Published in final edited form as:

Med Res Rev. 2019 July ; 39(4): 1372–1397. doi:10.1002/med.21578.

A Review of the Molecular Design and Biological Activities of RXR Agonists.

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Abstract

An attractive approach to combat disease is to target the regulation of cell function. At the heart of this task are nuclear receptors (NRs); which control functions such as gene transcription.

Arguably, the key player in this regulatory machinery is the retinoid X receptor (RXR). This NR associates with a third of the NRs found in humans. Scientists have hypothesized that controlling the activity of RXR is an attractive approach to control cellular functions that modulate diseases such as cancer, diabetes, Alzheimer's disease (AD) and Parkinson's disease (PD). In this review we will describe the key features of the RXR, present a historic perspective of the first RXR agonists, and discuss various templates that have been reported to activate RXR with a focus on their molecular structure, biological activity, and limitations. Finally, we will present an outlook of the field and future directions and considerations to synthesize or modulate RXR agonists to make these compounds a clinical reality.

Keywords

Retinoid X receptor; 9-*cis*-retinoic acid; bexarotene; anticancer agent; rexinoids

1. INTRODUCTION

Nuclear receptors (NRs) are proteins that bind to DNA, regulating cellular functions such as growth, differentiation, and metabolism.¹ The activity of NRs can be modulated by the binding of small molecule ligands such as heterocyclic compounds or peptides, by cytokine signaling cascades, or by their interactions with other proteins such as growth factors.

Because of their ability to control gene expression, they are considered transcription factors.

² There are 48 different human NRs³ identified. All have a similar structural organization and typically contain five structural domains: (1) the *N*-terminal domain, which varies considerably among the receptors; it contains a transactivation domain known as Activation Function 1 (AF-1) and is ligand independent; (2) the DNA-binding domain (DBD), which is conserved across various receptors; this region has four cysteines that coordinate to two zinc atoms and contains the "P-box," which binds to DNA response elements; (3) the hinge, a highly flexible connecting region believed to regulate the cellular distribution of the NR; (4)

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⁶ CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

the ligand-binding domain (LBD), the largest domain within the transcription factors, containing a binding pocket to identify small molecule ligands and coregulatory regions; and (5) the C-terminal domain that contains functional motifs such as the activating function 2 (AF-2), which confers ligand-dependent transcriptional activity to some NRs.⁴⁻⁷ The LBDs usually recognize coactivator peptide segments of sequence LXXLL, and corepressor segments of sequence LXXXLXXX[I/L] sequences (where L = leucine, I=isoleucine, and X= any amino acid).^{8,9}

Based on their DNA binding ability and their mechanism of action, the NRs can be categorized into 4 core groups.^{10,11}

Type 1 Receptors, usually known as steroid hormone receptors, are homodimeric, bind to DNA inverted repeats. These NRs are retained in the cytosol until there is a binding event to a ligand, which triggers a translocation to the nucleus. Some examples of this class are the androgen (AR), the estrogen (ER), the progesterone (PR), and the glucocorticoid (GR or GCR) receptors.

Type 2 Receptors, also called nonsteroidal NRs, are found in the nucleus forming complexes with co-repressor proteins.¹² This group is composed of heterodimers of the retinoic X receptor (RXR) and another NR including: retinoic acid receptor (RAR), vitamin D receptor (VDR), thyroid hormone receptor (TR) and peroxisome proliferator-activated receptors (PPAR). These heterodimers bind to DNA repeats and remain in the nucleus.

Type 3 receptors¹³ are pairs formed by the same NR (homodimers). These are similar to the type 1 receptors but bind to DNA direct repeats.

Type 4 receptors bind DNA directly as monomers or dimers to half-site hormone-response elements.²

These last two categories are related to the orphan receptors (OR), proteins that do not have an identified endogenous ligand. Some identified orphan receptors are: the small heterodimer partner (SHP), the testicular receptor 2 and 4 (TR2 and 4), the photoreceptor-specific NR (PNR), and the estrogen-related receptor (ERR). It is worth to mention that some authors consider RXR an OR since the presence of a natural ligand agonist remains controversial.¹⁴ There is, however, a report that shows 9-*cis*-13,14-dihydroretinoic acid is a RXR ligand in mice.¹⁵

2. THE RETINOID X RECEPTOR

The favored heterodimerization partner for approximately one third of the NRs is RXR.^{16,17} Three isoforms of RXR have been identified in humans: (i) the α isoform, which is found in the kidney, liver, and intestine and is the major RXR isotype present in human skin; (ii) the β isoform, which is present in virtually every human tissue; and (iii) the γ isoform, which is mainly found in the pituitary gland, brain, and muscles.¹⁸⁻²² The literature indicates that there is a functional overlap between these three isoforms and that their malfunction is damaging to human health. Deficiencies in the α subtype have been shown to have a more detrimental impact on health compared to the other isoforms.^{23,24,25} For example, knockout

of the α isoform in mice is lethal due to cardiac failure and ocular malformations. In addition, the inactivation of the α subtype presents consequences that are similar to those observed in vitamin A-deficiency, which suggests this isoform is a key player in retinoid signaling.²⁶

The RXR heterodimers can be divided in two main groups as seen in Figure 1:

1. permissive heterodimers,²⁷ are dimers whose activation can be triggered by binding of RXR agonists, binding of the agonists of the NR partner, or binding to both NRs. Some of these NR pairs are: RXR/Liver X receptor (LXR), RXR/PPAR, and RXR/Farnesoid X receptor (FXR) heterodimers. The binding of ligands to both partners could lead to an additive or synergistic biological response. The associated corepressor proteins are released when binding of any agonist takes place.
2. non-permissive heterodimers, are systems whose transcriptional activation is triggered only by agonist binding to the heterodimerization partner. In this situation the RXR is considered a silent or subordinated partner. The RXR/TR and the RXR/VDR are examples of this group. In this case, the RXR can still bind to a ligand and interact with coactivator proteins. Meanwhile, corepressor proteins can only be released when the agonist of the RXR partner produces a binding event. In some cases, binding of a RXR agonist, when the heterodimer ligand is present, may lead to synergistic action.^{27,28} Some special cases of heterodimers are the pairs formed by RXR/RAR and RXR/VDR. While some authors considered these duos as RXR non-permissive partner, others group them into a special category: conditional permissive partnerships, due to the mixed status of the activation. The heterodimer is not affected by the binding of a RXR ligand but binding of the RAR or VDR agonists promote transactivation and permissivity, which allows binding of the RXR agonist leading to greater activation of the RAR or the VDR.^{29,30}

The structure of RXR (Figure 2A) is similar to other NRs, including its N terminus, DBD, connecting (hinge) region, and LBD (Figure 2B).^{31–33} The Nterminal domain possesses a ligand independent activation function (AF-1), and is followed by the DBD. This segment comprises residues 130–209, and the Zn finger domains are located within this region at residues 135–155 and 171–190 (Figure 2C). The two key residues in this area are Lys160 and Arg164, and interact with the phosphate backbone and nucleotides of the DNA major groove. The T-box is positioned after residue 198, and associates with a Zn finger of the upstream 5' of the RXR partner, yielding a DBD dimerization interface that allows the cooperative dimerization on DNA.¹⁴ It is important to mention that in the case of RXR heterodimers the promoter site is defined by the binding of the partner's agonist. Most RXR/partner heterodimers recognize and bind to direct repeat (DR) DNA sequences as their response element (RE), with the DR sequence consisting of two direct repeat half-sites separated by a number of nucleotides (x), as follows: (5'-AGGTCA-N_x-AGGTCA-3'). For example, in the case of RXR/PPAR, the heterodimer preferentially binds to the DR1 PPAR response element (PPRE)³⁴, which consists of two DR half-sites separated by a single base pair. However, in the case of RXR/VDR, the separation is three nuclear bases, binding

preferentially the DR3.³⁴ The heterodimers can bind to RE with RXR occupying either the 5' upstream or 3' downstream half-site.³⁴ Normally, RXR is located on the upstream half-site, with the exception of RAR/RXR/DR1 and PPAR/RXR/DR1³⁵, which have a reversed polarity with RAR and PPAR located on the 5' half-site, while RXR occupies the 3' position.^{34–36} In the RXR/VDR/DR3, the RXR occupies the 5'-end position.³⁶ Meanwhile, in the RXR/RAR heterodimer, the separation can be two or five bases, with RXR located in either the 3' or 5' position.

Next to the DBD is the hinge area, which comprises residues 200–229. This flexible area allows the movement of the LBD of RXR to shift to a position that permits the accommodation of the LBD of other NRs (such as the PPAR γ). RXR is composed of 12 α -helices (when in Apo form) and a β -sheet (more like a β -turn) region found between helices 5 and 6 (Figure 2B). The helices are arranged in a barrel; five helices: H4, H5, H8, H9, and H11 are flanked by H1-H3 on one side and H6, H7, and H10 on the other. H12 extends outside of the main domain. An interesting observation relates to the integrity of H2, upon ligand binding, this helix unwound to create a longer loop between H1 and H3. This allows H12 to move 13Å creating a surface that can bind to coactivator proteins such as TIF2, p/CIP³⁷, or TRAP220.³⁸ A key aspect of RXR is that the position of H12 vary between heterodimers. Only binding to an agonist and a coactivator protein leads to a “fixed” (agonist) conformation of H12. Upon agonist binding, the homodimers experience a conformational change where the alpha helices rotate, “trapping” the agonist (an event that has been termed mousetrap mechanism) and exposing the DNA binding domain.³⁹ Lately, a comprehensive study of current literature has questioned this mechanism and the role of H12.⁴⁰ Those authors favor a disorder-to-order transition mechanism, also known as the H12 dynamic stabilization model,⁴¹ which is induced by ligand agonist binding as the reason of RXR activation. For a current discussion of the topic, we suggest the reader to analyze the report by Rastinejad *et al.*⁴⁰ The conformational changes underwent by RXR and the rotation of some of its alpha helices are shown in Figure 2. This spatial rearrangement exposes Phe450, Glu453 and Glu 456 (all in H12) to the solvent favoring interactions with coactivator peptides.²⁷ This conformational change also promotes the dissociation of corepressor proteins such as SMRT (corepressor silencing mediator for RXR and TR)⁴² and NCoR (NR co-repressor)⁴³ and binding to RE.⁴⁴ The bound coactivator peptides recruit regulatory proteins, which then control biological functions such as gene expression.

The binding pocket of RXR is hydrophobic and restrictive to elongated molecules that have many degrees of freedom. Traditionally, RXR agonists have a non-polar surface that interacts with ligands through a network of van der Waals interactions. Figure 3A shows the structure of 9-*cis*-retinoic acid (9cRA, **1**) and its key interactions with residues inside the RXR binding pocket (Figure 3B). As can be seen, the ligand folds to maximize interactions with residues inside the hydrophobic cavity. A common characteristic of RXR ligands is the presence of hydrogen bonds with the Arg316 and Ala327 residues within the hydrophobic binding pocket.^{14,45–47} It is noteworthy to remark that **1** occupies about 60% of the pocket in RXR α LBD, suggesting that molecules designed to fill the unoccupied space may show better affinities.⁴⁸ There is some controversy regarding **1** as the endogenous ligand of RXRs because this molecule has not been detected *in vitro* or *in vivo* unless all trans-Retinoic acid

(ATRA) is present.⁴⁹ It is not clear if the *C*termini segment, a common feature in NR, exists in RXR. It is proposed that this region is part of the LBD. Regardless of its existence and function, which at the moment is unknown, it is clear this domain is shorter in RXR than in the other receptors.

Given its interactions with various NRs, RXR has become an intriguing target for drug discovery.²⁷ For example, RXR overexpression is found in ~70% of ductal breast carcinomas and the RXR α subtype is up-regulated during cancer progression. The activation of RXR, by its agonists, leads to p21^{WAF1/CIP1} (cyclin-dependent kinase inhibitor) overproduction. Several studies indicate that over expression of p21^{WAF1/CIP1} causes apoptosis in cancer cells.^{50–52} RXR also has a role in other physiological processes.⁵³ For example, Domingo and coworkers reported **1** disrupts normal differentiation from monocyte to immature dendritic cells (DC).⁵⁴ One experiment indicated that treatment with **1** (10 nM) increased the levels of the DC maturation markers CD83 and, CD86 while decreasing CD1A.⁵⁴ The authors concluded that **1** affects the proliferative capacity of the DC. RXR γ has also being linked to the function of working memory in animal models,⁵⁵ can promote oligodendrocyte precursor cell differentiation, and enhance CNS myelin regeneration.⁵⁶ Another study has shown that the heterodimers RXR/LXR and RXR/PPAR γ are involved in glucose/lipid metabolism and insulin resistance.^{45,57,58} The RXR/Nurr1 heterodimer is linked to PD. In contrast to other NRs, the ligand binding domain of Nurr1 adopts a “closed conformation” and becomes difficult to target. However, Nurr1 can be indirectly activated by targeting its permissive heterodimerization partner RXR. Figure 4 shows a simplified RXR activation scheme with the RXR ligand bexarotene (**2**); basically, **2** binds to the NR, which interacts with DNA-RE triggering a biological response.

It has been shown that RXR possess other (non-genomic) functions. For example, RXR shuttles TR3 (an orphan receptor) from the nucleus of the cell to the cytoplasm. This transport allows the TR3-mitochondrial Bcl-2 interaction promoting apoptosis.⁵⁹ In another report, Ray and colleagues found that RXR α modulates platelet activation and that treatment with **1** can inhibit platelet aggregation.⁶⁰ Various reports have indicated that RXR modulates inflammation, as recently reviewed by Zhou et al.⁶¹ For example, anti-inflammatory compounds can act as RXR α ligands, which suggest RXR may be an anti-inflammatory intracellular target. Some of these effects may be related to modulation of NF- κ B function or activation of JNK followed by c-jun phosphorylation.^{62,63} The group of Zhang has identified a link between RXR α and the activation of the PI3K/AKT pathway.^{64,65} Further, RXR has been associated with other diseases including: leukemia,^{66,67} AD,⁶⁸ schizophrenia,⁶⁹ and multiple sclerosis (MS).⁷⁰ Because of its role in gene control and non-genomic functions, the development of RXR ligands is a blossoming area of medicinal chemistry research. Various common molecular scaffolds shown to activate RXR and their biological actions are discussed on the next section.

3. RXR AGONISTS

3.1. 9-*cis*-Retinoic acid

Compound **1** (Figure 3A) is a retinoid. Other members of the retinoid family include ATRA, retinol, retinal, and 13-*cis*-retinoic acid. Retinoids play important roles in cell differentiation,

growth, and apoptosis.⁷¹ Mechanistic investigations of retinoids have attributed their actions to binding to RARs and RXRs.^{72–74} ATRA binds to RARs only on RAR/RXR complexes. When ATRA isomerizes into **1**, however, it acts as an agonist of RXR and also as a ligand for RAR.^{48,75} Upon ligand binding, the RAR/RXR dimer interacts with retinoic acid response elements (RARE), triggering events such as cell differentiation, arrest, and apoptosis.⁷¹ The biological role of RARs in the heterodimer is to regulate cellular differentiation and proliferation, whereas RXRs generally regulate cellular apoptosis.⁷²

Retinoids such as **1** have been investigated as chemo-preventers and for chemotherapy.⁴⁸ For example, Koeffler reported that **1** at 1 μ M inhibited the growth of Human myeloid leukemia HL-60 cells after 4 days, and that the compound was more potent than ATRA.⁷⁶ In the same report, the ED₅₀ (effective dose that inhibited 50% of colony formation) of **1** was 2.9 nM compared to 40.0 nM for ATRA in HL-60. Molecule **1** also induced differentiation and inhibited proliferation in fresh leukemic cells from 28 patients.⁷⁶ Soprano and colleagues found that **1** inhibits the growth of ER⁺ MCF-7 breast cancer cells at 1 μ M concentration by blocking entrance in the S-phase. Further, treatment with this molecule did not affect the levels of RXR α or β mRNA.⁷⁷

Since RAR and RXR response elements are found in almost all cell types, they can trigger biological cascades affecting multiple organ systems. As a consequence of its RAR activation properties, **1** produces side effects such as headache, hypertriglyceridemia, teratogenicity, mucocutaneous toxicity and hypercalcemia. In addition, retinoids such as **1** may have catastrophic consequences in pregnancy and toxic effects on embryonic tissue, liver and bone.⁷⁸ This fact has limited the use of **1** as a therapeutic agent. Molecule **1** is currently used only for Kaposi's sarcoma of the skin⁷² when applied as gel (Toctino® or Panretin®) and for chronic hand eczema.^{79104, 159}

3.2. 9cRA related compounds

Nevertheless, **1** has been used as a template for the design of rexinoids,⁸⁰ which are RXR specific ligands that modulate gene transcription without the serious side effects associated with retinoids. Atigadda *et al*⁸¹ synthesized the molecule UAB 8 (**3**) and related analogues **4–7**, all containing a 9-*Z* tetraenoic acid chain bounded to a disubstituted cyclohexenyl ring.⁸¹ Biological studies showed that rexinoid **4** has a half maximal effective concentration (EC₅₀) of 22 ± 5 nM and was able to activate RXR α with more potency than **2** (EC₅₀ = 40 ± 3 nM) in Human embryonic kidney (HEK)-293 cells. This molecule also exhibited high effectiveness for prevention of mammary cancer in rats at doses of 50 mg/kg.⁸¹ However, **4** also caused triglyceride accumulation, which is the same side effect observed in treatments based on other rexinoids. Interestingly, compounds **4**, **7** and **8** all showed higher affinity than **1** for RXR-LBD by ITC; K_d (μ M) = 1.21 ± 003 , 0.47 ± 0.02 , 1.33 ± 0.12 , 1.81 ± 0.06 , respectively. The tumor size decreased to 68%, when rats were fed **4** (50 mg/kg diet) in combination with the selective ER modulator (SERM) tamoxifen (0.4 mg/kg diet), suggesting an additive effect. Rexinoid **5** alone was effective, preventing 62% of mammary cancers.⁸¹ Although **5** was clearly less potent than **4** and equipotent with **1** (Table 1), this molecule showed substantially reduced serum triglyceride levels (71% vs 430% vs 326%). A highlight of the study was the fact that triglyceride levels can be modulated by increasing the

steric bulk of the R₁ substitution (see molecules **4–7**). Furthermore, tumor reduction does not correlate to RXR binding affinity, which suggests other pathways may be present. Moreover, the study showed that **4**, in combination with tamoxifen, and **5** are promising therapeutics against human mammary cancer, with good tumor reduction and low triglyceride levels.⁸¹

A similar molecule, 9cUAB30 (**8**, Figure 5),⁸² was designed by substituting the trimethylcyclohexenyl ring of **1** with a tetralin ring. Compound **8** showed selective RXR agonist activity with IC₅₀ = 284 nM and EC₅₀ = 118 nM in CV-1 (monkey kidney) cells. The same molecule displayed an EC₅₀ of 820 ± 70 nM in RK3E (rat kidney) cells.^{82,83} Molecule **8** did not show any induction (increase of activity) against the three RAR isoforms. Importantly, the compound did not substantially elevated serum triglycerides (63%). Compound **8** was tested in the *N*-methyl-*N*-nitrosourea (MNU)-induced mammary cancer model and was found to be highly active in the prevention of mammary carcinogenesis in rats. Doses of 200 and 100 mg/kg of **8** reduced the number of mammary tumors by 63% and 29%, respectively. Meanwhile **1** at the 100 mg/kg dose level (a level below the toxic dose) decreased the multiplicity of cancers by 65%.⁸⁴ **8** is currently undergoing clinical trials for the treatment of breast cancer.^{83,85}

Atigadda and co-workers⁸⁶ synthesized four 9cUAB30 homologues with methyl substitutions at the 4-, 5-, 6-, 7-, or 8-position of the tetralone ring **9–13** to evaluate the influence of a methyl group in potency and lipid toxicity. Substitution at those positions led to more potent agonists than **8** (Table 1). The EC₅₀ values for RXR α activation in RK3E cells of 6-methyl-UAB30 (**11**) and 7-methyl-UAB30 (**12**) were similar to **1** but with a lower increase in triglyceride levels in the case of **11**. **12** induced severe hyperlipidemia, increasing serum triglycerides 642% in rats. Compound **11** presented a K_d value similar to **1**, 15 ± 2 nM vs 14 ± 3 nM. This molecule also displayed identical inhibition of the oncogene KLF4 when compared with **1**: 110 ± 50 nM vs 110 ± 13 nM. In a mammary rat model, compound **11** (200 mg/Kg dose) caused a 79% decrease in proliferation index (PI) of the cancer when compared with the control experiment (PI indicates the ability of a compound to prevent mammary cancer development). However, **11** was not able to reduce established mammary tumors (table 1),⁸¹ suggesting that **11** may work as a chemopreventive agents. Two rexinoids, 4-methyl-UAB30 (**9**) and 7-methyl-UAB30 (**12**) were more effective than **8** at reducing cancer; 78% (for **9** at a dose of 200 mg/kg), 61% (for **12** at a dose of 100 mg/kg) and 63% (for **8** at a dose of 200 mg/kg), respectively. However, **9** and **12** increased triglycerides to 560–642% in serum and produced hypervitaminosis A at the same diet dose.⁸¹ Thus, toxicity limited their *in vivo* utility since cancer prevention requires dosing for an extended period of time.

The X-ray crystallography structure of **9** (Figure 6C) and 7-methyl-UAB30 (**12** Figure 6D) bound to hRXR α -LBD showed that the methyl groups occupied a similar space in the LBD as the C23 and C24 methyl groups of **2** (Figure 6A). These groups displayed van der Waals interactions with some residues of helix 7 (F346 and V349), revealing the site of lipid toxicity. Notably, these interactions are missing in the crystal structure of 9cUAB30 (**8**, Figure 6B).⁸⁶ The study demonstrated that the addition of a single methyl group increases

the agonist activity nearly 10-fold when compared with **8**, presumably due to additional van der Waals forces within the RXR binding pocket.

3.3. Bexarotene

Perhaps the most popular synthetic retinoid is **2**, which has a better therapeutic index than ATRA and **1** in skin diseases and several types of cancer.⁸⁷ In fact, **2** was the first clinically approved synthetic retinoid for refractory cutaneous T-cell lymphoma (CTCL) treatment by the United States Food and Drug Administration and the European Agency for the Evaluation of Medicinal Products.

The compound was first reported by Boehm *et al* as part of a larger study investigating the effect of RAR and RXR ligands that could activate gene expression on CV-1 cells using a co-transfection assay.⁸⁸ Compound **2** selectively binds and activates RXR subtypes with $EC_{50} = 33 \pm 2$ nM (α), 24 ± 4 nM (β) 25 ± 2 nM (γ) and $K_d = 14 \pm 2$ nM, 21 ± 4 nM, and 29 ± 7 nM for the different subtypes. On the other hand, **2** has low affinity for RARs ($EC_{50} > 10000$ nM).⁸⁸ These data contrast with **1**, which non-selectively activates all 6 NRs with $EC_{50} = 191 \pm 20$ nM for RAR α ; $EC_{50} = 50 \pm 17$ nM for RAR β ; $EC_{50} = 45 \pm 5.0$ nM for RAR γ ; $EC_{50} = 100 \pm 25$ nM for RXR α ; $EC_{50} = 200 \pm 30$ nM for RXR β ; and $EC_{50} = 140 \pm 13$ nM for RXR γ in the same CV-1 co-transfection assay.⁸⁸ Since its approval to treat all stages of the lymphoma in 1999, **2** is still used as a second line drug to treat CTCL. Molecule **2** exerts its therapeutic action in three CTCL cell lines by inhibiting malignant T-cell proliferation and preferentially inhibiting cell growth via induction of apoptosis instead of cell cycle arrest.⁸⁹

Molecule **2** was also reported to be effective in limiting the proliferation of leukemic (HL-60) cells.⁷³ It was observed that **2** (identified as LGD1069 on that paper) inhibited the proliferation of HL-60 cells by 37% at 1 μ M while **1** and ATRA inhibited the HL-60 cell proliferation by 97% at the same concentration. A combination of **2** and ATRA (both at 0.01 nM concentration) lead to an 8-fold induction of the integrin CD11b when compared with an ATRA (0.01 nM) control.⁷³ **2** also inhibits the proliferation and stimulates the differentiation of HL-60 leukemia cell lines as well as leukemic cells from patients with acute myeloid leukemia (AML), an outcome that cannot be attained with ATRA. Indeed, **2** has shown significant clinical activity and was well-tolerated in cases of non-M3 AML. Because **2** leads to minimal bone marrow suppressive side effects, it is a promising cytotoxic chemotherapy for those patients.^{89,90}

Molecule **2** also activated RXR/Nurr1 heterodimers and restored behavioral function in a rat model of PD.^{70,91} Nurr1 is essential for both development and maintenance of dopamine neurons and a reduction of its expression is associated with PD in humans. **2** was found to interact more potently with RXR/Nurr1 heterodimers ($pEC_{50} 8.3 \pm 0.1$) than RXR/RXR homodimers ($pEC_{50} 7.5 \pm 0.1$), exhibiting 7-fold selectivity in a bioluminescence resonance energy transfer (BRET) assay using HEK-293T cells. The bexarotene analogue LG100268 (**18**) also showed good results with $pEC_{50} 8.6 \pm 0.1$ for RXR/Nurr1 and $pEC_{50} 7.6 \pm 0.1$ for RXR/RXR.⁹¹ In addition, **2** at 1 mg/kg/day was effective in blocking the development of

behavioral deficits and dopamine neuron degeneration in a rat model of PD producing significantly reduced changes in both triglycerides and T4 serum.⁹¹

The PPAR γ plays an important role in adipocyte differentiation, insulin resistance, and atherosclerosis.⁹² Since PPARs forms dimers with RXR, rexinoids may be potential antidiabetic drugs. In fact, **2**⁵⁸ is also an insulin sensitizer, and has been shown to decrease hyperglycemia, hypertriglyceridemia and hyperinsulinemia, in mouse models of type-2 diabetes and obesity.⁵⁸ That report from the group of Heyman et al. indicated that after a 2-week treatment, **2** (30 mg/kg) diminished glucose levels by 55% and insulin by 70% when compared with the control without affecting the weight of the animals.

However, the toxicity of oral **2** has limited its clinical utility for chronic administration. Molecule **2** caused hyperlipidemia (both elevated serum triglycerides and serum total cholesterol), which is postulated to occur due to the activation of LXR/RXR heterodimers.^{86,93} Thus, CTCL patients receiving treatment centered in **2** also need lipid-lowering drugs to control the hyperlipidemia. If the lipid levels continue to be high, the administration of molecule **2** is discontinued until the levels return to normal ranges.⁸⁶ Although the activation of LXR is reported to improve the glucose metabolism in type 2 diabetes, the excessive activation of LXR induces the sterol regulatory element-binding transcription factor 1 (SREBP-1c) expression, resulting in elevated blood triglycerides. Researchers have hypothesized that selective agonists, without the ability to activate LXR/RXR, may still be promising candidates for the development of new drugs without the side effects listed above.
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Unfortunately, **2** also suppresses the thyroid hormone causing hypothyroidism, inducing hepatomegaly, and weight gain as adverse effects. The mechanism by which retinoids and rexinoids induce reversible central hypothyroidism is unknown. Studies have shown that **1** and **2** decreased serum thyroid-stimulating hormone (TSH), T4, and T3 levels in humans.⁹⁴⁻⁹⁶ For example, the TSH level of 6 patients decreased from 1.5 mU/L to less than 0.5 mU/L after 48 hr when treated with 400 mg/m² of molecule **2**. The cortisol and glucose levels were not affected after 8 hr (post-meal), while insulin levels increased 63% (from 11.6 mU/L to 18.6 mU/L) when compared with the control. It is not clear if the activation of RAR, RXR, or both, is responsible for the thyroid suppression. However, evidence indicates that RXR-mediated suppression of TSH gene expression could contribute to rexinoid-induced hypothyroidism.⁹⁶

It has been noted that the effects of molecule **2** are linked to a myriad of signaling activities related to cell arrest, differentiation and apoptosis.⁸⁹ Moreover **2** also downregulates tumor-promoting genes such as cyclin D1, EGFR, COX-2. For a summary of the bexarotene field including various ongoing clinical trials in the oncological area, we suggest a recent review from Shen *et al.*⁸⁹ That report shows the therapeutic effects of **2** are not limited to CTCL, but also extend to: non-small cell lung cancer (NSCLC), AML, breast cancer, thyroid cancer, AIDS-related Kaposi sarcoma, and Cushing's disease (CD). Compound **2** has also shown promising activities in other diseases including diabetes⁵⁸, and AD.⁹⁷ In fact, current clinical trials are exploring the ability of **2** to treat AD, schizophrenia, and MS.⁸⁹ A special issue review discusses the therapeutic applications of rexinoids to target the genetic risk for AD,

the apolipoprotein E4 (APOE4).⁹⁸ Currently, there is no cure for AD and treatments are palliative. Since persistence of liver hepatomegaly and steatosis adverse effects of rexinoid treatment, **2** and related molecules could negatively affect the central nervous system (CNS) function. Thus, it is imperative to develop rexinoid therapies that improve and protect CNS without affecting other organs such as the liver. The rexinoids induce apoE lipidation, and this would be particularly beneficial for APOE4 carriers, although few studies have been reported and further work should be done to understand the effects of APOE on rexinoid therapeutics.⁹⁸

3.4. Bexarotene-related compounds

The ABC transporter A1 (ABCA1) mediates the biogenesis of high-density lipoprotein (HDL) from helical apolipoprotein acceptors (apoA), cellular cholesterol and phospholipids. The ABCA1 expression is stimulated by LXR/RXR activation. The RXR agonists PA024 (**14**, Figure 7) and HX 630 (**15**), both analogues of **2**, were reported to activate LXR/RXR. Both agonists (**14** and **15**) enhanced ABCA1 mRNA expression and apoA-I-dependent cellular cholesterol release, in differentiated THP-1 cells. However, **15** did not increase ABCA1 mRNA in RAW264.7 cells and undifferentiated THP-1 cells. These results suggest the possibility of obtaining heterodimer-selective agonists.^{45,99} Aza-rexinoids, such as **16**, were described as RXR specific agonists (EC_{50} 's of 64 nM, 117 nM and 125 nM respectively for RXR α , β , and γ).¹⁰⁰ The nicotinic acid analogue **17** (EC_{50} = 5–9 nM for RXR α , β , γ and Kd = 22–61 nM in CV-1 cells) has been reported to be equipotent to **2**. Analogue **17** was able to induce ~ 60% apoptosis in CTCL Hut-78 cells for after 48 h treatment. For comparison, molecule **2** lead to ~ 50% cell death during the same time period.

The combination of the most structural favorable features; introduction of nicotinic acid isostere and incorporation of a cyclopropyl bridging group, resulted in the highly potent and selective cyclopropylnicotinic acid derivative **18**. This compound, also known as LGD100268, produced an increase in RXR transcriptional activation (EC_{50} = 3–4 nM for RXR α , β , γ) and displayed a high binding affinity (Kd = 3 nM) in CV-1 cells. As comparison, **2** possess an EC_{50} = 20–28 nM for RXR α , β , γ with a Kd = 21–36 nM for the same cell line.¹⁰¹ Compound **18** did not promote apoptosis of the human promyelocytic leukemia HL-60 cells as observed by a TUNEL assay. However, a combination of **18** and the RAR selective ligand TTNPB promoted cell death, suggesting that the presence of both RAR and RXR activating components is required for induction of apoptosis.¹⁰¹ Highly selective compounds like **18**, which exhibited > 1000-fold selectivity for the RXRs, provide tools for identifying new biological pathways which may provide therapeutic utility for control of cellular disorders. LG100268 rapidly decreased plasma TSH levels, and 24 h after a single oral administration (10 or 30 mg/kg) significantly suppressed total T3 levels in rats.^{95,96} It also reduces glucose and insulin levels by 52% and 41% respectively after 2 weeks (20 mg/kg). Further, while triglyceride levels were ~ 100 mg/dL after 2 weeks treatment at 20 mg/kg, the addition of thiazolidinones (1 mg/kg), reduced the levels by half. This results suggest that a combinatorial therapy may ameliorate the side effects caused by **18**.⁵⁸

Given its promising pre-clinical results in various tests and limited serious side effects reported, it is surprising **18** has not been further studied in the clinic. For example, the

combination of the **18** and Selective ER Modulators (SERM) such as Arzoxifene (Arz) enhances the production of transforming growth factor β (TGF- β) in 3T3 cells and inhibits the expression of inducible nitric oxides synthase (iNOS).¹⁰² *In vivo* studies from the Sporn group in an ER negative mouse model have shown that the combination of these two drugs was synergistic in breast cancer treatment.¹⁰³ While Arz (20 mg/kg) was able to prevent tumor growth in 50% of the mice after 60 weeks, **18** (30 mg/kg) was inactive during the same time frame. However, the combination of both compounds completely prevented the tumors from developing. Based on this study, it would be beneficial to survey this combination for the prevention and treatment of breast cancer.

The addition of two fluorine atoms to **2** gave the RXR agonist **19**. This molecule presented an EC₅₀ value of 34 nM in HCT-116 colon cancer cells, which is superior to the EC₅₀ value for **2**, 55 nM.¹⁰⁴ The acrylic acid analogue CD3254 (**20**) was also reported as a potent and selective RXR agonist (EC₅₀ = 13±3 nM in HCT-116 cells).^{105,106} Another set of similar molecules reported by the group of Jurutka, compounds **21–24**, appear to be more potent, demonstrate higher efficacy, and are likely to cause side effects than **2**.¹⁰⁵ The analogues **21** (EC₅₀ = 44±12 nM), **22** (EC₅₀ = 50±10 nM), **23** (EC₅₀ = 42±3 nM) and **24** (EC₅₀ = 15±2 nM) showed RXR activity compared with **2**, EC₅₀ = 55±6, in transfected HCT-116 cells, demonstrating that improvements in potency and selectivity of **2** can be achieved through drug design. Noteworthy are compounds **13** and **15**, with presented a 20% increase in RXR transcription when compared with the control, molecule **2** (all tested a 100 nM). An apoptotic assay of treated CTCL cells using sodium butyrate as a control (100% dead) indicated that, after treatment with 100 nM of the compounds, **2** and molecules **22–24** killed ~50% of the cells while **21** caused ~70% cell death. The results also indicated that modification of **2** with more polar atoms in the aromatic ring that bears the carboxylic acid may increase the ability to activate RAR (% RAR agonistic activity at 100 nM = 27±6, 28±5, 31±6 and 24±4 for **21–24** in transfected HCT-116 cells, respectively) and the ability to activate RXR (analogue **21**).¹⁰⁵

Aiming to evaluate the influence of the carboxylic acid moiety on RXR heterodimer activation, Fujii *et al.*¹⁰⁷ synthesized NEt-TMN (**25**, Figure 8) derivatives containing carboxylic acids isosteres **25a–c**. Neither of the new molecules displayed RAR α activities even at 10 μ M. The carboxylic acid analogue **25** (EC₅₀ = 5.28 nM) was more potent than **2** (EC₅₀ = 19.8 nM). While the phosphonic acid did not show RXR-agonist activity up to 10 μ M, the tetrazole (EC₅₀ = 205 nM) and hydroxamic acid (value not reported) showed RXR full-agonistic activity in COS-1 cells, but were less potent than carboxylic acid. The lower potency of hydroxamic acid (pK_a ~ 9.0) as compared with tetrazole (pK_a ~4.5) may reflect the importance of the acidic character of the compounds. Importantly, a docking simulation with RXR α (PDB code: 1MVC) showed that tetrazole and hydroxamic acid do not hydrogen bond to Ala327 of RXR α while the carboxylic acid group does. The authors also found that the modification in the acid domain of the RXR agonists did not have significant influence on permissive RXR/PPAR γ and RXR/LXR α activation (less than 60% of **2**).¹⁰⁷ Unfortunately, **25** was reported to cause several of the side effects observed with **2** such as increase in liver weight, cholesterol, and triglyceride levels.^{108–110} However, the obtained results may be useful for the development of RXR agonists with better bioavailability since

RXR agonists are lipophilic molecules, and their pharmacokinetics characteristics are not optimal.¹⁰⁷

Compounds **26–31** (Figure 8) were developed by Heck and co-workers and their potential for causing hypertriglyceridemia and inhibit cancer cell proliferation, and mutagenicity were evaluated.¹⁰⁹ Among the prepared molecules, the rexinoids **26** ($EC_{50} = 7.9 \pm 0.4$ nM), **27** ($EC_{50} = 13.8 \pm 1.5$ nM), **28** ($EC_{50} = 40.9 \pm 0.6$ nM), **29** ($EC_{50} = 18.2 \pm 0.4$ nM), and **31** ($EC_{50} = 33.8 \pm 0.1$ nM) were found to possess lower EC_{50} than compound **2** ($EC_{50} = 52 \pm 2.1$ nM) in a RXR mammalian two-hybrid assay using HCT-116 colon cancer cells. Compounds **25**, **27** and **28** were efficient at inhibiting growth (>85%) of the CTCL cell line Hut-78, a result comparable to **2** (>90%). More important, compounds **25**, **27–30** exhibited less side effects with and enhanced therapeutic potential than **2**. For example, the RXR-mediated transcriptional activation in HCT-116 cells at 100 nM was ~150% for **28–30** (the control, molecule **2**, was 100%). The molecules also presented, at least, a 2-fold preference for activating RXR/RXRE transcription versus sterol regulatory element-binding proteins (SREBPs).¹⁰⁹ More encouraging, all the ligands presented 20% the RAR activation of **1** (which was 100%) and **2** (~30%). None of the ligands more mutagenic in a *Saccharomyces cerevisiae* cell-based system up to 0.1 mg/mL

Many RXR agonists contain a lipophilic domain (1,1,4,4-tetramethyltetralin) and an acidic group (benzoic acid or nicotinic acid) connected by a linking domain.^{45,80,107} Aiming to develop potent, less-lipophilic and subtype-selective RXR agonists, The group of Kakuta^{45,72,111,112} prepared RXR agonists by modifying the lipophilic (**32**, **33**, **35–37**)¹¹¹ and the linking group domains (**34**, **38**).¹¹² The lipophilic group was modified with alkoxy and isopropyl groups, which impart polar character.¹¹¹ As potent RXR activity requires a lipophilic moiety on the linking amino group, the *N* atom was alkylated with an ethyl group while nicotinic acid was inserted into the acidic domain. One of the synthesized molecules, **32** (Figure 9) was identified as the first selective, dual potent RXR α / β agonist reported. Molecule **32** presented more than a 10-fold selectivity for the RXR isoforms α or β when compared with γ with EC_{50} values, in COS-1 cells, of 32 ± 0 nM (α), 36 ± 8 nM (β), and 376 ± 13 nM (γ).

The analogue NEt-3IB (**33**) showed similar RXR α agonist activity ($EC_{50} = 19 \pm 6$ nM)¹¹³ to **2** ($EC_{50} = 20 \pm 3$ nM) but the activity towards PPAR γ /RXR α was weaker, showing that changes in the lipophilic domain can influence the ability of RXR-heterodimer activation. The ratio of RXR EC_{50} values for RXR α : β : γ was 0.58:23:3 respectively, indicating that molecule **33** agonist prefers RXR α / γ over RXR β . This is an important result because it indicates that selectivity for the different subtypes can be modulated by altering the substituents on the hydrophobic segment. Compounds **32–33** did not show RAR activity. However, they showed synergistic retinoid activity when combined with the RAR agonist Am 80 (Tamibarotene),¹¹⁴ EC_{50} 16 ± 1 nM and 25 ± 1 nM in HL-60 cells, respectively. The cLogP value of **32** (5.61) is lower than the values of **33** (6.23) and **14** (7.23), indicating that lipophilic properties lead to subtype-selective RXR agonists (cLogP values were calculated using ChemDraw Ultra 7.0).

In order to design less lipophilic RXR agonists, a sulfonamide was introduced into the linker area (**34**, Figure 9). The EC₅₀ values in COS-1 cells between RXRα:RXRβ:RXRγ were 1.0:11.5:3.1, indicating that this agonist prefers RXRα/γ over RXRβ (EC₅₀ = 195±25 nM for RXRα, EC₅₀ = 2250±0 nM for RXRβ and EC₅₀ = 620±50 nM for RXRγ) and a lower cLogP (6.55) compared to PA024 (**14**). Conversely, compound **34** did not presented activity in HL-60 cells at 1μM when administered alone. However, in combination with Am80 (0.3 nM), the EC₅₀ value was 300±30 nM, indicating a synergistic effect when RXR and RA agonists are used together. This research group also made the cyclopropylmethoxy (**35**), benzyloxy (**36**) and *n*-propoxy (**37**) analogues.¹¹³ These molecules showed reduced RXRα agonistic activity with EC₅₀ in COS-1-cells of 290±40 nM (**35**), 160±60 nM (**36**), and 160±20 nM (**37**). The compounds displayed similar agonistic activity towards RXR and LXR/RXR but **35** showed more potent PPARγ/RXRα activity than **36** or **37**. The compounds **35–37** displayed different patterns of RXR-heterodimer activation depending on the nature of alkoxy group. Agonists that activate PPAR/RXR moderately are potential candidates for clinical applications because activation of PPARγ improves insulin resistance but excessive activation can cause edema and obesity.¹¹³

Inspired by the partial RXR agonist CBt-PMN¹¹⁰ (**38**, Figure 9), a compound reported to significantly lower glucose levels, Kawata *et al*¹⁰⁸ sought to develop partial RXR agonists. The group synthesized the RXR partial agonist NEt- 4IB (**39**) by exchanging the isobutyl and isopropyl groups at the lipophilic moiety of the RXR full agonist **33**. Compound **39** showed 50–60% activation towards RXRα,β and γ with dose-dependency and produced partial inhibition of the activity of **2**, confirming its properties as partial agonist. In addition, **39** induced permissive activation of LXRα/RXRα and PPARγ/RXRα heterodimers.¹⁰⁸ Molecule **39** (EC₅₀ = 169 nM) has antitype 2 diabetes activity with reduced side effects compared to full RXR agonists in animal models and gave a higher blood concentration than full agonist **33**, after oral administration.¹⁰⁸ Kobayashi and coworkers⁶⁸ investigated the pharmacokinetic difference of **39** and **33** (compounds are regioisomers) by positron emission tomography (PET). PET images revealed that **39** was easily absorbed from the digestive tract and excreted slowly than **33**. The partial RXR agonist **39** may be a promising candidate for the treatment of Parkinson's and Alzheimer's diseases as well without the serious side effects caused by **2**.

As discussed previously, the RXR/Nurr1 heterodimer is a promising target for the treatment of neurodegenerative diseases such as PD. Sundén *et al*⁷⁰, reported the design and synthesis of sterically constricted benzofurans as RXR agonists. Because of its good overlay with **2**, it was hypothesized the dihydrobenzofuran scaffold could be a promising starting point for the synthesis of RXR and RXR/Nurr1 ligands. The dihydrobenzofuran acid enantiomer (**40**, Figure 10) was found to be a full RXR agonist with pEC₅₀ (negative logarithm of EC₅₀, in mols) of 8.2 and an efficacy of 119% (when compared to **2**, which was equal to 100%), and a RXR/Nurr1 pEC₅₀ of 7.9 and efficacy of 111%.⁷⁰ The X-ray structure of **40** showed hydrophobic interactions with Val 342, Val 349, Ile 310, Ile 268, and Leu 436, and H-bond interactions of the carboxylic acid group with Arg 316 and Ala 327 (Figure 10). The superpositions of **40** and **2** (Figure 10C) showed the two molecules overlay nicely. The *ent*-**40** derivative displayed a steric clash (Figure 10B) with Ala 272 and failed to make H

bond contacts with Ala 327 and Arg 316, which may explain why the molecule is 100-fold less potent in the biological assays. The sterically constricted dihydrobenzofuran scaffold presented exactly the right molecular topology to activate RXR α , providing a new platform to further RXR/Nurr1 exploration.⁷⁰

3.5. Diarylamines

Amano and associates developed a series of diarylamines molecules, that contain hexahydrophenalene or octahydrobenzoheptalene, as potential RXR-specific agonists.⁶⁶ Initially, the authors reported several metacyclophane, phenalene, and benzoheptalene derivatives **41–43** (Figure 11) that selectively activated RAR α and RAR β , but did not activate RAR γ or the RXR isotypes.⁶⁷ The diarylamines, however, showed agonistic activity towards RXRs and the *N*-ethyl- and *N*-cyclopropylmethyl-pyrimidine-5-carboxylic acid compounds **44–47**. These compounds showed potent and selective activity towards RXR α with 35–47 fold inductions (vs DMSO control) at 100 nM in a transcriptional activation assay using COS-1- cells.⁶⁶ Compound **45** showed subtype selectivity for the $\alpha/\beta/\gamma$ isoforms presenting transcriptional activities of 25:7:15 at 100 nM. Molecule **47** also showed selectivity with values of 32:7:19 respectively at 100 nM (selectivity diminished at higher concentrations). The compounds did not activate RARs at concentrations above 1000 nM in COS-1 cells, nor did they induce the differentiation of HL-60 cells at 100 nM. Nevertheless, the combination of Am80 (1 nM) with compounds **45–47** promoted HL-60 cell differentiation of 77%, 85% and 88%, respectively. This value is similar to the one observed for compound Am80 + molecule **14** (86%) and Am80 + **15** (73%). It is noteworthy to mention that compounds **45–47** are more potent than compound **15**, which presented transcriptional activities of ~ 2 at the same concentration, and **1** (10:4:5 for the RXR subtypes). The study also reported that compounds **45–47** were more selective than **2** or **18** towards RXR. In summary, the inclusion of ethyl or butyl chains into the 2^{ty} amine of the core was found to enhance RXR activity. Conversely, hydrogen or methyl substituents were found to provide weak RXR activity but enhanced RAR activation.⁶⁶ This suggest that careful molecular design, based on the different structures of the RAR and RXR binding pockets, can lead to selectivity for either of the receptors and their isoforms.

3.6. Indenoisoquinolines

During their search for novel cancer chemopreventive scaffolds, the Cushman and Pezzuto groups screened a library of 5000 compounds to find potential RXR agonists. The indenoisoquinoline **48** (Figure 12), also known as AM-6–36, was found to activate RXR α with an induction ratio (IR) of ~5.5 at 40 μ M in COS-1 cells (IR= the fold increase in transcriptional activity when compared with a control). As a comparison, **1** produced a 14.6-fold increase at 100 nM and **2** yielded a 48.9 increase at the same concentration.¹¹⁵ Another experiment using MCF-7 cancer cells showed that **1** presented IRs of 3.7 (120 nM) and 6.1 (400 nM) while **48** presented IRs of 3.2 (625 nM) and 16.8 (1250 nM). Further studies showed that **48** stopped the proliferation of MCF-7 breast cancer cells with an IC₅₀ of 1.09 μ M after 24 h (**2** IC₅₀ = 82.3 μ M).^{116,117} In addition, molecule **48** inhibited HL-60 cell proliferation with an IC₅₀ of 92 nM after a 96 h incubation. Further experiments indicate

that these results are linked to the upregulation of the CDKN1A gene and the consequent expression of p21^{WAF1/CIP1}.¹¹⁵

Those results were surprising because compound **48** lacks a carboxylic acid functional group. Thus, Cushman and co-workers decided to include a carboxylic acid functional group within the indenoisoquinoline core with the goal of maximizing the interactions with Arg316 and Ala327. Over 100 indenoisoquinolines were evaluated, and it was found that the carboxylic acid was not essential for activity (Table 2). The biological data suggested that electron-withdrawing groups at the 3-position provided inactive molecules, but neutral or electron donating groups were tolerated. The data showed that a 1st, 2nd or 3rd aminopropyl side chain was key for biological activity, and more or less methylene units affect the agonist response. The replacement of the amino group for alcohols, imidazole, carbamates or conversion into a methyl ammonium group resulted in inactive compounds. The authors concluded that a basic, positively charged amine is necessary for activity. Even though the obtained results indicate these molecules are not as potent as the NEt-TMN compounds (see **25–39**), the novelty of the core, the ease of functionalization when compared to **25**, and the fact that some members of the family are currently undergoing clinical trials to treat cancer,¹¹⁸ make the indenoisoquinolines an attractive platform for further development of RXR agonists.

The obtained results made the Cushman group question the importance of Arg316 in RXR activation. At the time, other publications questioned the importance of the hydrogen bond interaction between a carboxylic acid and the mentioned amino acid. For example, the molecule bigelovin was able to activate RXR/PPAR heterodimers by binding to the RXR even though it does not possess a carboxylic acid substituent.¹¹⁹ Thus, a new docking study was performed assuming that RXR conformational rearrangement occurs once the ligand binding has taken place and not before.^{120–123} The authors found two hydrogen bonds between the indenoisoquinolines and the residues Gln275, Leu309, and Ala327. These interactions, as well as hydrophobic interactions within the RXR binding pocket, are believed to cause receptor activation after agonist binding. Thereafter, a conformational change takes place within the RXR molecule, and the ligand is transported to a more hydrophobic environment. This model is more consistent with the biological data and partially explains the favorable results seen with the indenoisoquinolines. A recent molecular dynamics simulation paper from the van der Vaart group suggests that Arg316 may not be key for RXR activation¹²⁴ supporting the observation made by Cushman.

3.7. Other RXR agonists

An interesting retinoid scaffold is the stilbene core. Gronemeyer, de Lera, and coworkers prepared the stilbene retinoids **49** and **50** (Figure 13), which displayed dual activity with strong activation of RXR β and strong antagonism of RARs as seen by luciferase experiments, which is an unusual property for these ligands. Molecule **49** achieved a ~17-fold increase in RXR β expression at 100 nM while **50** achieved ~20-fold increase in induction at the same concentration. The natural ligand **1** promoted less than 10-fold induction, when compared with control, at the same concentration. The data combined with molecular modeling studies indicate that the RXR ligand binding domain possesses some

degree of elasticity to support binding of bulky stilbenes.¹²⁵ As is the case with the retinoic acids, natural products are a key source of potential RXR agonists. Inoue *et al.* have reported two flavonones **51–52** isolated from *Sophora tonkinensis*, a traditional Chinese plant. Both isolated compounds activated RXR α with a ~15-fold induction at 10 μ M concentration, while the **2** control promoted a 30-fold induction at 0.1 μ M. These compounds were able to activate PPAR γ /RXR, PPAR δ /RXR, RXR/LXR, and RXR/RAR heterodimers alone with potencies similar to **2**. These compounds can also potentiate the activation achieved by agonists of the partner NRs⁹³ and induced the expression of mRNAs at 10 μ M. For example, when combined with the LXR agonist T0901317, **51** and **52** increased mRNA levels of ABCA1 100-fold, ApoE 7-fold, and LPL 5-fold (both compounds presented comparable values). Compounds **51** and **52** are some of the most potent naturally occurring rexinoids, yet with lower activity than **2**. These two molecules induced expression in genes associated with heart diseases, diabetes, and atherosclerosis.

In another investigation, 86 crude herbal drugs used in the traditional Japanese Medicine were screened for RXR α agonist activity. Honokiol (**53**), the methanol extract of *Magnolia obovate*, was reported to activate RXR α (EC₅₀ of 11.8 μ M)¹²⁶ but not RAR, PPAR or LXR.¹²⁶ The RXR α binding activities were evaluated using a yeast two-hybrid assay, and **53** showed much higher binding affinity to RXR α than **1**.¹²⁶ The same year, Jung and coworkers found that **53** induced RXR-mediated ABCA1 expression.¹²⁷ ABCA1, a member of ATP-binding cassette transporter family, promotes the efflux of cholesterol and phospholipids from intracellular to extracellular acceptors. ABCA1 is upregulated by the activation of LXR and PPARs, both of which form heterodimers with RXR. Molecule **53** showed RXR β affinities of 1.5- and 2-fold at 10 and 30 μ M concentration, respectively in the binding assay¹²⁷, and **53** also increased ABCA1 mRNA and protein expressions levels in human glioma cell line U251-MG cells and THP-1-cells.¹²⁷ Compound **53** was found to also upregulate ABCA1 and ABCG1 mRNA levels in macrophage like RAW264.7 cell line up to 1.8-fold induction compared with 1.3-fold induction of **2** (both results are relative to vehicle-treated cells).¹²⁶ Another study showed that **53** was able to activate PPAR γ /RXR, RXR/LXR and RXR/VDR heterodimers with slight efficacy on its own.¹²⁸ Yet, this efficacy was synergistically potentiated by activation of the NR partner. Altogether, the results showed that the activation of RXR/LXR heterodimers and the upregulation of ABCA1 genes in the absence of the partner agonist are dependent on the cell type involved.¹²⁸

A computational screening of the Dictionary of Natural Products (DNP) done by Merk *et al* identified three RXR-targeting natural products; valerenic acid (**54**, Figure 14), dehydroabietic acid (**55**), and isopimaric acid (**56**). Preliminary *in vitro* characterization indicated the RXR α agonistic activity of **54–56** at a concentration of 30 μ M. The compounds were also able to induce ABCA1 and ApoE expression.¹²⁹ Then, the software Design of Genuine Structures (DOGS) was used to generate structures based in **53**, and compounds **54–56**. The mimetic **57** (EC₅₀= 23.6 $\pm$ 0.8 μ M for RXR α , 13.9 $\pm$ 0.1 μ M for RXR β and 27.2 $\pm$ 0.3 μ M for RXR γ in a Gal4-Hybrid Reporter Gene Assay using HEK-293T cells) showed a similar potency to **53**. Molecule **58** was the most potent analogue activating RXRs with low micromolar potency and upregulating ABCA1 and Apo E

expression. This study indicates that natural products are excellent templates for designing synthetic drugs against the RXRs.¹²⁹

4. Conclusion

Although only two RXR agonists have been approved by the FDA; *alitretinoin*- to topical treatment of Kaposi's sarcoma and chronic hand eczema, and *bexarotene*- for the treatment of CTCL; there are many ongoing clinical trials suggesting there is a promising future for RXR agonists. At this stage, targeting and modifying the molecular cores discussed in this review are key toward optimizing the structures of RXR agonists (Figure 15). Even though isosteres have been incorporated into some of those cores, isosteric replacements can be further investigated in those molecules (Figure 15). Perhaps, the first potential direction of the field includes the exploration and modification of the scaffolds that have not being widely investigated: **40, 48–52, 57–58**. Because their structures are different to traditional retinoids, a systematic exploration of substituent effects will be needed (in addition to isosteric replacements). Arguably, computational studies have been under-utilized. Most reports perform molecular docking to position ligands and comprehend binding affinity. However molecular dynamics simulations that aim to understand the role of these agonists in inducing conformational changes are scarce. Perhaps, the biggest challenge is to design agonists that can be selective for only one RXR sub-class, using the information obtained by testing **32–34**¹¹¹ **46–47**⁶⁶ and applying it to other templates will be a logical approach. This will help to further understand the role of each member of the RXR family and target better biological control of activity.

An interesting opportunity is in the realm of nanotechnology; because of their nature, the retinoids can be easily encapsulated inside the hydrophobic core of a nanoparticle. This approach will lead to nanosystems that can overcome the biodistribution and solubility problem faced by some of these molecules. This will lead to opportunities for synergistic treatment by virtue of multiple drug encapsulation and targeted drug delivery. Finally, not much has been done regarding the incorporation of a targeting element to these molecules. A logical next step is the inclusion of targeting elements such as peptides, aptamers, and small molecules, to the core of selected agonists to improve their localization within a disease such as cancer. The future is promising for these compounds. Much has been done, but even more is yet to be accomplished.

ACKNOWLEDGMENTS

This project was funded by The University of Nebraska Medical Center (Start-up funds, MC-S) and by the National Institute of Health-NIGMS, Nebraska Center for Molecular Target Discovery and Development (1P20GM121316-01A1, PI: Robert Lewis, Project Leader, MC-S).

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8.

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ABBREVIATIONS

9.

9cRA	9- <i>cis</i> -retinoic acid
ABCA1	ABC transporter A1
AD	Alzheimer's disease
Ala	Alanine
AML	Acute myeloid leukemia
ApoA	Apolipoprotein acceptors
Arg	Arginine
ATRA	All <i>trans</i> retinoic acid
DBD	DNA binding domain
DC	Dendritic cells
DR	Direct repeats
EC₅₀	Half maximal effective concentration
HDL	High-density lipoprotein
IC₅₀	half maximal inhibitory concentration
IR	Induction ratio
LBD	Ligand binding domain
MS	Multiple sclerosis
PD	Parkinson's disease
PPAR	Peroxisome Proliferator-Activated Receptor
RA	Retinoic acid

RAR	Retinoic Acid Receptor
RXR	Retinoid X

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Figure 1.

Transcriptional activation by nonpermissive and permissive heterodimers. (A) Transcriptional activation by the nonpermissive heterodimeric partner VDR. Agonist binding to VDR leads to the recruitment of a coactivator protein (CoA). The RXR/VDR/CoA complex interacts with a histone acetylase, and this leads to gene transcription from the nonpermissive-ligand responsive gene transcriptional start site. Agonist binding to RXR agonist would not augment the response caused by the VDR ligand. (B) Transcriptional activation by the permissive RXR heterodimeric partner and PPAR. An agonist can bind to either RXR or PPAR to initiate the recruitment of a CoA; then, transcription is initiated. Ligand binding to the second NR would increase the transcriptional response induced by first NR–agonist complex. Figure and legend adapted from reference.14

Figure 2.

(A) cartoon representation of RXR binding to DNA (PDB 3dzy). DNA in yellow, DBD in magenta, Tbox in pink, hinge region in purple, LBD in green, zinc atoms in orange. (B) Conformational changes induced in hRXRα upon agonist binding. Apo hRXRα monomer is in blue while the holo structure is in green. The arrows indicate the structural changes of the various segments. The ligand is depicted in yellow. Used, and modified, with permission from Ref. 44 (C) Structure of the RXR-DBD showing the two fingers (I and II), which are stabilized by complexation of a zinc (II) ion with four of its cysteine sulfhydryl groups. The Recognition helix and Helix II (alpha-helical) are illustrated in blue and purple. The T-box (pink) is required for cooperative dimerization on DNA. The protein-DNA interactions are shown along the half-sites at the DR1 sequence. Arrows indicate the direction of the hydrogen bonds. Adapted from Ref.14

Figure 3.

(A) Structure of 9cRA. (B) Crystal structure of 9cRA inside the binding pocket of RXR (pdb 1FBY). Proposed hydrogen bonds with key residues are shown in red. Used, and modified, with permission from Ref.48

Figure 4. Molecule 2 binds RXRs to activate multiple pathways through heterodimerization with other nuclear receptors to regulate the expression of important genes in apoptosis, cell growth inhibition and differentiation. Reproduced from Ref.90

Figure 5.

9cRA related compounds: UAB 8 (3) and analogues 4–7, 9cUAB30 (8), 4-methyl-UAB30 (9), and 9cUAB30 homologues 10–14.

Figure 6.

Interactions between the rexinoid rings with helix 7 residues, F346 and V347, with van der Waals surfaces of rexinoid and protein residues shown as dots: (A) bexarotene, and (B) 9cUAB30. Used, and modified, with permission from Ref.86

Figure 7.
Structures of PA024 (14), HX 630 (15), 16 and bexarotene-related compounds 17–24.

Figure 8.
Structures of diarylamine compounds 25–31.

General structure of well-known RXR agonists

Figure 9.

Strategy used for the design of subtype-selective RXR agonists and structures of compounds 32–39.

Figure 10.

“(A) Crystal structure of RXR (green) in complex with 40. (B) Interactions of 40 in the ligand binding pocket of RXR. (C) Structural superposition of RXR in complex with bexarotene (2) (purple) and (40) (orange) in the LBD of RXR. (D) Overlay of 40 (orange) and its enantiomer ent-16 (red) in the LBD of RXR. The arrow indicates how the enantiomer would sterically clash with RXR residue A272 (PDB 5EC9)”. Used, and modified, with permission from Ref.70

Figure 11.
Structure of RAR agonists 41–43 and RXR agonists 44–47.

48, AM6-36; R = NH₂, R₁ = CH₂NH₂

Figure 12.
Structure of indenoisoquinolines with RXR agonist activity.

Figure 13.

Stilbene rexinoids 49 and 50. Natural RXR ligands: flavonones 51 and 52, isolated from *Sophora tonkinensis*, and Honokiol (53), isolated from *Magnolia obovate*.

Figure 14.
RXR-modulating natural products 54–56, and their mimetics 57–58.

Figure 15.

9cRA related compounds: UAB 8 (3) and analogues 4–7, 9cUAB30 (8), 4-methyl-UAB30 (9), and 9cUAB30 homologues 10–14.

Table 1:

Summary of Biological data for 9cRA and related compounds.

Retinoid	RXR α activation, EC ₅₀ (nM)	Increase in serum triglyceride, 200 mg/kg diet (%)	Reduction in mammary cancers. Number (%)
1	120 $\pm$ 30 ^a	326 ^{c,*}	65 ^{c,*}
2	40 $\pm$ 3 ^a	456 ^a	70 ^a
4	22 $\pm$ 5 ^b	430 ^b	99 ^b
5	19 $\pm$ 5 ^b	71 ^b	62 ^b
6	23 $\pm$ 5 ^b	341	69 ^b
7	80 $\pm$ 15 ^b	76 ^b	1 ^b
8	820 $\pm$ 70 ^a	63 ^a	63 ^a
9	120 $\pm$ 5	560	78
10	720 $\pm$ 20	31	0
11	100 $\pm$ 30	51	10
12	160 $\pm$ 10	642	61 (100 mg)

^aData reported in Ref^{70,81,86} at 200 mg/kg diet for (R)-4-methyl-UAB30.^{*} 100 mg/kg diet for compound **12**.

Table 2.

RXR induction ratio caused by indenoisoquinolines

R₁	R₂	IR
CO ₂ CH ₃	CH ₂ N(CH ₃) ₂	25
CO ₂ H	CH ₂ NH ₂	<1
CO ₂ CH ₃	CH ₂ NH ₂	<1
CH=CHCO ₂ CH ₃	CH ₂ NH ₂	<1
Br	CH ₂ Imid	<1
Br	CH ₂ N(CH ₃) ₂	12
Br	CH ₂ N ⁺ (CH ₃) ₃	<1
Br	CH ₂ NHCH ₃	33
H	CH ₂ Imid	<1
H	CH ₂ N(CH ₃) ₂	44
H	CH ₂ N ⁺ (CH ₃) ₃	<1
H	CH ₂ NHCH ₃	106
H	CO ₂ H	<1
H	CONH ₂	<1
H	CH ₂ OCH ₃	<1
H	CH ₂ CH ₃	<1
NO ₂	CH ₂ N(CH ₃) ₂	<1
NO ₂	CH ₂ OCH ₃	<1
NH ₂	CH ₂ N(CH ₃) ₂	34
NO ₂	CH ₂ NHCH ₃	<1
NH ₂	CH ₂ NHCH ₃	59
CN	CH ₂ Imid	<1
CN	CH ₂ N(CH ₃) ₂	7
NH ₂	CH ₂ OH	<1
NHAc	CH ₂ NH ₂	19
NHAc	CH ₂ OH	<1
NO ₂	CH ₂ Cl	<1
NO ₂	CH ₂ N ₃	<1
NO ₂	CH ₂ NH ₂	<1
NO ₂	N ₃	<1
NH ₂	CH ₂ NHBoc	<1
NH ₂	CH ₂ OH	<1
NO ₂	CH ₂ NHAc	<1
NH ₂	CH ₂ NHAc	<1
NO ₂	CH ₂ OH	<1
NHCH ₃	CH ₂ NH ₂	23

IR: induction Ratio; Testing Concentration: 50 mM; Imid: N-imidazolyl

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