examine the progress that has been made toward identification of ligands for retinoic acid receptor-related orphan receptors (RORs) and the liver receptor homolog 1 (LRH1) as well as a novel class of ligand for peroxisome proliferator-activated receptor γ (PPARγ) that holds promise for an improved pharmacological profile over older glitazone agonists.

Pharmacological Targeting of Retinoic Acid RORs for Autoimmune Diseases

As the canonical domain structure and conserved sequence of members of the NR superfamily became apparent in the 1980s, several laboratories isolated additional members of this superfamily that had no identified ligands. The first member of the ROR subfamily of receptors (RORα) was based on sequence similarities to the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) yielding the name "retinoic acid related-related orphan receptor" (Becker-André et al., 1993; Giguère et al., 1994). Similar receptors, RORβ and RORγ, were identified shortly after RORα was identified (Carlberg et al., 1994; Hirose et al., 1994). RORα, RORβ, and RORγ display significant sequence similarities and each *Ror* gene generates multiple isoforms based on alternative promoter usage and splicing. All of the isoforms vary only in the amino-terminal A/B regions of the receptors. RORα, RORβ, and RORγ display distinct patterns of expression. RORα is widely expressed and is found in the liver, skeletal muscle, skin, lungs, adipose tissue, kidney, thymus, and brain (Hamilton et al., 1996; Steinmayr et al., 1998). RORβ displays a very restricted pattern of expression and is limited to the central nervous system (André et al., 1998a, 1998b). RORγ is most highly expressed in immune tissues including the thymus, but significant expression is also found in the liver, skeletal muscle, adipose tissue, and kidney (Medvedev et al., 1996). RORγt, is exclusively expressed in key cells within the immune system (Miller and Weinmann, 2009). When bound to their specific DNA response elements within the promoter of a target gene, all three RORs constitutively recruit coactivators resulting in activation of transcription of their target genes. Interestingly, another group of orphan nuclear receptors, REV-ERBs, recognize the same response elements as the RORs and are coexpressed in many tissues (Duez and Staels, 2009; Solt et al., 2011a; Yin et al., 2010). REV-ERBs are ligand-dependent

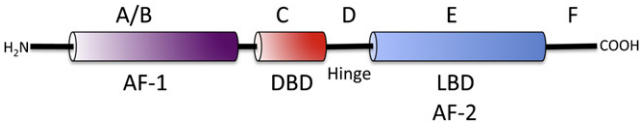

Figure 1. Structural Domain Organization of Nuclear Receptors
Regions (A–F) are indicated above the schematic and domains are indicated below the schematic. AF, activation function; DBD, DNA binding domain; LBD, ligand binding domain.

transcriptional repressors and in many cases, functionally antagonize the action of the RORs (Burris, 2008; Raghuram et al., 2007; Yin et al., 2007).

Distinct adaptive immune responses directed toward protection against various classes of pathogens are facilitated by differentiation of $CD4^+$ T cells into specific types of effector T cells (T_H1, T_H2, and T_H17 cells). RORα and RORγ have garnered significant attention over the past several years due to their essential role in development of T_H17 cells. Prior to the discovery of T_H17 cells, T_H1 cells were considered the effector T cell type responsible for the pathology of autoimmune diseases including psoriasis, multiple sclerosis (MS), and rheumatoid arthritis among others. Key experiments in interferon γ receptor-deficient mice suggested that this may not be the case because these mice, deficient in T_H1-IFNγ signaling, were actually more susceptible to autoimmune disease (experimental autoimmune encephalomyelitis (EAE)—an animal model of MS) rather than less susceptible to disease (Ferber et al., 1996; Willenborg et al., 1996). These data suggested that there may be another, as of yet unidentified, cell type responsible for at least a portion of the autoimmune pathology associated with EAE. Later studies demonstrated that the IL23/IL17 cytokine axis was crucial for autoimmune disease progression in the EAE and collagen induced arthritis (CIA) models and progression was independent of the T_H1 and T_H2 cells (Cua et al., 2003; Murphy et al., 2003; Zhang et al., 2003). Indeed, a distinct lineage of T effector cells that produce IL17 (T_H17 cells) was soon identified (Harrington et al., 2005; Park et al., 2005). T_H17 cells have since become recognized as critical mediators of autoimmune pathology (Bettelli et al., 2008; Littman and Rudensky, 2010). Thus, it became apparent that one might be able to modulate autoimmune disease progression by regulation of T_H17 cell development.

One key factor in development of the T_H17 cells has been shown to be expression of RORs. RORγt was demonstrated to play a critical role in development of T_H17 cells because overexpression of RORγt in naive $CD4^+$ T cells resulted in induction of development of IL17 producing T_H17 cells and RORγt-deficient mice display impaired T_H17 cell differentiation (Ivanov et al., 2006). The transcriptional cascade leading to T_H17 differentiation is complex and additional transcription factors involved in metabolic regulation such as HIF-1 and RORα have also been demonstrated to play an important role (Dang et al., 2011; Yang et al., 2008). Mice deficient in either *Rorγ* or *Rorα* or both receptors displayed resistance to development of autoimmunity (Ivanov et al., 2006; Yang et al., 2008) suggesting that one mechanism to reduce autoimmune pathology may be to inhibit these receptors leading to decreased T_H17 cell differentiation. At the time of these discoveries, ligands had not been identified for either of these receptors but developments in 2010 suggested that both RORα and RORγ were indeed "druggable."

Crystallographic studies of RORα suggested that a sterol such as cholesterol or cholesterol sulfate may function as a natural ligand of this receptor (Kallen et al., 2004, 2002). We later found that hydroxycholesterols were high affinity ligands for both RORα and RORγ and that some of these such as 7-oxygenated sterols (Figure 2) functioned as inverse agonists suppressing the constitutive activity of both of these receptors (Wang et al., 2010a, 2010b). The structure of the RORγ LBD bound to several oxysterols provided a clear structural basis for these sterols as natural ROR ligands (Jin et al., 2010). A key breakthrough with this discovery was the ability to develop a radioligand binding assay for RORα and RORγ using high affinity radiolabeled oxysterols and this allowed our group to identify the first synthetic ligands for these two "orphan" receptors. The characterized liver X receptor (LXR) agonist, T0901317 (T1317) was identified as an inverse agonist for both RORα and RORγ and shown to directly bind to the LBDs of these receptors and inhibit coactivator binding leading to suppression of their constitutive transcriptional activity (Kumar et al., 2010). T1317 displays promiscuous activity at several NRs including LXR, farnesoid X receptor, pregnane X receptor, and RORα and RORγ limiting its ability to be used as a chemical tool to probe the utility of a selective RORα/RORγ inverse agonist in models of T_H17 cell differentiation; however, a focused medicinal chemistry effort was able to develop a analog of T1317 (SR1001) that retained activity at both RORα and RORγ but lacked activity at any other NR (Solt et al., 2011b).

Unlike the natural ligands that have been identified, SR1001 does not contain a sterol or steroid-like structure (Figure 2). We were able to demonstrate that SR1001 directly binds to the LBDs of RORα and RORγ (K_i = 111–172 nM) leading to a conformational change resulting in loss of affinity for the receptors for coactivator (SRC-2) and increased affinity for corepressor (NCoR) (Solt et al., 2011b). SR1001 effectively suppressed IL17 expression and T_H17 cell development without affecting other T cell lineages (Solt et al., 2011b). Most importantly, SR1001 was effective in delaying the onset and severity of EAE in mice demonstrating proof-of-principle that a small molecule inhibitor of ROR transcriptional activity is effective in suppressing autoimmunity (Solt et al., 2011b).

Huh et al. (2011) also identified a small molecule inhibitor of RORγ and this compound was the well-known cardiac glycoside, digoxin (Figure 2). Digoxin is used clinically for treatment of atrial fibrillation where it directly alters electrical conduction in the heart targeting the Na^+/K^+-ATPase. Digoxin displayed a potency (IC_{50}) of ~2 μM for suppression of RORγ-mediated activity (Huh et al., 2011). Digoxin displayed RORγ specificity and did not affect RORα, DHR3, DAF-12, or the androgen receptor (Huh et al., 2011). Similar to SR1001, digoxin inhibited T_H17 cell differentiation and delayed the onset and severity of EAE in mice (Huh et al., 2011). One significant issue of digoxin is the toxicity associated with this drug and the small therapeutic window. These authors examined analogs of digoxin and demonstrated that these compounds also demonstrate activity against RORγ and T_H17 cell differentiation and that the anti-T_H17 cell activity does not correlate with the cardiac glycoside

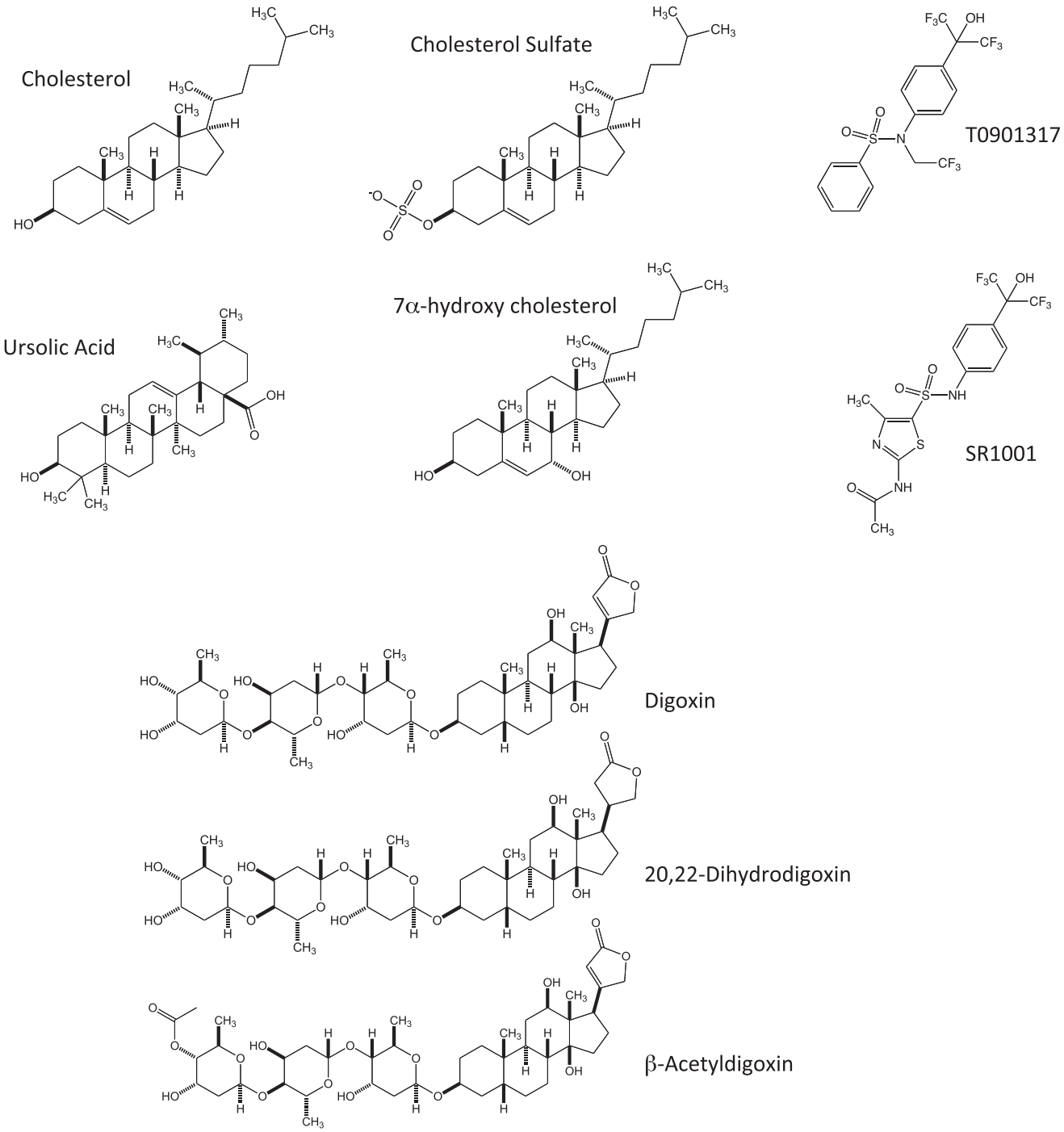

Figure 2. Structure of ROR Ligands
Chemical structure of several ROR ligands including sterol derivatives (cholesterol, cholesterol sulfate, 7α-hydroxycholesterol, ursolic acid, and digoxin as well as its derivatives) and nonsteroidal compounds such as T0901317 and SR1001.

activity suggesting that there may be opportunities for optimization of the RORγ activity with removal of the potential cardiac toxicity (Huh et al., 2011). A crystal structure of the RORγ LBD bound to digoxin demonstrating the molecular mechanism of inhibition of coactivator binding was reported soon after the original study identifying dixogen as a RORγ inhibitor (Fujita-Sato et al., 2011). Given the severity of the toxicity associated with and the limited intellectual property space associated with this chemical scaffold, it is unlikely that this would be pursued by pharmaceutical companies even though Huh et al. (2011) indicate that some digoxin derivatives (Figure 2) do display RORγ inhibitory activity at levels where they do not display

cellular toxicity. However, of critical importance is the fact that these data demonstrate the feasibility of targeting RORγ with small molecule inhibitors for treatment of autoimmune disorders.

Ursolic acid has also been recently shown to inhibit T_H17 cell differentiation via targeting RORγ (Xu et al., 2011) (Figure 2). Ursolic acid also delayed the onset and decreased the severity of EAE in mice (Xu et al., 2011). Ursolic acid, like digoxin, displays a steroid-like chemical structure and, in fact, several have reported that ursolic acid modulates the activity of the glucocorticoid receptor (GR) (Cha et al., 1998; Kassi et al., 2009). Biochemical assays indicate that ursolic acid effectively bindings to the RORγ LBD and blocks coactivator binding, but was considerably less active on RORα (Xu et al., 2011). The possibility that ursolic acid exhibits activity at the GR complicates the interpretation of the results because glucocorticoids are very effective in the EAE model.

Given the validated results in the development of small molecule ligands for RORα and RORγ by multiple teams it is clear that these orphan receptors are druggable. The data also clearly indicate that these compounds have efficacy in suppression of T_H17 cell development and an in vivo model of autoimmunity (EAE model). Thus, targeting the RORs represents a very promising path forward for novel treatments for autoimmunity that offers potential significant advantages over current therapies that broadly target the immune system and/or are typically biologics that must be injected.

Revival of PPARγ Ligands for Type 2 Diabetes

The incidence of diabetes is increasing rapidly as the percentage of the population ages and becomes more obese. According to the National Center for Health Statistics (http://www.cdc.gov/nchs/) diabetes is now the seventh leading cause of death in the United States. Adipose tissue is at the center of the metabolic syndrome, which encompasses an array of medical disorders including insulin resistance and diabetes. Excessive body fat defines obesity and leads to insulin resistance, dyslipidemia, type 2 diabetes (T2D), and cardiovascular disease. Understanding the molecular pathways that link adipose tissue biology to these pathologies is of critical scientific and medical importance. Adipose tissue secretes a variety of cytokines and cytokine-like molecules, called adipokines, which have both positive and negative effects on peripheral insulin sensitivity (Kershaw and Flier, 2004). In obesity, TNFα, IL-1, and resistin are secreted from adipose tissues and suppress insulin action on peripheral tissues (Hotamisligil et al., 1993; Lagathu et al., 2006; Steppan et al., 2001). Conversely, adipose tissues from lean individuals secrete higher levels of adiponectin, a circulating protein that has insulin-sensitizing effects on liver and other tissues (Berg et al., 2001; Hu et al., 1996; Yamauchi et al., 2006).

The nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) is viewed as a master regulator of fat cell biology and differentiation, being both necessary and sufficient to drive conversion of fibroblastic precursors into fat cells (Morrison and Farmer, 2000; Tontonoz et al., 1994; Willson et al., 2001). Transcription factors such as C/EBPs and EBF proteins regulate the expression of PPARγ (Jimenez et al., 2007; Wu et al., 1996). The glitazones or thiazolidinedione (TZDs) class of drugs that include rosiglitazone and pioglitazone are synthetic ligands that demonstrate high affinity binding to PPARγ, and these compounds function as full agonists of the receptor (Day, 1999; Reginato and Lazar, 1999) (Figure 3). Treatment of animal models of diabetes and diabetic patients with TZDs results in potent insulin-sensitizing activity (Lehmann et al., 1995) concomitant with decreased expression of insulin resistance-inducing adipokines including TNF-α, IL-1, and resistin, and increased production of the insulin-sensitizing hormone, adiponectin (Sharma and Staels, 2007; Trujillo and Scherer, 2006). Unfortunately, PPARγ agonists can have long-term adverse effects on the health of certain patients, such as increased body weight, fluid retention, and increased risk of heart failure (Lipscombe et al., 2007). This is unfortunate, as TZDs have consistently shown robust efficacy for treatment of T2D. More recently concerns have increased on the association of TZD use with bone loss (Schwartz et al., 2006; Wei et al., 2010). The latter risk is troublesome as detection is typically only made when a patient suffers a fracture. The biguanide metformin is now the first-line medication used for treatment of T2D as safety concerns over the use of TZDs has grown.

Interestingly, studies in preclinical models and in clinical trials have shown that weight gain and plasma volume expansion can be minimized without loss of insulin sensitization by the use of modulators that are weak or partial agonists of PPARγ. Partial agonists have been referred to as selective PPARγ modulators or SPPARγMs and this class of ligand has been shown to have a different binding mode in the PPARγ ligand binding pocket (LBP) as compared to the full agonists (Berger et al., 2005). Selective recruitment of transcriptional coactivators by partial agonists has also been demonstrated. A combination of different ligand binding mode and distinct coactivator recruitment profile may explain the change in gene expression patterns compared to that of full agonists (Berger et al., 2003). These findings highlight that several important aspects of PPARγ action remain unclear. First, there is no general deficiency in PPARγ function in obesity or insulin-resistant states. Hence, it is not clear why synthetic activation of a receptor should afford anti-diabetic effects. Second, although antidiabetic potency of the PPARγ ligand drugs correlates very well with their binding affinities (Willson et al., 1996), some ligands with full agonist action, like rosiglitazone, have powerful insulin-sensitizing actions, whereas other compounds with poor agonist activities, such as the benzyl indole MRL24, retain very good antidiabetic effects (Acton et al., 2005).

A recent study by Choi et al. (2010) demonstrated that many PPARγ-based drugs can activate the receptor by recruitment of coactivators but these compounds have a separate biochemical activity involving blocking the obesity-linked phosphorylation of PPARγ by Cdk5 on S273 of the receptor. In this study, it was shown that the insulin sensitization efficacy of a rosiglitazone and the partial agonist MRL24 correlates with their ability to block S273 phosphorylation. Although it is likely that other kinases can phosphorylate PPARγ on S273, this study demonstrates that activation of CDK5 by proinflammatory cytokines leads to an increase of phosphorylation of S273 in cellular models and increased S273-P was observed in obese diabetic mice (Choi et al., 2010). The level of S273-P correlates with the repression of a subset of PPARγ target genes that are dysregulated in obesity and associated with insulin resistance (Choi et al., 2010) and interestingly, a recent study indicates that

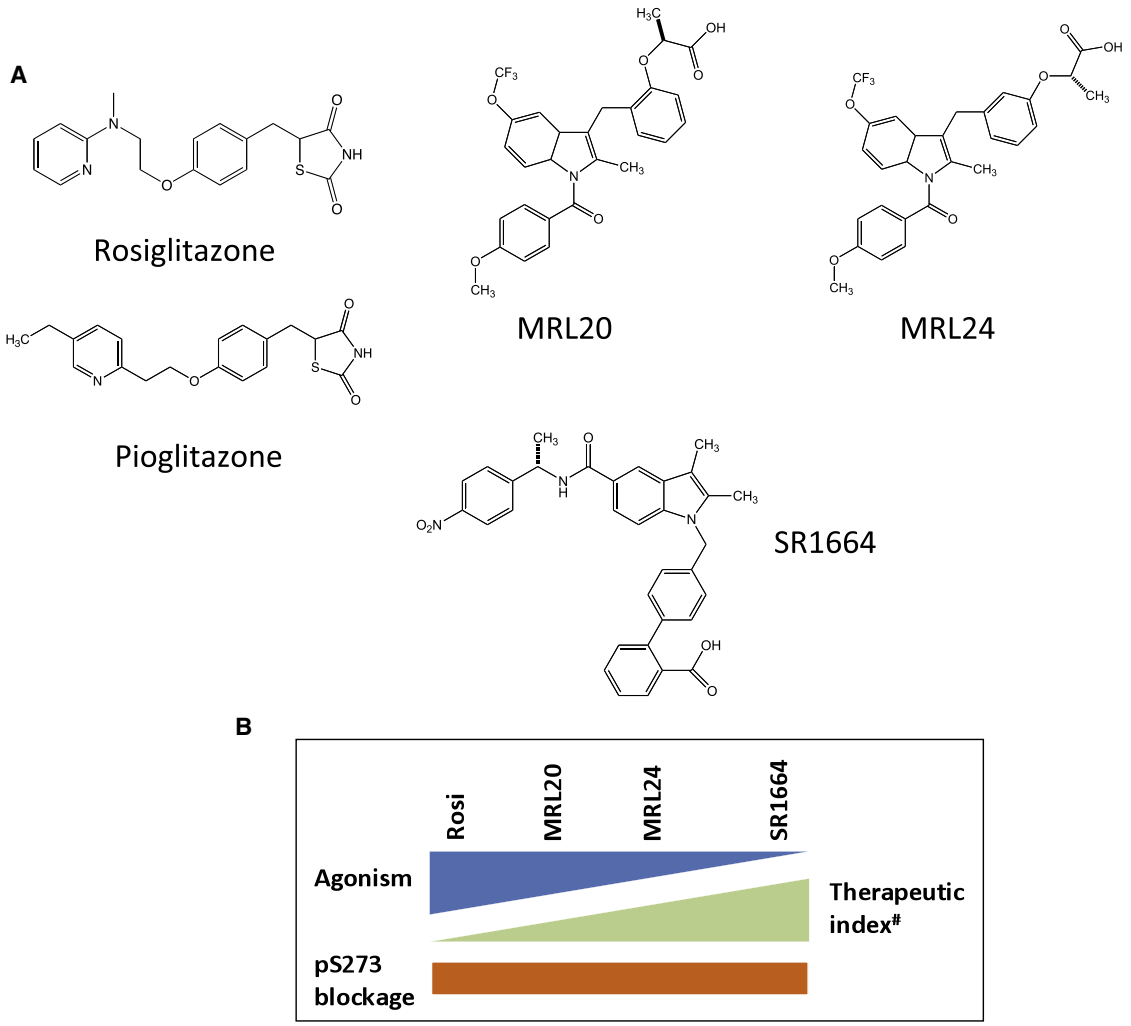

Figure 3. Structure of PPARγ Ligands

(A) Chemical structure of various PPARγ agonists, partial agonists, and "non-agonists."

(B) The desired profile of a PPARγ modulator is high affinity binding with minimal to no classical agonism and potent blockage of S273 phosphorylation by CDK5. A compound with this profile will result in improved therapeutic index (maximal efficacy with minimal side effects).

deletion of NCoR in adipocytes facilitates S273 phosphorylation by CDK5 and mimics the action of the TZD treatment. These studies suggested the possibility that the mechanism driving efficacy of PPARγ drugs can be separated from classical activation of the receptor, which if not required for efficacy likely contributes to the adverse event profile of TZDs.

Efforts to discover high affinity PPARγ ligands that lacked classical activation of the receptor while retaining the ability to block S273 phosphorylation in cultured adipocytes and in diabetic mice led to the discovery of SR1664 (Figure 2). SR1664 has similar binding affinity as rosiglitazone for PPARγ in a lanthascreen ligand displacement assay (K_i ~160 nM). In a well established cell-based cotransfection promoter/reporter assay, rosiglitazone induces significant transcriptional activation of the reporter whereas SR1664 failed to transcriptionally activate the reporter even at the highest concentrations tested. On purified PPARγ, both rosiglitazone and SR1664 demonstrated dose-dependent inhibition of the CDK5-dependent phosphorylation of S273. The blockage of phosphorylation of S273 is a direct action of the ligands that requires binding to the LBD of the receptor to induce a conformational change that interferes with the ability of Cdk5 to phosphorylate serine 273. Adipogenesis was the first known biological function of PPARγ and PPARγ agonists have been shown to potently stimulate the differentiation of pre-adipose cell lines (e.g., 3T3-L1 cells). In this study, rosiglitazone potently stimulated fat cell differentiation, where in contrast, SR1664 did not stimulate increased lipid accumulation or changes in morphology characteristic of differentiating fat cells. More importantly, glucose tolerance tests were markedly improved with both rosiglitazone and SR1664. Whereas SR1664 demonstrated potent antidiabetic activity in diabetic mice, there was no measurable increase in fluid retention or weight gain. Not surprising, significant plasma volume expansion and weight gain was observed in the rosiglitazone-treated animals. Unlike rosiglitazone, SR1664 did not interfere with bone formation in cultured cells.

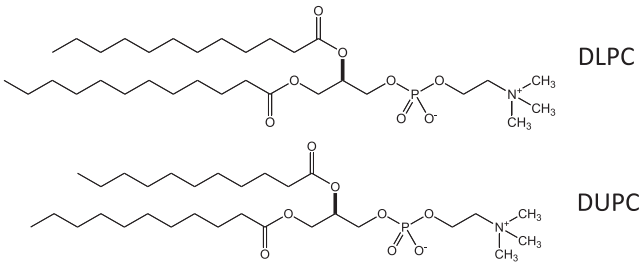

Figure 4. Chemical Structure of Two LRH-1 Ligands
Chemical structure of dilauroyl phosphatidylcholine (DLPC) and diundecanoyl phosphatidylcholine (DUPC), two LRH-1 ligands.

These data illustrate that it is possible to develop a new class of antidiabetes drugs that allosterically block the phosphorylation of PPARγ at S273. Even though SR1664 is only a proof of concept compound that needs to be optimized for improved pharmaceutical properties, these studies represent a significant advancement toward developing insulin sensitizers with improved therapeutic index. Several questions need to be addressed to facilitate development of compounds like SR1664. First, what is the mechanism by which phosphorylation of S273 represses the transcriptional output of a subset of genes that are dysregulated in obesity? Second, what are the molecular and structural determinants that facilitate high affinity binding to PPARγ without inducing classical activation yet maintaining potent blockage of phosphorylation of S273? And third, will non-activating/S273 phosphorylation blockers like SR1664 completely dissociate efficacy from the major side effects associated with TZDs? Although the answer to the last question will require testing compounds in man, it seems highly plausible that, as shown in Figure 3, that the therapeutic index will improve as agonism if removed while maintaining blockage of S273 phosphorylation.

Synthetic Agonists for LRH-1 Treatment of Diabetes and Metabolic Diseases

Liver receptor homolog-1 (LRH-1) is a member of the NR5A family of nuclear receptors that also includes the close homolog, steroidogenic factor-1 (SF-1). Although these receptors are considered orphan receptors, a number of reports have identified the presence of bacterial phospholipids in the binding pockets of both SF-1 and LRH-1, but it remains to be determined whether these represent merely structural or fortuitous ligands or if they can modulate receptor activity (Krylova et al., 2005; Li et al., 2005; Ortlund et al., 2005). LRH-1 plays important roles in embryonic development and is highly expressed in the intestine liver, pancreas and ovary (Lee and Moore, 2008). In addition, it is well established that LRH-1 regulates critical enzymes involved in cholesterol homeostasis and bile-acid biosynthesis in the liver in addition to regulating the expression of aromatase in the breast and ovaries (Lee et al., 2008; Santen et al., 2009).

A report published recently in *Nature* by Lee et al. (2011) identified two unusual phosphatidylcholine (PC) species as LRH-1 agonists that also uncovered a previously unknown role for LRH-1 in the control of glucose homeostasis and insulin sensitivity. Dilauroyl PC (DLPC; C12:0/C12:0) and diundecanoyl PC (DUPC; C11:0/C11:0) were shown to function as direct ligands of LRH-1 and modulate the receptor's activity (Figure 4). Both phospholipids were able to induce activation of LRH-1 specific promoters such as *Shp* and *Oct4* whereas closely related phospholipids such as dipalmitoyl PC (DPPC; C16:0/C16:0) did not. In addition, the authors demonstrate that these phospholipids were specific to NR5A family members and did not cross react with other families of nuclear receptors such as PPARα. Mammalian two-hybrid assays confirmed the ability of these two phospholipids to bind to LRH-1 and selectively recruit important coactivators such as SRC-3. Moreover, coactivator peptide association assays with purified LRH-1 suggested that the binding of DLPC to LRH-1 is subnanomolar. The effects of these LRH-1 agonists were then explored in vivo where they were administered to normal mice and it was shown that both ligands produced significantly reduced levels of nonesterified fatty acids and glucose levels in serum as well as a reduction of hepatic triglycerides. These effects were further explored in two mouse models of insulin resistance, *db/db* and diet induced obese (DIO) mice and the results were equally striking. DLPC treatment of insulin-resistant leptin-receptor deficient *db/db* mice for 2 weeks saw improvements in glucose homeostasis as well as lower fasting serum insulin levels and significantly lowered hepatic triglyceride levels in these animals compared to vehicle control.

To further confirm the role of LRH-1 in this pathway, DIO mice, that either expressed wild-type hepatic LRH-1 or were knocked out for hepatic LRH-1, were fed a high fat diet to induce obesity and insulin resistance. These animals were then continued on the diet with the addition of either DLPC or vehicle control for an additional 3 weeks and then the same parameters of glucose homeostasis were measured. It was determined that consistent with the *db/db* mice, the DIO mice containing wild-type hepatic LRH-1 and treated with DLPC showed marked improvement of glucose homeostasis that was absent from the mice containing the LRH-1 knockout. Moreover, these animals displayed increased insulin sensitivity, decreased hepatic triglyceride levels, decreased nonesterified fatty acids and their livers appeared less pale and fatty than the animals with the LRH-1 knockout given DLPC. These data illustrate the utility of these two novel LRH-1 agonists in further elucidating the mechanisms that control this pathway and identifies a potential new target for the intervention into metabolic disease. Furthermore, the results suggest that a targeted synthetic chemistry approach to targeting LRH-1 is warranted.

Summary

Defining both natural and synthetic ligands for orphan NRs continues to be a rich area and discoveries in the areas of RORs and LRH-1 over the past 2 years suggests that this group of NRs as well as additional orphan NRs may develop into validated drug targets. Beyond autoimmunity, the RORs offer the potential for development of drugs targeting a range of disorders. 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 (Kang et al., 2007; Lau et al., 2008; Mamontova et al., 1998). During the investigation of the effects of SR1001, we reported that metabolic genes known to be regulated by ROR were also affected (Solt et al., 2011b). Furthermore, we also demonstrated that a RORα-selective

agonist, SR3335, suppresses hepatic glucose output in vivo (Kumar et al., 2011). Thus, there are clear opportunities for assessing the ability of synthetic RORα/γ ligands for treatment of metabolic disorders such as type 2 diabetes. Currently, much less is known about targeting RORβ with small molecule synthetic ligands. RORβ expression is limited to the CNS and plays a critical role in regulation of the central circadian rhythm, but RORβ null mice also display reduced anxiety (Masana et al., 2007). Although RORα also plays a critical role in regulation of the circadian rhythm, the restricted pattern of expression of RORβ suggests that one may be able to develop RORβ-selective compounds that would modulate the central circadian rhythm without affecting the periphery.

The utilization of the TZD class of PPARγ agonists in the clinic has been declining significantly due to the association of these compounds with a range of side effects that has even led to the removal of drugs from the market. The observation that a new class of "modulator" compounds that selectively alter the post-translational modification of this receptor leading to "selective" pharmacology offers potential for a new class of PPARγ ligands that would offer the beneficial insulin sensitization effects without the detrimental side effects that are associated with typical agonists. This approach, although clearly promising, is in early stages and it is unclear if this will yield a superior drug in the clinic. With the array of selective modulators being developed for various nuclear receptors, it is interesting to speculate whether this method of selective modulation of posttranscriptional modification of the receptor leading to selective pharmacology will be applicable to a wider array of receptors.

The observation that DLPC regulates LRH-1 activity in vivo and is therapeutic for type 2 diabetes begs for a classical medicinal chemistry approach to be applied to this orphan receptor. With this recent publication from the Moore lab (Lee et al., 2011), it is clear that there will be several groups investigating the potential for development of synthetic ligands that target LRH-1. This class of nuclear receptor ligands may also offer a unique method for treatment of metabolic diseases.

ACKNOWLEDGMENTS

T.P.B. and P.R.G. have filed a patent application on SR1001 and related compounds. P.R.G. has filed a patent application on SR1664 and related compounds and licensed this to Adipothermics, a company of which P.R.G. is both a founder and consultant. T.P.B. is on the scientific advisory board of Phenex Pharmaceuticals, AG.

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