

# Review paper on Orphan Receptors as Drug Targets

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**Abstract** - Orphan receptors is a unique resource for discovering novel regulatory systems that impact human health. These provide excellent drug targets for a variety of human diseases. Ligands of nuclear receptors have been used in number of important therapeutic area, such as breast cancers, skin disorders and diabetes. Orphan nuclear receptors; therefore, represent a tremendous opportunity in understanding and treating human diseases. Orphan nuclear receptors using in particular PPARs a strategy for drug discovery in diabetes and obesity, neurodegenerative disease and other related disorders. If an endogenous ligand is there, the orphan receptor is assumed or de-orphaned. An example is the nuclear receptor Farnesoid X receptor (FXR) and the GPCR TGR5/G-protein coupled receptor-19/G protein-coupled bile acid receptor, both of which are activated by bile acids. Adopted orphan receptors in nuclear receptor group contain FXR, liver X receptor (LXR), and peroxisome proliferator-activated receptor (PPAR). One example of an orphan receptor site is the PCP binding site in the NMDA receptor, a type of ligand-gated ion channel. This site is where the recreational drug PCP works, but no endogenous ligand is known to bind to this site. Orphan GPCR exhibited sequence similarities with the opioid receptors. OFQ/N was isolated as its natural ligand and shown to also share sequence similarities to the opioid peptides.

**Index Terms** - Nuclear receptors, Orphanin FQ/nociception, Farnesoid X receptor.

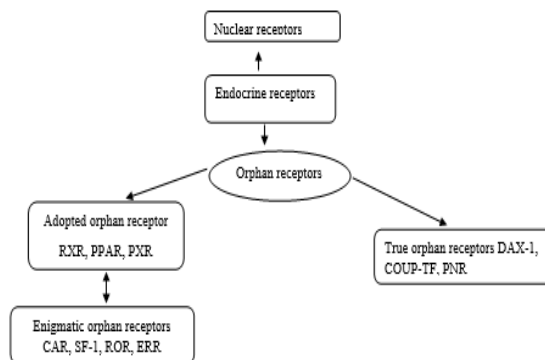
## 1. INTRODUCTION

The pharmaceutical industry has readily included genomics to provide new targets for drug discovery. Large scale DNA sequencing has allowed the identification of a excess of DNA sequences distantly related to known G protein-coupled receptors (GPCRs), a superfamily of receptors that have a proven history of being excellent therapeutic targets. In most cases the extent of sequence homology is insufficient to assign this orphan receptors to a particular receptor subfamily {1}. Consequently, reverse molecular pharmacological and functional

genomic strategies are being employed to identify the activating ligands of the cloned receptors. Briefly, the inverse molecular pharmacological methodology includes cloning, expression of orphan GPCRs in mammalian cells and screening these cells for a functional response to replace agonists present in biological extract preparation, peptide libraries and complex compound collections. The functional genomics includes the use of humanized yeast cells, where the yeast GPCR transduction system is planned to permit functional expression and coupling of human GPCRs to the endogenous signaling machinery. These two systems provide an excellent platform for identifying novel receptor ligands. Once the activating ligands are identified these can be used as pharmacological tools to explore receptor function and relationship to disease {2}.

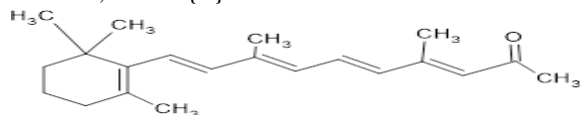
## 2. SCOPE

Orphan receptors is an apparent receptor that have similar structures to other identified receptors but whose endogenous ligand has not yet been identified. If a ligand for an orphan receptor is discovered later, then the receptors is referred as “adopted orphans”. Examples of orphan receptors are found in GPCR and nuclear receptors families: GPCR orphan receptors are usually given the name GPR followed by a number e.g.: GPR1.



### 3. ORPHAN GPCRS

The first GPCR is identified was rhodopsin in 1878. It was later proven that rhodopsin consists of the GPCR protein opsin and a reversibly covalently bound cofactor, retinal {3}.



- After completion of the human genome sequence in 2004, the number of human GPCRs increased to about 800 based on the screening approaches, such as low-stringency hybridization, PCR-derived methods, and bio-informatics analyses.
- Besides the olfactory receptor family, more than 140 GPCRs have not yet been linked to

endogenous ligands. These are the so-called orphan GPCRs.

#### Dimeric Orphan receptors:

The orphan receptors derive their name from the fact that cognate hormones for these receptors were originally unknown or little understood. Orphan receptors can bind homodimers to recognition sequences arranged as direct repeats.

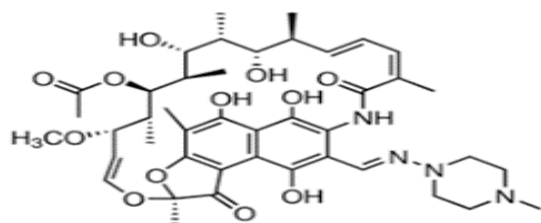
#### Monomeric Orphan Receptors:

Orphan receptors is also known as (e.g., Retinoic Z receptor, RZR; NGF- induced clone B, NGFI-B) which bind as monomers to asymmetric recognition sequences.

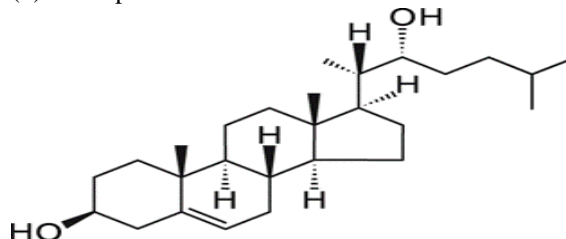
Table: 1

ORPHAN NUCLEAR RECEPTOR (ONR)	NRNC SYMBOL	DISEASE ASSOCIATION	AGONIST	ANTAGONIS T
Pregnanex receptor (PXR)	NR112	1. Colon, Breast and Ovarian Cancer (91,93,94) 2. Drug Drug Interactions (91,92) 3. Alcoholic and non-alcoholic hepatitis (101) 4. Acetaminophen-induced hepatotoxicity (103,104) 5. Osteomalacia (102) 6. Intestinal inflammation (109) 7. Osteoporosis (106) 8. Bile acid induced cholestasis (99)	Rifampicin PCN, PB, SSR12813	Ketoconazole, Suphoraphane, ET-743, Coumesterol
Constitutive androstane receptor (CAR)	NR113(α) NR114(β)	1. Liver cancer (125) 2. Hyperthyroidism (126) 3. Obesity (127) 4. Metabolic disorders (128-130)	PB, TCPOBOP	Clotrimazole, androstenol
Farnesoid X receptor (FXR)	NR1H4	1. Hyperglyceridemia (153) 2. Cholesterol gall stone disease (151) 3. Colon and breast cancer (158,159) 4. Hypo-hdlcholesterolemia (152)	Chenodeoxycholic acid, 1,1-bisphosphonate esters, GW4064	Guggulsterone
Liver X receptor (LXR)	NR1H3(α) NR1H2(β)	1. Atherosclerosis (138-140) 2. Inflammation (145-146) 3. Prostate, breast and colon cancer (142-144) 4. Cholesterol related 5. Neurodegenerative disorders (148) 6. Hepatic lipogenesis (136,137)	Oxysterol, 22@-hydroxycholesterol, GW3965	Fenofibrate esters

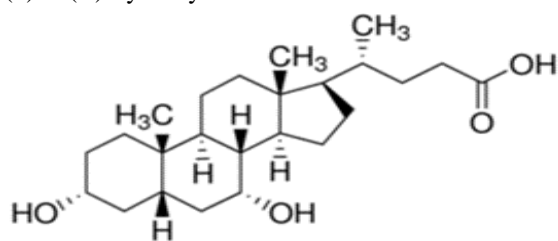
#### Agonist structures



#### (2) Rifampicin

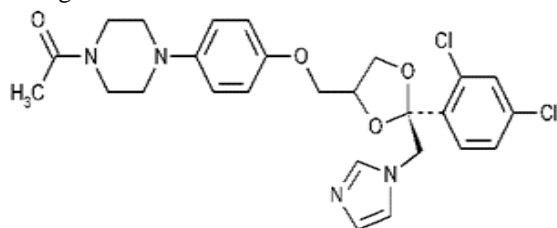


(3) 22(R)-hydroxycholesterol

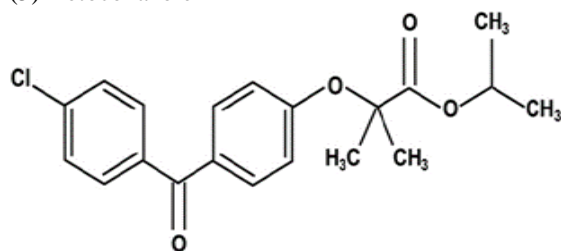


(4) Chenodeoxycholic acid

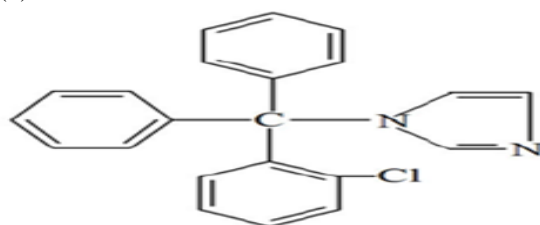
Antagonist structures



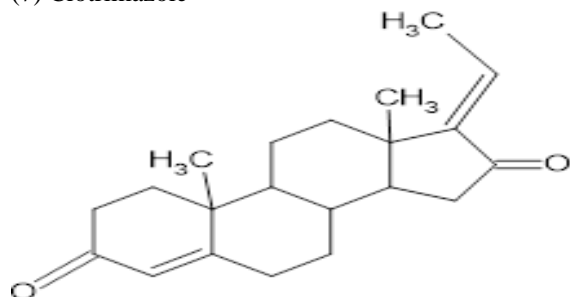
(5) Ketoconazole



(6) Fenofibrate esters



(7) Clotrimazole



(8) Guggulsterone

On the basis of degenerated-oligonucleotide PCR analysis of HUT 102 cells designed since the conservative regions of the human chemokine receptor using primers GPR18, Orphan receptor, is replicated. GPR18 was expressed significantly in

lymphoid cell lines, but not in non-lymphoid hematopoietic cells. GPR18 gene expression was higher in peripheral lymphocyte subsets (CD4+, CD4+CD45RA+, CD4+CD45RO+, CD8+, and CD19+) than in monocytes and lymphoid cell lines, and was increased after stimulation with Phytohemagglutinin [4]. By using screening, a lipid library, N-arachidonylglycine (NAGly) induced an increase in intracellular Ca<sup>2+</sup> concentration in GPR18-transfected cells, which was significantly greater than that in mock-transfected cells. Skolin-induced cAMP production is inhibited by NAGly in a pertussis toxin-sensitive manner in the GPR18-transfected CHO cells. It is the first study to demonstrate NAGly which is a natural ligand for GPR18.

#### 4. ORPHAN RECEPTORS AS DRUG TARGETS

A) Orphan Receptors as Drug Target for the Treatment of Parkinson's Disease (Pd):

The NR4A subfamily of nuclear receptors consists of three members, Nurr1, Nur77, and Nor1.

They largely function as immediate-early genes, the expression and activation are regulated in a cell-type specific manner in response to a range of signals, such as mitogenic and apoptotic stimuli.

Nurr1 is expressed predominantly in the central nervous system (CNS), especially in substantia nigra, ventral tegmental area, midbrain and limbic areas.

Several lines of evidence have indicated that Nurr1 and this is essential for the development, migration, and survival of dopaminergic neurons.

As Parkinson's disease results the loss of dopaminergic neurons, the prospect as a drug target for PD is promising of using Nurr1.

B) Orphan Nuclear Receptors as Drug Targets for the Treatment of Prostate and Breast Cancers: {6}

Nuclear receptors (NRs), it belongs to a family of 48 transcriptional factors, it has been studied intensively for their roles in cancer development and progression. NRs attractive targets for developing cancer therapeutics made by the presence of distinctive ligand binding sites capable of interacting with small molecules.

Over the last few years, so many drugs have been developed to target human androgen- and estrogen

receptors for the treatment of prostate cancer and breast cancer.

In contrast, orphan nuclear receptors (ONRs), in many cases lack known biological functions or ligands, are still largely under investigated.

C) Targeting Orphan G Protein-Coupled Receptors for The Treatment of Diabetes and Its Complications: C-Peptide and GPR146: {7}.

G protein-coupled receptors (GPCRs) is the most abundant receptor family encoded by the human genome and the targets of a high percentage of drugs currently in use or in clinical trials for the treatment of diseases such as diabetes and its associated complications.

Orphan GPCRs, where the ligand is unknown, represents an important untapped source of therapeutic potential for the treatment of many diseases.

It was identified that, previously an orphan GPCR, GPR146, and the putative receptor of proinsulin C-peptide, which may prove to be an effective treatment for diabetes-associated complications.

For example, a monolayer of cells in the retina is surveyed as part of the blood-retinal barrier and is disrupted in diabetic macular oedema by the potential role of C-peptide and GPR146 is regulating the function by retinal pigment epithelium,

Though, C-peptide signaling in this cell type appears to depend in the part of extracellular glucose concentration and show its interaction with insulin.

D) GPCRS, ORPHAN GPCRS in the treatment of Diabetes {5}:

Traditionally, patients with diabetes have been treated with injectable insulin or oral agents.

Even though insulin therapy is the mainstay treatment of type 1 diabetes which lowers the blood glucose levels, it must be carefully administered to approximate physiological insulin delivery.

Although many formulations exist (regular, intermediate- and long-acting insulin, human and animal origins, etc.), the use of these products requires substantial empirical adjustment.

Significant side effects limiting their therapeutic effectiveness can include hypoglycaemia, allergic reactions and antibody-mediated insulin resistance.

Oral agents are typically the first-line therapy in patients with type 2 diabetes.

These makes move by either stimulating insulin secretion from the beta cells of the pancreas (sulphonylureas such as glipizide or gliburide) or increasing insulin sensitivity, enhancing glucose uptake and decreasing gluconeogenesis in peripheral tissues (biguanides).

These approaches are not there without complications, and it also include side effects such as hypoglycemia, abdominal pain, nausea or diarrhea stemming from their effects on either the endocrine or peripheral tissues {8}.

Table 2 {7}.

GPCR	Targeting Compound	Trade Name (If Applicable)	Action	Approval Status
GLP1R	Exenatide	Byetta, Bydureon	Incretin mimetic	Approved
	Liraglutide	Victoza, Saxenda	Incretin mimetic	Approved
	Albiglutide	Tanzeum	Incretin mimetic	Approved
	Dulaglutide	Trulicity	Incretin mimetic	Approved
	Semaglutide		Incretin mimetic	Phase III
	Lixisenatide	Lyxumia	Incretin mimetic	Phase III completed
	Taspoglutide		Incretin mimetic	Phase III terminated
	Insulin degludec and liraglutide	iDeg-Lira	Combination	Phase III
	Insulin glargine and lixisenatide	LixiLan	Combination	Phase III completed
DPP4 inhibitor	Alogliptin	Nesina	DPP4 inhibitor	Approved
	Saxagliptin	Onglyza	DPP4 inhibitor	Approved
	Sitagliptin	Januvia	DPP4 inhibitor	Approved
	Vildagliptin	Galvus	DPP4 inhibitor	Phase III
	Linagliptin	Tradjenta	DPP4 inhibitor	Approved
	Gemigliptin		DPP4 inhibitor	Phase III
	GPR40	Fasiglifam (Tak-875)		GPR 40 agonist
D2R	Bromocriptine	Cycloset	Reduces HbA1C	
CpepR	C-peptide	Ersatta	CpepR agonist	Phase IIb

E) Nuclear Receptors as Drug Targets in Obesity, Dyslipidemia and Atherosclerosis: {9}

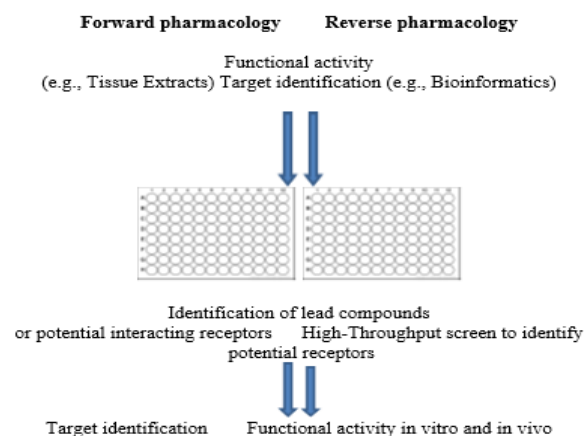
Nuclear hormone receptors contain peroxisome proliferators- activated receptors (PPARs), liver X receptors (LXRs) and the farnesoid X receptor (FXR), are transcription factors involved in the regulation of

essential metabolic functions, it also consists of glucose and lipid metabolism, reverse cholesterol transport, and the regulation of bile acids.

This develops in the use of PPARs, LXR and FXR agonists for the treatment of obesity and cardiovascular diseases, including dyslipidemia and atherosclerosis.

### 5. DEORPHANIZATION STRATEGIES: PAST, PRESENT AND FUTURE

- Classically, identification of ligand–receptor pairs depended upon purification of biologically active compounds from large quantities of tissue extracts, which be used to assess activity of cloned receptors overexpressed in null cell lines.
- With the advent of the Human Genome Project and the formation of readily accessible databases containing the genome sequences of humans and many other species, targeted drug development, or ‘reverse pharmacology’ became possible.
- Using this there is a strategy, a potential drug target is identified based on sequence homology or gene expression, and synthetic ligands replacement of purified tissue extracts which is used in traditional or ‘forward pharmacology’ {14}.
- In recent years, reverse pharmacology has been used by the pharmaceutical industry to develop synthetic substances based on natural molecules to treat a variety of conditions.
- Most recently, companies have turned to computer modelling systems to predict the molecular activity of a drug at a particular receptor, which has allowed more, focused in vitro experimentation.



### 6. DRUG TARGET DISCOVERY AND RECEPTOR DEORPHANIZATION STRATEGIES

- Both forward and reverse pharmacology depend upon high-throughput assays, which have been a backbone of receptor deorphanization for many years.
- Robotic screens, in which hundreds of receptors are overexpressed with reporter molecules (e.g., SRE-luciferase reporter proteins) in separate cell populations are exposed to potential ligands or drugs, are in use by several pharmaceutical companies {13}.
- Although this strategy had some success in identifying ligand–receptor pairs, particularly when used in combination with reverse pharmacology and PCR based homology screening technologies.
- For example, the orexins were identified based on the ability of orexin-containing tissue extracts, these will stimulate calcium release, and this will apply to separate cell cultures expressing over 50 different GPCRs.
- The orexins and their receptors are now known to play important roles in sleep and arousal, as well as in metabolism, and orexin receptor-based drugs are currently in development for the treatment of narcolepsy.
- Other examples include ghrelin, which is produced in the gut and stimulates appetite and was found to bind the growth hormone secretagogue receptor (GHSR), and the adipokine apelin, which binds the previously orphan receptor APJ and plays important roles in glucose homeostasis as well as exerting cardiac and vascular effects {11}.

### 7. CONCLUSION

In last few years, A numbers of new orphan GPCRs have increased and there is several members have been relatively well characterized. These is used for ligands of orphan nuclear receptors has led to the discovery of many signaling pathways and revealed a direct link of nuclear receptors to human diseases such as diabetes, obesity, and neurodegenerative diseases. Ligand identification of orphan receptors will lead to the discovery of novel hormone response systems and

open many new therapeutic avenues for a variety of human diseases. Identifying these compounds with selective activity for specific orphan receptors is consisting of clinical and pharmacological importance and promises a bountiful harvest in the near future.

However, the orphan GPCR function research is hampered by the lack of identified ligands and by the unique structures of the GPCR themselves. Further there show investigation of their signaling pathways is valuable to understand the physiological and pathological roles of these new orphan GPCRs. The development of orphan GPCR knockout mice has also been shown to be a successful method for the characterization of their physiological and pathological functions. The knockout approach of orphan GPCRs is essential for our understanding of these receptor functions and their potential pathways. Probes can serve functional and specific antibodies not only for the ligands, but also for emerging therapies for tumors and genetic disorders in orphan GPCRs are involved. Although progress is very difficult, searching for the ligands of orphan GPCRs and identifying their physiological functions will continue. With recent discoveries of more and more orphan GPCR signaling pathways, understanding of their particular physiological functions and deorphanization for therapeutic purposes should accelerate in the coming years.

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