Retinoids as Therapeutic Agents: Today and Tomorrow

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Abstract: Retinoids have shown beneficial therapeutic effects in pre-clinical and animal models for multiple pathologic indications, however severe adverse effects, restricting dosage and efficacy of oral formulations limit their use in patients. The focus of this review includes the actual medicinal use of retinoids and chemical efforts to generate highly selective and less toxic synthetic retinoids.

Keywords: RAR, RXR, transcription factors, cancer, skin, type II diabetes, retinoids, rexinoids.

INTRODUCTION

Retinoids and retinoid signaling have been extensively studied for more than two decades**,** revealing promising therapeutic potentials for a number of indications including cancer, dermatology, HIV infection, and type II diabetes. Over the last two years, several focused reviews have comprehensively covered the therapeutic benefits and limitations of the natural and synthetic retinoids, and at this date no major breakthrough has impacted the content of these reports (see below for references). The objective of this short review is to provide the reader with an overview of the functions, mechanisms of action and medicinal potential of the retinoids, as well as the recent evolution of retinoid chemistry and tomorrow's potential new drugs.

RETINOID FUNCTIONS AND CELL SIGNALING

Retinoids are a class of natural ligands which are synthesized *in vivo* from the irreversible oxidation of vitamin A naturally derived from the diet, and which include mainly all-trans-, 9-cis-, and 13-cis- retinoic acid (tRA, 9cRA and 13cRA, respectively). The physiological functions of retinoids are pleiotropic at all stages of life: embryogenesis, fetal development, post-natal and adult life. The diversity of retinoid functions was first inferred from studies on the pathologies associated with vitamin A deficiency (VAD) and from the developmental defects occurring after administration of supra-physiological doses of tRA [1]. Thereafter, multiple groups have carried out in depth genetic, cellular, molecular and macromolecular studies to develop a comprehensive understanding of retinoid cell signaling and biological functions.

In the 90's, the cloning of two types of nuclear receptors, the retinoic acid receptors (RAR) and the retinoid X receptors (RXR) indicated that the retinoid signaling involved the modulation of genetic networks. Several levels of complexity accumulated with the discovery, for both RAR and RXR, of multiple isotypes (α, β and γ) and several isoforms generated by alternative splicing or promoter usage, raising the question of why such a diversity of receptors is necessary for mammalian retinoid signaling and functions [2]. Retinoic acid receptors have similar high affinity for tRA and 9cRA and a very weak response to 13cRA; RXRs bind to and are activated only by 9cRA. Retinoic acid receptors and RXRs bind to DNA predominantly as RAR-RXR heterodimers, which recognize specific short DNA response elements organized in direct repeats of the consensus sequence AG(G/T)TCA [3]. The mechanisms underlying the regulation of gene expression by retinoid are different depending on the ligand status. In the absence of retinoic acids (RA), heterodimers of RAR-RXR bound to the promoter region of specific target genes form multiprotein complexes with transcriptional co-repressors, including nuclear receptor co-repressor (NcoR), silencing mediator for retinoid and thyroid receptors (SMRT), and histone deacetylases (HDAC), in order to silence gene expression by inducing a condensation of the chromatin through deacetylation of histone "tails". In the presence of RA, the receptor conformation changes that occur after ligand binding disrupt the co-repressor complex and permit the sequential recruitment of co-activator complexes. Histone acetyl-transferase (HAT)-containing complexes relieve chromatin silencing in the target gene promoter region, while large multiprotein units variously called thyroid hormone receptor associated proteins (TRAP), vitamin D3 receptor interacting proteins (DRIP), or activator recruited cofactors (ARC) bind to the heterodimer, thereby allowing the communication of RAR/RXR heterodimers with the basal transcriptional machinery to initiate target gene expression [4,5, see also ref. in 6]. Additionally, RXRs are promiscuous dimerization partners for multiple nuclear receptors, suggesting that selective ligands for RXR (rexinoids) can impact various transduction pathways independently of RAR. Retinoid X receptors can also form transcriptionally active homodimers in transfected cells, however, no physiological function of RXR homodimers has been reported.

Genetic studies targeting RARs and RXRs in mice demonstrated that integrity of RA signaling was crucial for embryogenesis, normal development and viability of the

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pups. Remarkably, the abnormalities observed in the fetal VAD syndrome were reproduced in animals depleted for RAR genes or combinations of RAR-RXR genes, substantiating that RA is the biologically active derivative of vitamin A and the effects of vitamin A are mediated by RAR-RXR heterodimers [7,8]. In the post-natal life, vitamin A is indispensable for survival, growth, reproduction, vision and the maintenance of various tissues. Hallmarks of the postnatal VAD syndrome include widespread squamous metaplasia of various epithelia, which is consistent with *in vitro* and *in vivo* studies showing the critical role of RA in cell homeostasis, proliferation, differentiation and apoptosis. The pleiotropic functions of RA in mammals therefore positioned natural and synthetic ligands as promising therapeutic candidates for various indications, although severe toxic side effects (see below) generate strict limitations in the dosage of these drugs and therefore in their efficacy.

RETINOIDS IN THE CLINIC

Cancer

The potentials for chemopreventive and chemotherapeutic applications of natural and chemically synthesized retinoids were recently comprehensively reviewed [4,6]. As of today, while a large number of molecules of various selectivities are still in the phases of discovery and clinical trials for the treatment or the prevention of hematopoeitic, solid and metastatic tumors in humans, three drugs are actually used clinically, including two natural retinoids and one synthetic rexinoid: tRA (tretinoin) is currently orally administrated to patients afflicted with acute promyelocytic leukemia (APL) in multiple countries including the US, Australia, Japan, UK, France and Germany; 9cRA (alitretinoin) has been launched in the US for the topical treatment of Kaposi's Sarcoma (KS), the most frequent cutaneous malignancy occurring in AIDS patients; and bexarotene (targretin) is prescribed in the US and UK for conditions of cutaneous T-cell lymphoma as an oral formulation. Bexarotene is currently under investigation in phase III clinical trials for non-small-cell-lung cancer (NSCLC) as combination therapy with cytoxic agents including paclitaxel (taxol), carboplatin, vinorelbine and cisplatin. Phase II clinical trials were also initiated for breast cancer indications as a single agent. The results of those studies are not yet available but it is likely that, similarly to NSCLC indications, trials using combination therapy may be initiated in the near future. Indeed, recent data suggest that the combination of bexarotene with paclitaxel prevented the development of paclitaxel-resistance in breast tumor cells both *in vitro* and *in vivo* models, which substantiates recent works showing that vitamin D3, tRA and a selective RARα/β agonists acted as sensitizers of paclitaxel in several tumor cell types [9-11]. Alitretinoin has been investigated in phase III trials for KS and APL indications. The results obtained with 15 patients afflicted with APL showed 40% complete remission for a duration of 2 to 18 months. The so-called atypical retinoid, fenretinide [4,6], was evaluated in phase III trials in combination with tamoxifen for post-menopausal breast cancer patients. However the poor accrual and high toxicity led to stopping the study. Fenretinide is currently under investigation for pediatric solid tumor indications, and in combination therapy with paclitaxel and cisplatin.

Skin Disorders

C. Zouboulis, in his remarkable review published in Skin Pharmacol. Appl. Skin Physiol. [12], described the potentials and clinic uses of the retinoids in dermatological indications. Tazarotene and Adapalene, two synthetic RAR selective retinoids have been launched in a wide variety of countries for severe acne conditions. The natural retinoids, tretinoin (tRA, microsponge formulation for topical applications) and isotretinoin (13cRA) are also prescribed in the US and Western Europe for acne conditions. Additionally, Tazarotene is clinically used in patients afflicted with mild to moderate psoriatic plaques. Interestingly, the tazarotenic acid metabolite of tazarotene is the species which binds with high affinity to the RARs and is responsible for the therapeutic effect**.** Acitretin is widely used in Europe, Australia, New Zealand and the US for the treatment of severe recalcitrant psoriasis. Acitretin is the active metabolite of Roche's psoriasis drug etretinate. Acitretin has a faster metabolic clearance than etretinate and therefore, it is thought to induce less of a long-term risk of causing birth defects.

Type II Diabetes

Because RXR is a promiscuous dimerization partner for a large number of nuclear receptors including PPARγ (peroxisome proliferating activating receptor γ), retinoid X receptor selective ligands, or rexinoids, have interesting potential as anti-diabetic agents for insulin-independent diabetes. Accordingly, bexarotene was shown to act as an insulin sensitizer. A comprehensive review on this topic was recently published [13], including a large section devoted to the chemistry leading to antidiabetic rexinoids. A phase II clinical was initiated by Ligand Pharmaceuticals in 1997 for the treatment of Type II diabetes using bexarotene. The 12-week trial was designed to assess the metabolic efficacy, safety and tolerability of four dose levels of bexarotene capsules in 48 patients with non-insulin-dependent diabetes. By September 1998, bexarotene was in phase III trials, however, it appears that the company had discontinued the development of bexarotene for this indication by December 2000.

Viral Infection

Several emerging reports support the anti-viral activity of retinoids in HIV infection. Viglianti's group has been focusing for several years on the mechanisms of action by which vitamin A or tRA inhibit HIV replication in monocyte-derived macrophages and the synergistic effects observed by the combination of tRA and interleukin-1β and interleukin-6 [14,15]. As far as has been reported, no pharmaceutical company has yet explored the potential of RA in this therapeutic area.

RETINOID CYTOTOXICITY, A STRONG BREAK FOR THEIR CLINICAL DEVELOPMENT

The beneficial therapeutic potential of retinoids is counterbalanced by their toxic side effects. Indeed, because vitamin A derivatives have pleiotropic physiologic functions in embryos and adults through the activation of widely

expressed RARs and RXRs, the administration of natural or synthetic retinoids is associated with severe toxic side effects with characteristics typical of hypervitaminosis A. These effects include varying degrees of teratogenicity depending on placenta permeability to the retinoid and embryo retinoid exposure [16]; mucocutaneous cytotoxicity including cheilitis, xerosis, desquamation and dryness of mucus membranes; ocular effects; hair loss; chondrogenesis inhibition and bone toxicity; and hyperlipidemia. The mucocutaneous cytotoxicity appears related to high levels of RARγ in skin. Similarly, the bone toxicity involves, at least in part, RARγ activity [17]. Hyperlipidemia is mostly induced by RARs, however rexinoids such as bexarotene were also associated with moderate lipid increments [18,19]. Note in this regard that bexarotene has weak RAR agonist activity which may play a role in the lipid-related toxicity. These major side effects have been the primary obstacle for the further development of these molecules as therapeutic agents, restricting their use (and their dosage) to the indications described above. Efforts at the chemistry level to increase selectivity and therefore decrease the extent of adverse effects, appear promising for future development of retinoids and rexinoids.

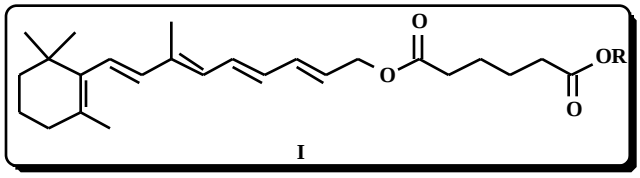
RETINOID CHEMISTRY TODAY

The pace of new retinoid synthesis has slowed since the mid-to-late 1990's. Excellent tools are now available for the elucidation of retinoid signaling pathways. A number of recent reviews have appeared [6, 20, 21, 22]. Ongoing synthesis is primarily aimed at RXR-selective compounds, with cancer as the primary (although not the exclusive) target. The following discussion covers new RAR structures followed by new RXR structures.

NEW RAR LIGANDS

Pan-Agonists

Workers from the Université de La Rochelle, France, have synthesized potential prodrugs of retinol, **I**, by lipase-catalyzed transesterification of retinyl palmitate [23]:



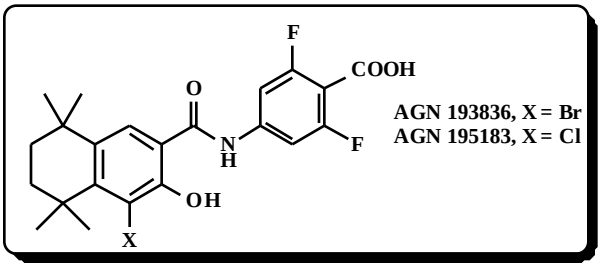
I

This was an attempt to impart aqueous solubility to the very non polar retinol, which would presumably be released by enzymatic hydrolysis. No biological data or release data were given. The most soluble diester contained R = saccharose (a disaccharide).

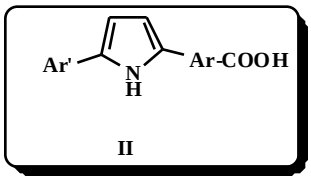
RARα-Specific Agonists

Chandraratna's group from Allergan reported that **AGN 195813** had improved RARα binding selectivity compared to the previously-reported **AGN 193836** [24]:

Both retinoids are highly selective for RARα; **AGN 193836** has weak but detectable binding to RARβ and γ, while **AGN 195813** has no detectable binding to the β or γ isotypes. Notably, **AGN 195813** was highly active at inhibiting proliferation of two human breast cancer cell lines known to express RARα, T47D and SK-BR-3 cells, and had no topical irritation activity, as expected from its complete lack of RARγ activity. In addition to breast cancer, this class of specific RARα agonist represents an attractive candidate to substitute for tRA in the treatment of acute promyelocytic leukemia or acute myeloid leukemia in the context of combination therapy with HDAC inhibitors [6].

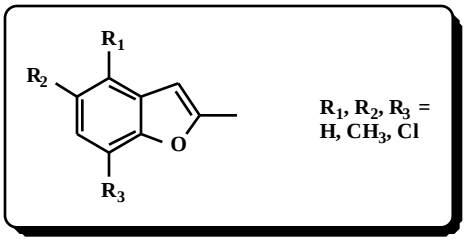


Workers at Eisai reported the solid-phase synthesis of a library of 57 2,5-disubstituted pyrroles, **II,** intended as selective RARα agonists [25]:



II

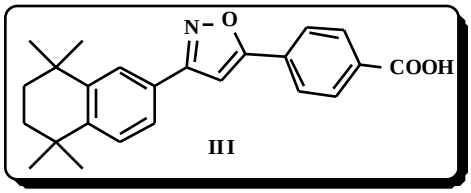
Ar = unsubstituted phenyl and Ar' = substituted benzofuran:



A few compounds had activity approaching that of retinoic acid in the inhibition of LPS-induced mouse B-lymphocyte proliferation, but most were inactive.

RARβ-Specific Agonists

Collaboration between the Universtià di Ferrara, Italy, and the University of Oklahoma retinoid groups developed a novel structure, **III,** in which the linker between the tetrahydro-tetramethylnaphthyl ("TTN") and benzoic acid fragments is an isoxazole [26]:



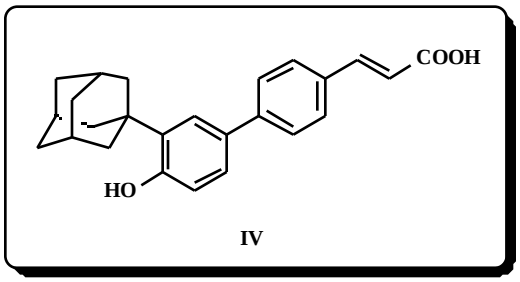
III

This compound was highly active in inducing apoptosis in HL-60 cells, in contrast to several more flexible analogs. It was even active against cell lines resistant to ATRA. It

showed strong activity in an RARβ test system, but the RARα and γ activities were not assayed. If the selectivity for RARβ was confirmed by further pharmacology studies, this class of compound in combination therapy with HDAC inhibitors (RARβ expression is silenced through hypermethylation and deacetylation of its promoter region during epithelial tumor development) may have promising applications in a wide variety of epithelial tumors.

RARγ-Specific Agonists

Researchers at the Università di Milano, Italy [27] reported the synthesis of **IV**, reminiscent of the "CD" (Galderma) series:



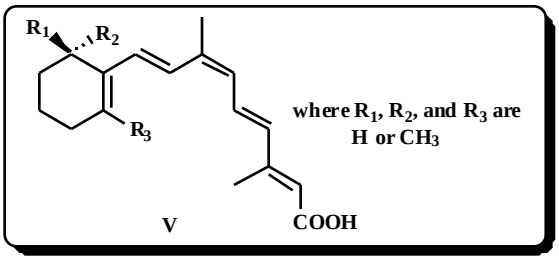
IV

IV is a weak but selective RARγ agonist with potent pro-apoptotic and antitumor activity *in vivo.* It is probable that, like other "atypical retinoids" [4,6], this activity may be partially RAR independent.

NEW RXR LIGANDS

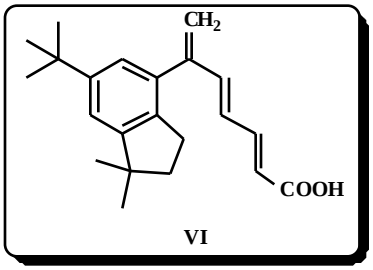
Agonists

Much of the recent RXR work follows the 9-cis-retinoic acid structure. A group at the Universidade de Vigo, Spain, reported the synthesis of ring-demethylated analogs, **V** [28]:



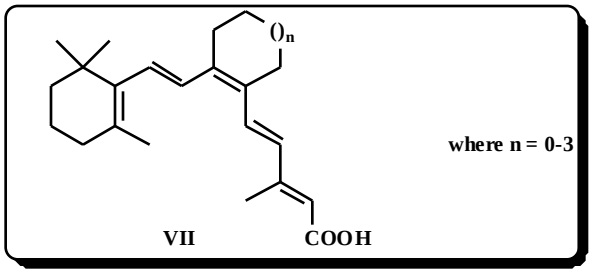
V

The series was intended as an investigation of the binding characteristics of systematically altered 9-*cis*-retinoic acid analogs. No biological activity was reported. They also reported, in collaboration with the University of Santiago de Compostela [29], a new RXR ligand, **VI**, with an unusual hydrophobic region showing a binding affinity for RXRα of 220 nM (compared to 50 nM for 9-*cis*-RA):



VI

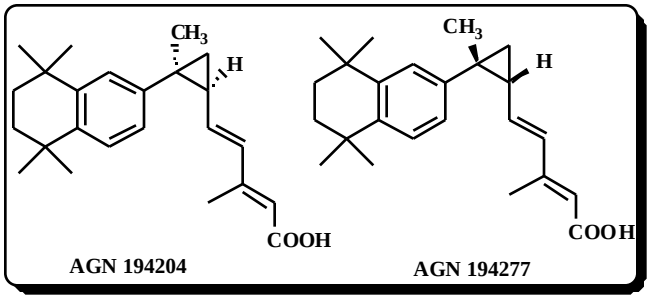
The same collaboration reported the synthesis and binding affinity of 9-*cis*-locked retinoic acid derivatives of general structure **VII** [30]:



VII

The binding affinities ranged from 89-1188 nM. The preferred ring size was 6>8>5>>7. The relative binding affinity was rationalized from the model by calculations of the van der Waals contacts and entropies of binding within the tightly constrained ligand binding pocket.

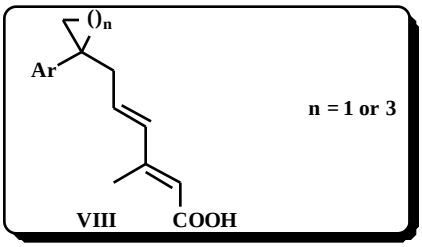
Chandraratna's group in Allergan also reported the activities of **AGN 194202** and **AGN 194277**, optical antipodes of an RXR agonist [31]:



AGN 194204 **AGN 194277**

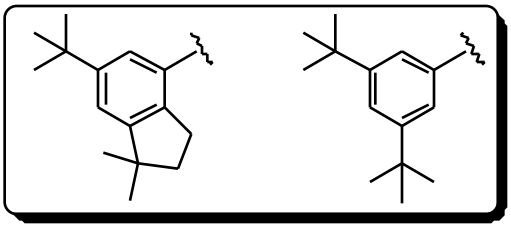
AGN 194204 is a high affinity, selective agonist for the RXR's, while **AGN 194277** shows much weaker binding affinity to RXR's and weak binding to the RAR's.

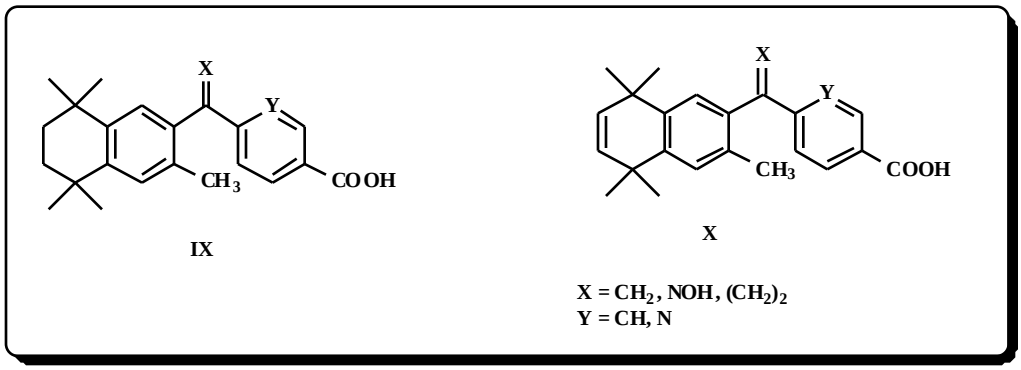
The Ligand Pharmaceuticals group synthesized a series of compounds, **VIII,** with a geminal cycloalkyl structure as a replacement for the *cis* double bond in 9-*cis*-RA [32]:



VIII

When n = 1 (cyclopropyl) and the aryl group was TTN, the compounds were pan-agonists for both the RAR's and the RXR's. In contrast, selective RXR binding and transactivation activities were observed when the TTN ring contained a 3-CH_3 group, or the aryl group was:



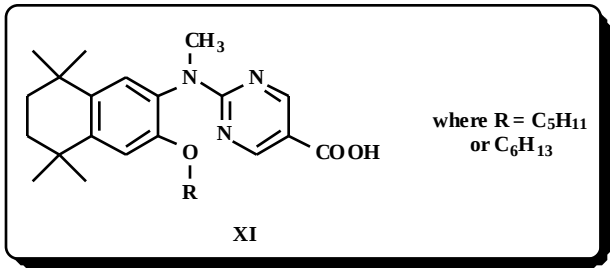


A group from Lilly reported the synthesis of compounds of general structures **IX** and **X** where the C=C in the non polar region was incorporated to influence the metabolism [33].

This class of compounds was shown to act as full agonists of the RXR-PPARγ heterodimers in transactivation assays, and demonstrated antidiabetic activity similar to that observed with PPARγ agonists (such as rosiglitazone) in db/db and ob/ob mice models [13]. Interestingly, the beneficial effects of those rexinoids on the diabetic condition of the mice were not associated with a gain in body weight as was observed with rosiglitazone.

RXR Antagonists

Compounds of general structure **XI** were shown by Kagechika's group at the University of Tokyo to be pure RXR antagonists [34]:



In transactivation assays, the compounds **PA451** (**XI**, R = C_5H_{11}) and **PA452** (**XI**, R = C_6H_{13}) dose-dependently inhibited agonist-induced RXR homodimer activity, while they did not affect **AM80**- (RARα agonist) or tRA- induced RAR-RXR heterodimer transcriptional activity. Similarly, PA451 or PA452 did not significantly affect HL60 differentiation induced by RAR agonists. The two antagonists reversed the synergistic differentiating effect obtained by the combination of RAR with RXR agonists. None of these RXR antagonists induced HL60 differentiation on their own. The therapeutic potential of RXR antagonists has not yet been evaluated.

CONCLUSIONS

The considerable efforts made to synthesize highly selective retinoids and rexinoids to diminish their toxicity is anticipated to open wider avenues for the clinical use of these molecules. Additionally, increased understanding of the molecular basis and epigenetic changes that occur during the development of pathologies such as carcinogenesis suggest that combination therapy involving retinoids/rexinoids with agents of differing modes of action, may be of synergistic therapeutic benefit. Candidates for combination treatments may include cytotoxic agents such as paclitaxel or other emerging therapies such as inhibitors of HDACs and/or DNA-, Arginine-, and Lysine- methyl-transferase, receptor tyrosine kinases, or pro-apoptotic biological agents such as TRAIL, tumor necrosis factor (TNF) or FASL [reviewed in 4,6]. Together, these observations support attractive opportunities for the use of selective retinoids with tumor suppressor, anti-diabetic, or anti-viral activities and tolerable side effects.

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