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Targeting xenobiotic receptors PXR and CAR for metabolic diseases

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Abstract

The pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) are two closely related and liver-enriched nuclear hormone receptors originally defined as xenobiotic receptors. Recently, an increasing body of evidence suggests that PXR and CAR also have endobiotic functions that impact glucose and lipid metabolism, as well as the pathogenesis of metabolic diseases. These new findings suggest that PXR and CAR not only regulate the transcription of drug-metabolizing enzymes and transporters, but also orchestrate energy metabolism and immune responses to accommodate stresses caused by xenobiotic exposures. The effectiveness of targeting PXR and CAR in the treatment of metabolic disorders, such as obesity, type 2 diabetes (T2D), dyslipidemia, and atherosclerosis, have been suggested in animal models. However, translation of these basic research results into clinical applications may require further investigation to determine the human relevance, and to obtain better understanding of the mechanisms through which PXR and CAR affect energy metabolism. Given a wide variety of natural or synthetic compounds that are PXR and CAR modulators, it is hoped that these two “xenobiotic receptors” can be harnessed for therapeutic potentials in managing metabolic diseases.

Introduction

Metabolizing and eliminating toxic chemicals in the liver and intestine is the first-line defensive system, which is conserved in nearly all animals from fruit flies to humans. Many of the chemicals absorbed into our body are lipophilic and need to be transformed into more water-soluble forms before being excreted. Pregnane X receptor (PXR/SXR, NR1I2) and constitutive androstane receptor (CAR, NR1I3), two members of the superfamily of nuclear receptors, have been well recognized as “xenobiotic sensors” that transcriptionally regulate the expression of Phase I and Phase II drug/xenobiotic metabolizing enzymes and transporters. In addition to its function in drug metabolism, the liver is also one of the major organs that regulate glucose and lipid metabolism. Recently, increasing evidence has suggested the endobiotic functions of PXR and CAR in regulating energy homeostasis under both physiological and pathological conditions. Furthermore, the immune modulating effect of PXR in the intestine and on immune cells also suggests a potential mechanism through

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which PXR may impact metabolic diseases. This review mainly focuses on the recent findings on the functions of PXR and CAR in energy homeostasis and immune regulation, and the implications of these functions in metabolic diseases.

PXR and CAR in hyperglycemia and diabetes

Type 2 diabetes (T2D) is a chronic disease characterized by uncontrolled hyperglycemia caused by insulin resistance in the initial stage followed by pancreatic β -cell failure at later stages. The liver plays a critical role in maintaining blood glucose homeostasis by controlling hepatic glucose production (HGP) in response to hormones, such as insulin, glucagon, and adipokines. In T2D, the high levels of HGP and the inability of insulin to suppress hepatic glucose output are major contributors to both fasting hyperglycemia and exaggerated postprandial hyperglycemia. It has been reported that hepatic insulin resistance and uncontrolled glucose output precede the onset of diabetes in several mouse models of T2D^{1, 2}. Moreover, increased gluconeogenesis, rather than glycogenolysis, is the predominant mechanism responsible for increased hepatic glucose output in T2D³. Indeed, suppressing hepatic gluconeogenesis has been proven to be an effective therapeutic strategy for T2D. Suppression of hepatic gluconeogenesis is believed to be the major mechanism to account for the antidiabetic effect of metformin.

Recently, the cross-talk between hepatic gluconeogenesis and PXR/CAR has gained increased attention. Phenobarbital, a prototypical CAR agonist, was known for its ability to decrease plasma glucose levels and improve insulin sensitivity in diabetic patients^{4, 5}. Later, it was reported that phenobarbital can suppress the expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), two rate-limiting enzymes of hepatic gluconeogenesis, in primary rat hepatocytes⁶ and mouse liver in a CAR-dependent manner⁷. Pregnenolone-16 α -carbonitrile (PCN), a PXR agonist, suppressed the expression of G6Pase in a PXR-dependent manner⁸. PXR transgenic mice also showed decreased expression of PEPCK and G6Pase⁹. The pathophysiological significance of CAR-mediated suppression of gluconeogenesis and its therapeutic implication potential in T2D have recently been independently reported by two groups. Both studies showed activation of CAR ameliorated hyperglycemia and improved insulin sensitivity in *ob/ob* mice and high-fat diet (HFD) fed C57BL/6J mice, likely mediated through the suppression of hepatic glucose production^{10, 11}. The metabolic benefit of CAR activation was most recently evaluated in the mouse model of gestational diabetes, in which the administration of a CAR agonist was shown to improve glucose tolerance and ameliorated gestational hyperglycemia and its associated symptoms of hypertension and proteinuria in pregnant mice fed with HFD¹². Compared to CAR, the *in vivo* effects of PXR activation on T2D are yet to be reported. Despite its function in suppressing gluconeogenesis, PXR activation is also known to cause hepatic steatosis^{9, 13} and increase serum corticosteroid levels¹⁴, conditions known to be positively associated with insulin resistance¹⁵. Based on these observations, it may not be a surprise that PXR activation may exert an adverse, rather than beneficial, effect on T2D, at least in the mouse models.

Mechanistically, PXR and CAR may suppress the gluconeogenic gene expression by targeting several key transcriptional factors involved in gluconeogenesis. It has been reported that both PXR and CAR can physically bind to FoxO1 and suppress its transcriptional activity by preventing its binding to the insulin response sequence in the gluconeogenic enzyme gene promoters⁸. Both receptors may also inhibit hepatocyte nuclear factor 4 α -mediated gluconeogenesis by competing for the DR1 (direct repeat spaced by one nucleotide) binding motif and its coactivators such as the peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α) in the gluconeogenic enzyme gene promoters^{16, 17}. It has also been reported that PXR can interact with cAMP-response element binding protein

(CREB) and prevent its binding in the promoter region of gluconeogenic genes upon cAMP stimulation¹⁸. However, most of these conclusions were based on *in vitro* studies and gene promoter analysis. It remains to be determined whether these *in vitro* scenarios are applicable *in vivo*. For example, CAR activation had much more significant inhibitory effect on gluconeogenic genes in HFD fed mice than their chow diet fed counterparts (our unpublished observations), which suggested a selective benefit of CAR activation in a diabetic condition. Further studies are necessary to focus more on pathophysiological conditions in order to fill the gap between *in vitro* observations and *in vivo* significance.

From the evolutionary perspective, the cross-talk between PXR/CAR and gluconeogenic pathway may represent a cellular adaptation mechanism to cope with energy deficiency in various pathophysiological conditions. Gluconeogenesis is an energy-demanding process through which lactate, pyruvate, and glycerol are converted into glucose and glycogen. In the meantime, drug metabolism also requires a large amount of ATP to generate sufficient NADPH, a reducing power for the cytochrome P450 enzymes, through the pentose phosphate pathway. By suppressing gluconeogenesis, such as those mediated by PXR and CAR, hepatocytes are able to maintain necessary NADPH levels and sustain a protective function of drug metabolism under energy-restricted conditions. A recent study has suggested that metformin inhibited hepatic gluconeogenesis in mice mainly through regulating the hepatic energy state¹⁹, which indicated an evolutionary conserved mechanism to cope with energy stress induced by external stimulus through the suppression of gluconeogenesis.

PXR and CAR in obesity and dyslipidemia

Excess nutrients and sedentary life style have led to the prevalence of obesity as a major health problem worldwide. The most common consequence of obesity is dyslipidemia, characterized by elevations of very low-density lipoprotein (VLDL) triglycerides and low-density lipoprotein (LDL) cholesterol and reduced concentrations of high-density lipoprotein (HDL) cholesterol, which significantly increased the risk of cardiovascular diseases, such as hypertension and atherosclerosis. Obesity also significantly increases the risk of cholesterol gallstones characterized by the formation of cholesterol oversaturation and crystallization in the gallbladder bile.

Our study has shown CAR agonist administration significantly suppressed or reversed the adiposity gain induced by HFD feeding in C57BL/6J mice without affecting the lean mass, and this reduced adiposity was associated with improved serum lipid profile and insulin sensitivity¹¹. The anti-obesity effect of CAR activation was supported by another independent study. Masuyama and colleagues showed that CAR agonist administration suppressed weight gain of pregnant mice in HFD condition, which was also associated with improved insulin sensitivity and serum lipid profile. Interestingly, maternal CAR activation ameliorated the increased fetal weight induced by maternal HFD feeding without affecting the average litter size and placental weight¹². Since fetal overgrowth resulting from maternal obesity significantly increased the risk of offspring developing obesity and metabolic syndrome in their later life²⁰, it is tempting for us to speculate that CAR activation may offer benefits in preventing juvenile obesity and insulin resistance. Indeed, the same group recently reported that treatment with a CAR agonist during pregnancy prevented weight gain and insulin resistance in offspring from HFD-induced obese pregnant mice²¹. CAR activation can also ameliorate dyslipidemia in mouse models of obesity. It has been shown that CAR agonist treatment can suppress lipogenic gene expression, reduce serum triglyceride levels, relieve hepatic steatosis, and reduce VLDL secretion from the liver^{10–12}. However, to what extent the anti-obesity effect of CAR can be attributed to its suppression of lipogenesis remains an open question.

Mechanistically, it has been reported that activation of CAR reduced the protein level of the active form of sterol regulatory element binding protein 1 (SREBP-1) through inducing Insig-1, a protein blocking the proteolytic activation of SREBPs²². Our own study showed CAR activation compromised the LXR agonist responsive recruitment of LXR α to the promoter region of Srebp-1c, a master lipogenic transcription factor²³. In addition, CAR activation has also been shown to decrease low-density lipoprotein cholesterol levels in Ldlr null mice and inhibit the development of atherosclerotic lesions²⁴, possibly through the stimulation of fecal bile acid excretion and reverse cholesterol transport, thus reducing systemic cholesterol loading over a long-term treatment²⁵. From the therapeutic perspective, suppressing lipogenesis by CAR activation offers additional benefits in the management of T2D since most of T2D patients also suffer from both hyperglycemia and hypercholesterolemia associated with insulin resistance and hyperinsulinemia.

In contrast to the beneficial effect of CAR activation on lipid metabolism, activation of PXR seemed to promote dyslipidemia. Transgenic mice expressing a constitutively activated PXR showed hepatomegaly and marked hepatic steatosis, and treatment of mice with a PXR agonist elicited a similar effect⁹. PXR-induced hepatic steatosis seemed to be attributed to a combination of several mechanisms. First, PXR activation increased fatty acid influx through inducing the expression of fatty acid translocase (FAT/CD36) in the liver⁹, while suppressing fatty acid oxidation through preventing the binding of Forkhead box A2 (FoxA2) to its target genes involved in lipid oxidation such as Cpt1 α and Hmgcs2²⁶. Promoter analyses revealed a direct repeat spaced by three nucleotides (DR-3)-type PXR-response element in the mouse CD36 gene promoter, establishing CD36 as a direct transcriptional target of PXR⁹. PXR can also activate the expression of PPAR γ , a master regulator of adipogenesis, and two DR-3-type PXR-response elements were found in the promoter region of PPAR γ gene¹³. It has also been reported that the thyroid hormone (TH)-responsive spot 14 protein, the expression of which correlates with lipogenesis, was a PXR target gene, which provides another plausible explanation for the lipogenic effect of PXR in liver²⁷. In animal models, PXR activation in wild type mice led to increased levels of the atherogenic lipoproteins VLDL and LDL in a PXR-dependent manner, and increased atherosclerosis in ApoE null mice²⁸. The atherogenic effect of PXR activation was likely mediated through reduced expression of antiatherogenic ApoA-IV in the liver, and more importantly through increased oxidized LDL uptake by inducing scavenger receptor CD36 expression in macrophages²⁸. On the contrary, PXR deficiency decreases atherosclerosis in ApoE null mice, which was associated with reduced expression levels of CD36 and oxidized LDL uptake in peritoneal macrophages²⁹. These results suggest that antagonizing PXR activity in the liver and macrophages may offer therapeutic benefits in the management of dyslipidemia and associated cardiovascular diseases such as atherosclerosis. However, PXR activation in monocytes/macrophages has been shown to exert an inhibitory effect on endotoxin-stimulated NF- κ B activation and tumor necrosis factor α (TNF α) expression³⁰. In addition, PXR may also protect the vasculature from endogenous and exogenous insults by inducing antioxidant genes in aortic endothelial and smooth muscle cells³¹. Whether antagonizing PXR can be used as a therapeutic strategy for dyslipidemia and associated cardiovascular disease requires further validation.

Anti-inflammatory activity of PXR and its potential role in metabolic disease

Excess nutrients and obesity can lead to a low-grade activation of the innate immune system, and this chronic low-grade inflammation has been widely recognized to play critical roles in the initiation, propagation, and development of metabolic diseases. There is emerging evidence suggesting a function of PXR in immune responses, and PXR agonists may have potentials in inhibiting inflammation related disease, including metabolic disease. PXR activation was known to suppress the activity of NF- κ B, a key regulator of inflammation

and the immune response, while PXR null mice had up-regulation of NF- κ B target genes and showed increased susceptibility to inflammatory bowel disease (IBD)³². Recent genome-wide association studies have also suggested the association between PXR gene polymorphisms and susceptibility to IBD^{33, 34}. More recent studies suggested a direct anti-inflammatory effect of PXR on immune cells. PXR has been shown to express in monocytes/macrophages, a major source of inflammatory mediators such as TNF α , and PXR activation inhibited endotoxin-induced NF- κ B activation and TNF α secretion from monocytes/macrophages in a PXR-dependent manner³⁰. PXR expression has also been detected in human T lymphocytes and the expression of PXR was significantly up-regulated upon immune activation. Activation of PXR inhibited T lymphocyte proliferation and activation upon immune stimulation, while PXR-null mice exhibited an exaggerated proliferation and hyper-responsiveness of T lymphocytes in response to inflammatory stimuli such as LPS³⁵. PXR has also been reported to negatively regulate B-cell development in the fetal liver and PXR null mice displayed B-cell lymphoma in an age-dependent manner^{36, 37}. The detailed molecular mechanisms through which PXR activation regulated immune response remain to be clearly defined. It has been shown that PXR activation inhibited MEK1/2 and NF- κ B signaling³⁵, and suppressed the binding of NF- κ B to its target genes³⁸. A recent study showed inflammatory response induced the SUMOylation of ligand-bound PXR and enhanced its inhibitory effect on TNF α -induced NF- κ B activation³⁹. Rifampicin, a potent human PXR activator, showed efficient immunosuppressive effect and has been used clinically for the treatment of psoriasis, an autoimmune skin disease⁴⁰. Rifaximin, a rifampicin derivative, showed PXR-dependent immunosuppressive effects in intestinal epithelial cells and is clinically used for the treatment of IBD^{38, 41}.

The well recognized causal relationship between inflammation and metabolic dysfunction highlights the potential utility of PXR agonist in metabolic diseases. A recent study suggested the intestinal inflammation induced by HFD precedes and correlates with obesity and insulin resistance⁴². Increased intestinal inflammation was likely the result of adaptive change of gut microbiota population in response to HFD⁴³. The lack of the host defense component, such as the Toll-like receptor 5 (TLR5) in the intestine, led to altered gut microbiota population, increased proinflammatory gene expression, and development of metabolic syndromes⁴⁴. Since PXR activation has been documented to suppress inflammatory response in the gut, it would be of interest to investigate whether intestinal PXR activation can offer benefit in relieving HFD-induced obesity and insulin resistance. It is conceivable that PXR activation specifically in the intestine would be desirable in order to avoid the adverse effect of PXR activation on hepatic lipid metabolism. Indeed, rifaximin, a clinically used rifampicin derivative, may represent a prototype of intestine-specific PXR agonist. Rifaximin has poor systemic bioavailability and cannot activate PXR in the liver, but it has high intestinal tract distribution and exerts the property of gut-specific human PXR activator⁴⁵. Given the complexity of PXR biology, both tissue-specific PXR agonists and antagonists may have their therapeutic potentials in managing metabolic diseases.

Challenges in harnessing the therapeutic potentials PXR and CAR in metabolic disease

CAR activation seemed to ameliorate most hallmarks of metabolic syndromes, including hyperglycemia, dyslipidemia, increased adiposity, and hypertension, which confer CAR desired properties as a promising therapeutic target for metabolic disease. However, a safety issue may be a hurdle for utilizing current CAR agonists for the treatment of metabolic disease. CAR activation by PB or its derivative TCPOBOP in mice has been known to mediate the hepatic toxicity of acetaminophen⁴⁶. Activation of CAR also promotes liver hyperplasia and carcinogenesis likely due to the induction of the growth arrest and DNA

damage inducible 45 (Gadd45) proteins⁴⁷. It remains uncertain whether the metabolic benefit of CAR activation can be separated from its adverse effects. Numerous chemicals from herbal medicines and polyphenols from alcoholic beverage, have been shown to activate CAR directly or indirectly^{48–50}. It is encouraging that most of these herbs have been widely used without obvious toxicity. PXR seems to have a more complex or even controversial role in metabolic disease in a tissue or cell type dependent manner. PXR activation in hepatocytes and macrophages may lead to hepatic steatosis, dyslipidemia, and increased atherosclerosis, therefore PXR antagonists may offer therapeutic benefits. On the other hand, PXR activation exerted anti-inflammation effects in immune cells and intestinal epithelial cells, so a PXR agonist may also ameliorate metabolic diseases known to be associated with chronic immune activation. Targeting dietary-induced intestinal inflammation by PXR agonist seems to be interesting and tempting, because the intestine-specific PXR agonist rifaximin has already been used clinically. As we know more about the PXR biology, the development of PXR modulators that can selectively target certain PXR downstream pathway may also become feasible. A recent study has shown that acetylation of PXR protein regulates its pro-lipogenic function independent of ligand activation⁵¹, which suggests the possibility of selectively regulating PXR downstream signaling through the manipulation of its post-translational modification. A similar concept has been adopted in the development of novel PPAR γ modulators as a new generation of anti-diabetic drugs with less adverse effect while retaining therapeutic efficacy^{52, 53}. Natural PXR agonists isolated from herbal medicine and marine species may also serve as potential leads for the development of novel PXR modulators. 4-methylenesterols and polyhydroxylated steroids from the marine sponge are natural PXR agonists that exerted robust immunomodulatory activity⁵⁴. Solomonsterols A and B, from a *Theonella swinhoei* sponge, have been shown to suppress pro-inflammatory cytokine secretion and trigger the secretion of anti-inflammatory cytokines in an animal model of IBD in a PXR- dependent manner⁵⁵. Further characterizing natural PXR agonists and their biological activities may hold promise of developing novel PXR modulators for the treatment of metabolic disease. The list of PXR and CAR ligands has been continuously growing in recent years^{56–72} and these small molecules with diverse structures may serve as starting points for future development of novel PXR and CAR modulators with therapeutic potentials (Tables 1 and 2).

Given the well-recognized role of PXR and CAR in regulating drug metabolism, pharmaceutical agents targeting PXR/CAR have the potential of causing untoward drug-drug interactions. However, we also recognize that PXR activation alone may not be sufficient to predict the propensity of drug-drug interactions. Sinz and colleagues published the evaluation of 170 pharmaceutical compounds in a PXR transactivation assay and compared these results to known clinical drug-drug interactions. Of the 170 agents tested, 54% of them demonstrated some level of PXR transactivation. However, by taking into consideration cell culture conditions (solubility, cytotoxicity, appropriate drug concentration in media), as well as *in vivo* pharmacokinetics (therapeutic plasma concentration or C_{max}, distribution, route of administration, dosing regimen, liver exposure, potential to inhibit CYP3A4), the risk potential of CYP3A4 enzyme induction for most compounds is reduced to approximately 5%, all of which are known to cause drug-drug interactions in the clinic⁷³.

Conclusion remarks

Recent findings from many laboratories have clearly suggested that PXR and CAR regulate not only drug metabolism but also energy homeostasis and immune response, which may establish PXR and CAR as potential therapeutic targets for metabolic diseases. The endobiotic functions of PXR and CAR, including those implicated in metabolic diseases, are expected to drive these “old” xenobiotic receptors into a new era. As summarized in Figure 1, targeting both gluconeogenic and lipogenic pathways by CAR confers desired properties

of this receptor as a promising therapeutic target for metabolic diseases. Although PXR activation may increase hepatic fatty acid influx, lipogenesis and lead to steatosis, its immunosuppressive effects in the intestine and on immune cells may offer beneficial effects for metabolic disorders by targeting chronic inflammation. The search for selective PXR and CAR modulators may provide novel therapeutic tools for the management of metabolic disorders.

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Abbreviations

CAR	constitutive androstane receptor
G6Pase	glucose-6-phosphatase
HFD	high-fat diet
HGP	hepatic glucose production
LDL	low-density lipoprotein
PCN	pregnenolone-16 α -carbonitrile
PEPCK	phosphoenolpyruvate carboxykinase
PXR	pregnane X receptor
T2D	type 2 diabetes
VLDL	very low-density lipoprotein
IBD	inflammatory bowel disease

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Figure 1. PXR and CAR as potential therapeutic targets for metabolic diseases

Activation of CAR suppresses both hepatic gluconeogenesis and lipogenesis, likely mediated through its inhibitory effect on transcriptional factors such as FoxO1, HNF4 α , PGC1 α , LXR, and SREBP1. Targeting both gluconeogenic and lipogenic pathways by CAR confers its desired properties as a promising therapeutic target for metabolic diseases. The *in vivo* benefit of CAR activation in relieving metabolic disease has also been reported. In the liver, PXR has a similar inhibitory effect on gluconeogenesis. However, PXR activation may increase fatty acid influx and lipogenesis directly or by activating PPAR γ , and suppress fatty acid oxidation through suppressing FoxA2. PXR activation also has immunosuppressive effects evidenced in the liver, intestine, and immune cells. The *in vivo* significance of PXR in metabolic syndrome remains to be demonstrated. CAR; constitutive androstane receptor; PXR, pregnane X receptor; FoxO1, Forkhead box protein O1; HNF4 α , hepatocyte nuclear factor 4 α ; LXR, liver X receptor; SREBP1, sterol regulatory element-binding protein 1; PPAR γ , peroxisome proliferator-activated receptor γ ; FoxA2, forkhead box protein A2.

Table 1

A partial list of PXR modulators and their biological activity

Name of Chemicals	Source and Biological Activity	Clinical Usage
Rifampicin	Semisynthetic human PXR agonist; Antibacterial and immunosuppressive	Used for tuberculosis, Psoriasis ⁴⁰
Pregnenolone-16 α -carbonitrile (PCN)	Synthetic mouse PXR agonist; Induce hepatic steatosis, Immunosuppressive	N/A
Rifaximin	Synthetic gut-specific human PXR agonist; Immunosuppressive ⁴⁵	Used for diarrhea and IBD
Hyperforin	Natural product from St. John's wort; Human PXR agonist ⁵⁶	Used for mild depression
Schisandrol B; Schisandrins A and B	Natural product from <i>Wu Wei Zi</i> ; Human PXR agonist ⁵⁷	A traditional Chinese medicine herb
Ginkgolide A	Natural product from <i>Ginkgo biloba</i> ; Human PXR agonist ^{58,59}	N/A
Guggulsterones	Natural product from guggul tree; Human and rodent PXR agonist ⁶⁰	A traditional Indian medicinal plant
Coumestrol	Natural phytoestrogen; Human PXR antagonist ⁶¹	N/A
4-Methylenesterols	Natural product from <i>Theonella swinhoei</i> sponge; Human PXR agonist with immunosuppressive activity ⁵⁴	N/A
Solomonsterol A, B	Natural product from <i>Theonella swinhoei</i> sponge; Human PXR agonist with immunosuppressive activity ⁵⁵	N/A
Ketoconazole	Synthetic PXR antagonist ⁶² ; Antifungal	Used for fungal infections
Sesamin	Natural product from sesame seeds, PXR antagonist ⁶³	N/A

Table 2

A partial list of CAR modulators and their biological activity

Name of Chemicals	Source and Biological Activity	Clinical Usage
Phenobarbital	Synthetic human, mouse CAR agonist	Used for seizures and anxiety
6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime (CITCO)	Synthetic human CAR agonist ⁶⁴	N/A
1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP)	Synthetic mouse CAR agonist ⁶⁵ ; Suppressing gluconeogenic and lipogenic genes, anti-obesity	N/A
cis-2,4,6-triphenyldioxane-1,3	Synthetic rat CAR agonist; Suppressing gluconeogenic and lipogenic genes ¹⁷	N/A
Meclizine	Synthetic mouse CAR agonist, and human CAR inverse agonist ⁶⁶	Used for nausea, vomiting, and dizziness
Di(2-ethylhexyl) phthalate	Synthetic human CAR agonist ⁶⁷	N/A
Diallyl sulfide	Natural mouse CAR agonist from garlic oil ⁶⁸	N/A
6,7-Dimethylesculetin	Natural human, mouse CAR agonist from Yin Chin ⁶⁹	Used for neonatal jaundice
Androstanol	Synthetic inverse CAR agonist ⁷⁰	N/A
1-(2-chlorophenylmethylpropyl)-3-isoquinoline-carboxamide (PK11195)	Synthetic human CAR antagonist ⁷¹	N/A
1-[(2-methylbenzofuran-3-yl)methyl]-3-(thiophen-2-ylmethyl) urea (S07662)	Synthetic human CAR inverse agonist ⁷²	N/A