Selective retinoids and rexinoids in cancer therapy and chemoprevention

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Natural and synthetic retinoids are effective inhibitors of tumor cell growth *in vitro* and *in vivo*. However, the toxicity of natural derivatives of vitamin A limits their therapeutic use. Recently, synthetic compounds selective for the different retinoid receptor isotypes have been generated that circumvent pan-retinoid toxicity. The tumor-suppressive activity of selective retinoid and/or rexinoid ligands has been established preclinically, and emerging clinical trials are supportive of the chemotherapeutic and chemopreventive potential of these compounds in multiple oncology indications, with reduced toxicity. Moreover, the combination of retinoids and/or rexinoids with chemotherapeutic agents for the synergistic modulation of specific pathways could also be of benefit in cancer therapy.

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▼ Retinoids are natural derivatives of vitamin A or retinol and include the all-*trans*-, 9-*cis*- and 13-*cis*-retinoic acids (ATRA, 9cRA and 13cRA, respectively). Vitamin A can be derived from a diet of animal products, including milk products, eggs and fish oils, or from the processing of pro-vitamin β-carotene, which is found in various vegetables. Synthesis of ATRA, the main biologically active derivative of vitamin A, involves the irreversible oxidation of retinol in target cells [1,2]. The biological activities of retinoids are mediated through specific nuclear retinoic acid receptors (RAR) and retinoid X receptors (RXR), encoded by separate genes. Retinoids have a wide variety of physiological functions during embryonic [3] and adult [4,5] life, and play important roles in modulating normal- or tumor-cell growth through the regulation of differentiation and/or apoptosis [6–8], thus supporting the potential use of retinoids in cancer therapy and/or prevention.

A wide variety of synthetic retinoids with various selectivities have been generated that retain many of the biological properties of the natural ligands, including the inhibition of tumor cell proliferation. This review outlines the chemotherapeutic and chemopreventive potential of several selective synthetic retinoids, with particular focus on the following questions:

(1) Why is retinoid receptor selectivity necessary?
(2) What is the structural basis for retinoid receptor selectivity?
(3) What is the future of these molecules in cancer therapy and/or chemoprevention?

Diversity and mechanisms-of-action of the retinoid receptors

The retinoid signal is mediated in target cells through RARs and RXRs, each of which comprise three isotypes designated α, β and γ as well as several isoforms generated by alternative splicing or promoter use [9]. Retinoic acid receptors and RXRs are divergent in their ligand specificity; ATRA can bind and activate only RAR receptors, with a similar affinity for all three isotypes. By contrast, 9cRA binds and activates all three RARs and RXRs, but with different affinities [10]. Retinoic acid receptors and RXRs are transcription factors that act predominantly as RAR–RXR heterodimers, positively or negatively modulating specific genetic programs. In the absence of RAR ligands or in the presence of certain RAR antagonists, DNA bound RAR–RXR heterodimers form multiprotein complexes with transcriptional co-repressors, including nuclear receptor co-repressor (NcoR), silencing mediator for retinoid and thyroid receptors (SMRT) and histone deacetylases (HDACs), to silence target gene expression [11–13] (Fig. 1). Agonist binding to RAR creates a conformational transition of the RAR ligand-binding domain (LBD) [14] that disrupts the co-repressor complex interaction and permits the sequential recruitment of various co-activator complexes [13,15,16]. The complexes containing histone

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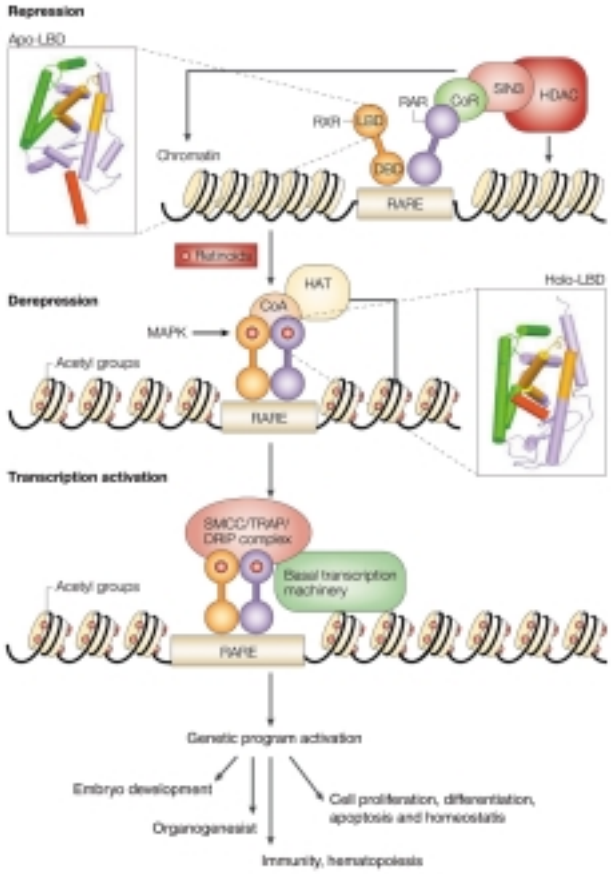

Figure 1. Mechanism of repression and activation of transcription by retinoic acid receptor (RAR)–retinoid X receptor (RXR) heterodimers. In the absence of RAR ligand, RAR–RXR heterodimers bind to specific DNA response elements (RARE [76]) and form multiprotein complexes with co-repressor and histone deacetylases (HDACs), resulting in deacetylation, condensation of chromatin, and transcription silencing. The binding of retinoids to RAR and, potentially, also to RXR generates a conformational transition of RAR and RXR ligand-binding domains, which disrupts co-repressor binding and enables the interaction of RAR–RXR with histone acetyl transferase-containing complexes, inducing derepression of the chromatin. Subsequently, the association of multiprotein complexes, comprising thyroid hormone receptor associated protein (TRAP), vitamin D3 receptor-interacting protein (DRIP) and activator-recruited co-factor (ARC) with RAR–RXR, enables the recruitment of basal transcription machinery to the promoter, and the activation of various genetic programs. Abbreviations: CoA, coactivators; CoR, corepressors; DBD, DNA binding domain; HAT, histone acetyl-transferase; LDB, ligand binding domain; MAPK, mitogen-activated protein kinase; RARE, RAR response element; SIN3, mammalian homologues of the yeast transcriptional repressor SIN3. Figure reproduced, with permission, from Ref. [13].

acetyl transferase activity, which relieve chromatin repression/silencing in gene promoter regions, are subsequently replaced by various large multiprotein units called thyroid hormone receptor associated proteins (TRAP), vitamin D3 receptor interacting proteins (DRIP), or activator-recruited co-factors (ARC), thereby enabling the communication of RAR–RXR heterodimers with the basal transcriptional machinery to initiate target gene expression [13,17] (Fig. 1). Retinoid X receptors are also promiscuous dimerization partners of a large number of nuclear hormone receptors, suggesting that specific RXR ligands (rexinoids) modulate multiple transduction pathways independently of RAR [18]. In addition, RXRs have been shown to form transcriptionally active homodimers in transfected cells. However, the affinity of RXR for homodimer complexes is significantly lower than its affinity for heterodimers with RAR [19,20], and no physiological function of RXR homodimers has been reported.

Why is selectivity needed for retinoid-based therapies?

The beneficial therapeutic potential of retinoids is counterbalanced by their toxicity. Taken together, studies on vitamin A deficiency (VAD) and genetic inactivation of the retinoid receptors indicate that vitamin A is essential during pre- and post-natal development, as well as in adult life, and that the retinoid receptors are responsible for mediating the physiological functions of retinoic acids derived from vitamin A. Indeed, similar abnormalities in fetal development of the respiratory tract, heart, ureter, genital tract and eye were observed in fetal VAD syndrome and in retinoid receptor mutant mice [21].

After birth, vitamin A is essential for the maintenance of various tissues and is required for survival, growth, reproduction, immunity and vision. Squamous metaplasia of a wide variety of epithelia, and degeneration of seminiferous tubules and photoreceptors are the common afflictions associated with postnatal VAD syndrome. With the exception of night blindness and photoreceptor degeneration, the effects of a post-natal VAD diet can be prevented or reversed by retinoic acid [21]. Thus, because vitamin A derivatives exhibit pleiotropic physiological 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. These effects include: (1) varying degrees of teratogenicity, depending on the permeability of the placenta to retinoid and embryo retinoid exposure [22]; (2) mucocutaneous cytotoxicity and chondrogenesis inhibition related to RARγ activity [23]; and (3) hypertriglyceridemia which, in rat, appears to be mediated mainly through RARs [24]. In humans, the hypertriglyceridemia observed in patients treated with Targretin (an RXR-selective agonist containing weak RAR agonistic selectivity) might also be partly attributed to RAR (see later).

These major side-effects have been the primary obstacle for the further development of these molecules as therapeutic agents, and have restricted their use to the treatment of acute promyelocytic leukemia (APL) [13].

The growing understanding of the molecular mechanisms involved in carcinogenesis has opened new avenues for the potential chemotherapeutic and/or chemopreventive use of selective retinoids and rexinoids. For example, APL results from a chromosomal translocation between the genes encoding RARα and promyelocytic leukemia (PML), or more rarely with genes encoding either promyelocytic leukemia zinc finger, nuclear mitotic apparatus, nucleophosmin, or signal transducer and activator of transcription 5B [reviewed in 13]. The oncogenic activity of the PML–RARα fusion protein results from: (1) the higher affinity of PML–RARα for HDACs, which is further enhanced by oligomerization of the fusion proteins, thus generating a 'super-repression' of RARα signaling pathways in APL blasts; and (2) the disruption of PML function as a transcriptional co-activator of the tumor suppressor protein p53 [13]. These observations suggest that the specific use of RARα-selective agonists in patients with APL could have a substantial advantage over ATRA by reducing the effects of retinoic acid syndrome. This syndrome results from ATRA treatment and side-effects include increased leukocyte counts, respiratory distress, weight gain, edema, pleural or pericardial effusions, hypotension and occasionally renal failure [13].

Retinoic acid receptor β is another example of the use of RAR-selective ligands for cancer treatment. Retinoic acid receptor β is unique among its family members in that its gene expression is lost during early development in a variety of tumors, including those derived from head and neck, lung, breast, cervix and prostate [25], thus suggesting a tumor suppressor role for RARβ. The loss of RARβ expression in cancer cells has been attributed to the silencing of the promoter region of its gene through histone hypermethylation and deacetylation [26–28]. Accordingly, HDAC inhibitor-induced reactivation of RARβ gene expression in breast cancer cell lines and xenograft tumors was associated with substantial growth inhibition [29]. The tumor-suppressive activity of RARβ was further supported by a large number of studies implicating RARβ as the main regulator of retinoid-induced inhibition of cell proliferation in teratocarcinoma cells [30], and in breast [31], lung [32] and cervical [33] cancer cells. Additionally, the tumorigenecity of RARβ-transfected lung cancer cells (CALU-1 and H157) in nude mice was substantially decreased, and the mean latency period for the tumors to appear was doubled [34]. Interestingly, the tumors derived from RARβ-transfected CALU-1 cells showed a striking reduction in RARβ message levels compared with the implanted stable clone [34], suggesting that downregulation of RARβ levels is necessary for the development of these tumors. Taken together, these data suggest that of the three RAR isotypes, RARβ specifically regulates essential pathways associated with the tumor-suppressive effect of retinoids in various epithelial cells. Furthermore, the combination of RARβ-selective retinoids with agents that relieve RARβ expression could also be of therapeutic benefit.

Structural determinants of ligand specificity between various retinoic acid receptors

General background

Synthetic retinoids have been the subject of chemical investigation since the early 1960s. Early attempts to overcome the air-, light- and metabolic-instability of retinoic acid led to the replacement of the polyene chain in the natural vitamin with aromatic groups (Fig. 2). Discovery of the individual retinoic acid receptors spurred further successful investigations into modifications conferring binding specificity. Several reviews of retinoid structural evolution have been published [35–37]. The following sections give a brief illustrative overview of the structural features of the ligands that confer specificity among and between the receptors.

RAR-isotype specificity

X-ray studies have shown details of several RAR and RXR LBDs, including RARγ bound to retinoic acid [14] and several other agonists [38,39], RARα bound to an antagonist [40], and RXRα both without ligand [41] and bound to 9-*cis*-retinoic acid [42]. Combining information from crystal and mutational studies with the structure–activity relationships of small-molecule ligands results in a coherent model for RAR-receptor specificity. A detailed analysis of the ligand binding pockets (LBPs) of RAR-α, -β- and -γ showed that the three isotypes differ by only three amino acids, which are located in helices 3, 5 and 11 (Table 1) [14]. Mutational studies revealed that these three amino acids are sufficient to confer the α-, β- and γ-selectivity of the RAR-specific compounds [43,44]. The polar ser-232 and the weakly polar met-272 are characteristic of RAR-α and -γ, respectively [44,45]. Ligands that interact with either of these amino acids thereby attain specific interactions with the individual receptors (Fig. 3).

RARα-specific compounds

Aromatic retinoids containing an amide in the linker region, such as AM580, AM80, AGN193836 and BMS-194753, were found to be RARα-selective (Fig. 2) [46–49]. The conformation of the linker region is important, and derivatives

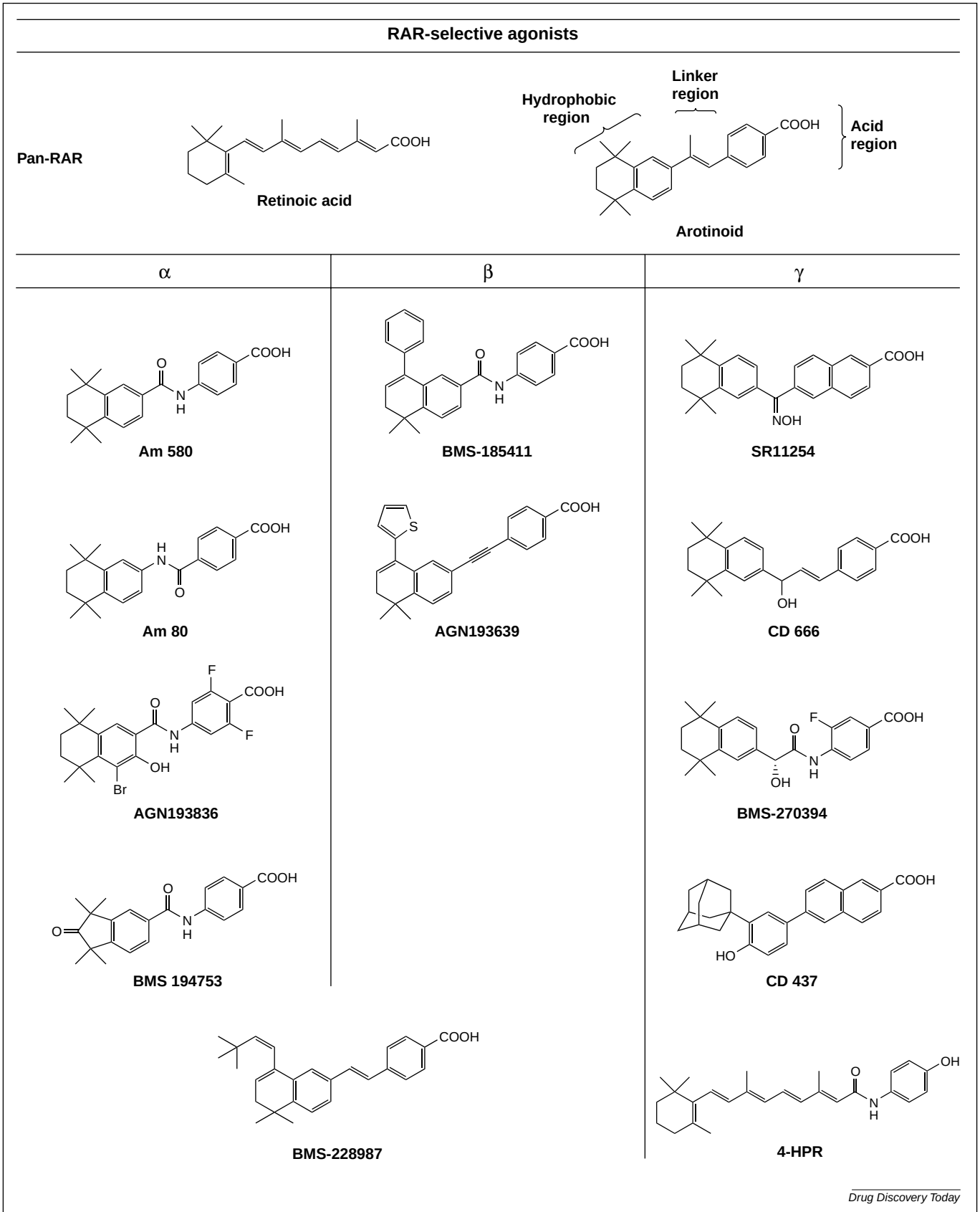

Figure 2. Chemical structures of retinoic acid receptors (RAR)-selective agonists.

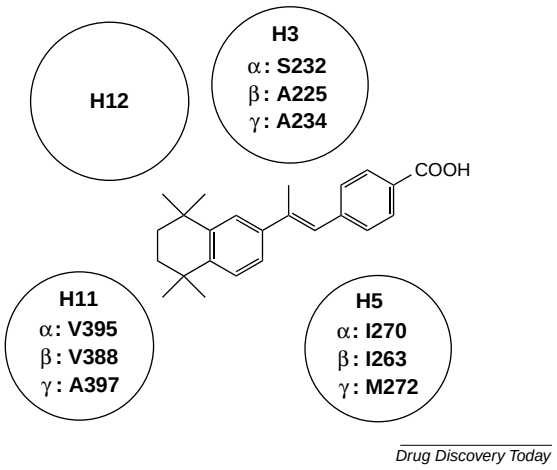

Figure 3. Schematic illustration of RAR ligand-binding pocket residues that are crucial for α-, β- and γ- binding specificity of the retinoic acid receptor (adapted from [14] and [44]).

constrained to *cis* geometry are inactive [50]. Mutation studies revealed the importance of ser-232 to the specific ligand-binding properties of RARα, and the existence of a hydrogen bond between the amide and OH of the serine was postulated [43–45]. Such a stabilizing interaction is not possible with the non-polar amino acids that can be found in the homologous positions in RAR-β and -γ. In the crystal structure of RARα bound to the pure α-antagonist BMS-195614 (see later), the postulated hydrogen bond was unexpectedly seen between the serine hydroxyl group and the benzoic acid ring [40].

RARγ-specific compounds

Many RARγ-selective compounds contain a longer linker group than RARα-selective retinoids. In addition, the γ-compound linker contains a hydrogen-bond-donating group near the hydrophobic-region aromatic ring, as exemplified by SR11254 [35], CD666 [51] and BMS270394 [38] (Fig. 2). The crystal structures of RARγ bound to specific ligands show the presence of a hydrogen bond between the ligand and the sulfur atom of the weakly polar amino acid, Met-272. Although probably weaker than a classical hydrogen bond, this interaction is still sufficient to confer γ-specificity. These compounds are also weak RARβ agonists. Other γ agonists, such as CD437 [52] (Fig. 2), are longer, and their increased hydrophobic bulk might dock better against helix H-11 of RARγ, which contains Ala-397 – a smaller residue than the Val in RAR-α and -β. However, this compound also has affinity for RARβ.

RARβ-specific compounds

The information given in Table 1 shows that there are no polar amino acids that are specific to RARβ, suggesting that different approaches were required to generate β-selective agonists, of which there have been only a few reports. However, the specific amino acids in the RARβ-binding cavity are smaller than the homologous residues in the α and γ isotypes (Ala<Ser and Ile<Met), indicating that slightly larger molecules can be accommodated. Accordingly, retinoids with a larger hydrophobic region, such as BMS185411 and AGN193639, have been reported to be RARβ-specific agonists [53,54] (Fig. 2). However, these compounds are not purely RARβ agonists as they also exhibit RARα and RARγ antagonist activity. Similarly, several compounds were generated with a slightly less bulky hydrophobic domain, such as BMS228987, and have been characterized as selective RARβ and RARα agonists of high affinity [7] (Fig. 2).

RAR antagonists

Modeling studies suggested that RAR-antagonist activity was related to additional bulky substitutions on the hydrophobic domain (Fig. 4), preventing the correct positioning of helix 12 [43,44] and interfering with the subsequent dynamic dissociation and/or association of co-factors with RAR–RXR heterodimers [12]. This model was also confirmed by the crystal structure of RARα bound to the α-selective antagonist BMS195614 [40]. The α-specificity of RAR antagonists can be achieved by incorporating an amide into the linker region (AGN194301 [55] and BMS195614), and very bulky groups are reported to give RARβ antagonists such as LE135 and LE540 [56] (Fig. 4).

RXR-selective agonists and antagonists

In contrast to RAR, the amino acids sequences of the RXR-α, -β and -γ LBPs are identical, making it unlikely that RXR-α, -β and -γ isotype-specific ligands can be found [42]. The natural derivative 9cRA was the first RXR ligand identified. More recently, docosahexaenoic acid was purified from adult mouse brain and identified as a specific RXR agonist [57] (Fig. 5). The crystal structure of RXRα bound to 9cRA shows that 9cRA is bent at the carbon C9, and twisted.

Table 1. Divergent amino acids in the ligand binding pockets of RAR α, β and γ.

RARα	RARβ	RARγ	Location
Ser 232	Ala 225	Ala 234	H3
Ile 270	Ile 263	Met 272	H5
Val 395	Val 388	Ala 397	H11

Abbreviations: H, helix; RAR, retinoic acid receptor; RXR, retinoid X receptor.

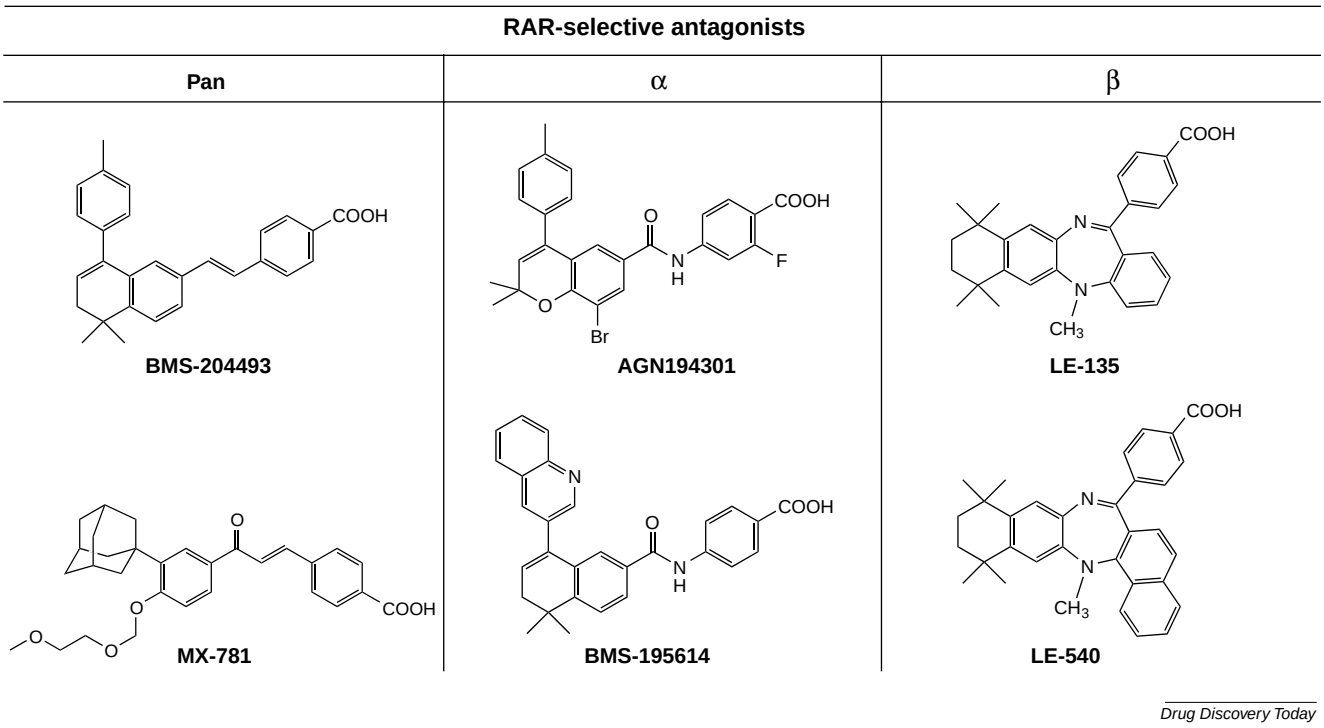

Figure 4. Chemical structures of retinoic acid receptors (RAR)-selective antagonists.

The plane of the diene near the carboxyl terminus is sharply twisted from the plane of the diene in the hydrophobic region. Accordingly, structures that can mimic this conformation will activate the RXRs. Highly active RXR ligands, such as LGD1069, SR11237 and LGD100268, have only one-atom linkers (tending to be slightly shorter than RAR agonists) and might optionally contain ortho-substituents on the hydrophobic aromatic ring to enforce a twisted conformation [58–60] (Fig. 5). Finally, LG100754 [61] and, very recently, PA452 [62] were reported as RXR antagonists (Fig. 5). In a similar way to the RAR antagonists, the extra bulk attached at the ether linkage probably prevents the proper conformation of H12 in the RXR LBD, thereby disrupting its interactions with the transcriptional co-factors.

Activity of selective retinoids for tumor growth inhibition

The use of RARα-selective agonists in the treatment of APL is a very attractive therapeutic approach because the underlying pathology involves oncogenic fusion proteins with the α-isotype of RAR. Accordingly, the selective RARα agonists, AM80, AM580 and BMS194753, were shown to induce differentiation of the acute promyelocytic leukemia cell lines HL60 and NB4, and this effect was reversed by the selective RARα antagonist BMS195614 [18,48]. Furthermore, AM80 successfully induced complete remission in APL patients who had failed ATRA therapy [63,64]. Additionally, in combination with inhibitors of HDAC activity, RARα selective ligands might successfully extend their therapeutic use to acute myeloid leukemia (AML), where the cells show a HDAC-dependent resistance to ATRA. This hypothesis is supported by a recent study showing that ATRA, in combination with the HDAC inhibitor, trichostatin (TSA), induces the nearly complete terminal myeloid differentiation of primary blasts isolated from 23 AML patients [65]. Selective agonists for RARα (AGN193835) have also shown efficacy in the inhibition of breast cancer cell proliferation independently of the estrogen receptor (ER) status, and this effect appeared to be directly correlated with endogenous levels of retinoid α receptors [66]. Furthermore, in combination with the anti-estrogen, 4-OH-Tamoxifen, RARα-selective agonists synergistically repressed the growth of ER positive breast cancer cells [66]. Taken together, these reports indicate the therapeutic potential of RARα-selective agonists in APL, AML and, possibly, breast cancers, and the activity of these compounds could be strengthened in association with other chemotherapeutic agents, including HDAC inhibitors and selective estrogen receptor modulators.

Dual RAR-αβ agonists have also shown therapeutic potential. A recent study monitoring the tumor-suppressive activity of the RAR-αβ-selective agonists BMS228987

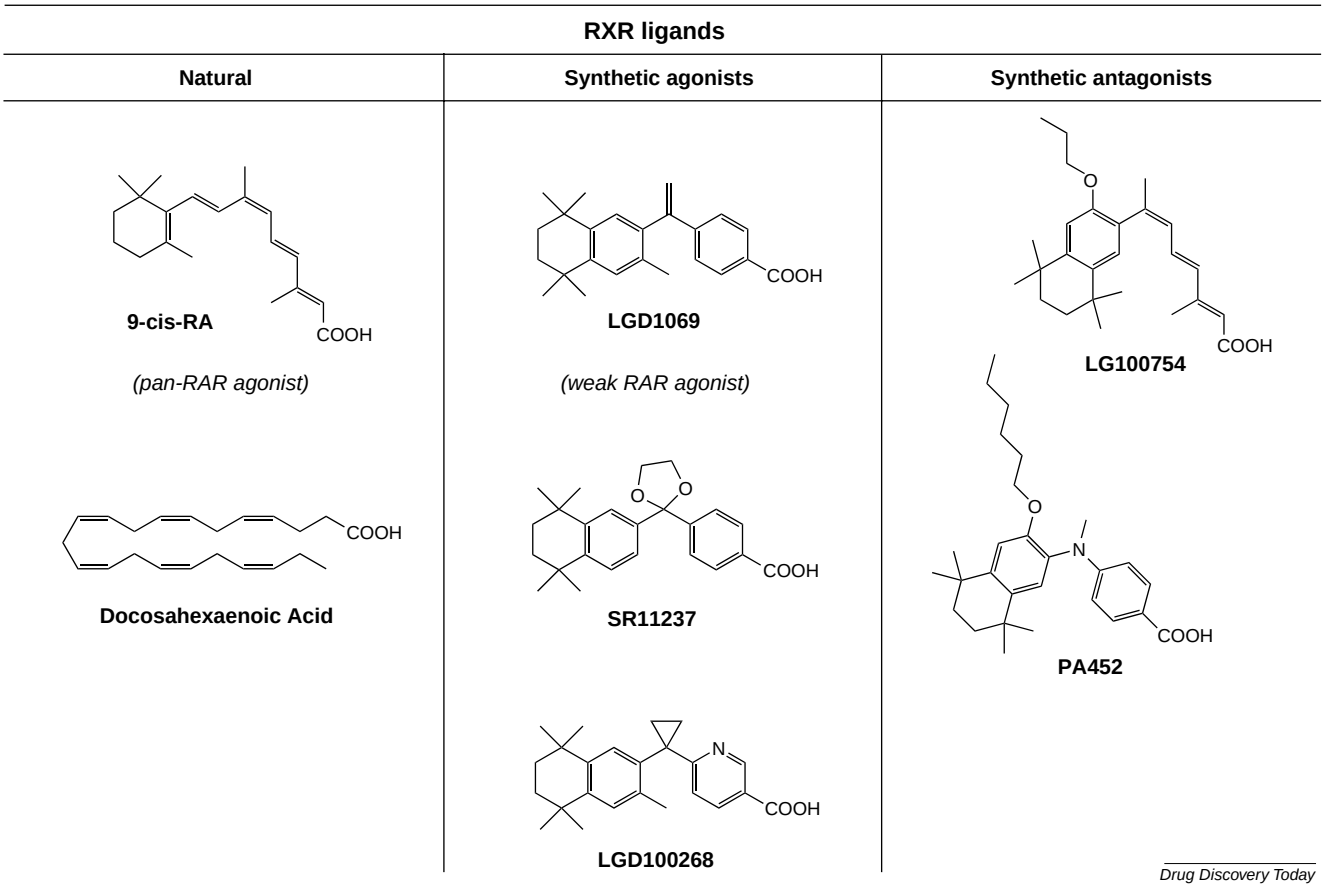

Figure 5. Chemical structures of retinoid X receptor (RXR) natural and synthetic ligands.

(Table 1) and BMS-276393 on a panel of 18 tumor cell lines of various origins demonstrated broad efficacy of these ligands at inhibiting tumor cell proliferation, comparable to that seen with ATRA or a pan-RAR agonists. These observations suggested that the γ-isotype of RAR, which is responsible for the retinoid-induced mucocutaneous cytotoxicity and chondrogenesis inhibition, is not critically required in retinoid-mediated growth inhibition [7]. Interestingly, RAR-αβ-selective agonists enhanced the cytotoxic effect of paclitaxel in several tumor cell types, including breast, head and neck, and ovarian cell lines. This combination effect was shown to result from complementary activities of retinoid and paclitaxel at the level of the anti-apoptotic factor Bcl-2 and on the JNK (Jun N-terminal kinase)–AP-1 transduction pathway [7], resulting in a synergistic induction of tumor cell cytotoxicity. The study highlighted the potential therapeutic benefit of combining selective retinoids with traditional cytotoxic agents as means to inhibit tumor cell growth.

Another class of selective retinoids that have interesting properties are the so-called 'atypical retinoids'. Two representative compounds of this class include 4-hydroxyphenylretinamide (4-HPR or fenretinide) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN or CD437) (Fig. 2). Although 4-HPR and CD437 bind to and transactivate RARγ (and weakly, RARβ), several observations suggest that the strong apoptotic activity of these compounds is RAR-independent. Indeed, 4-HPR- and CD437-induced tumor cell death was observed in ATRA resistant cells and was not compromised by RAR antagonists. 4-HPR-induced apoptosis involves the production of reactive oxygen species (ROS), followed by the release of cytochrome c from the mitochondria, activation of caspase 3, and induction of mitochondrial membrane permeability transition [67]. The mechanisms underlying CD437-induced apoptosis include: (1) induction of the death receptors FAS and DR5, which induce apoptosis after binding FAS ligand (FASL) and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), respectively; (2) relocation of TR3–NGFIB–nur77 (nuclear hormone receptor NR4A1) from the nucleus to the mitochondria to initiate the release of cytochrome c; and (3) activation of the p38 MAPK (mitogen-activated protein kinase) [reviewed in 13]. A recent report showed that in

non-small cell lung carcinoma (NSCLC), the RAR-independent induction of FAS by CD437, and the synergistic apoptotic response induced by the combination of CD437 and FASL, were dependent upon the wild-type p53 pathway [68]. Synergistic induction of apoptosis was also observed when CD437 was combined with TRAIL [69]. Based on these activities, 4-HPR and CD437 have shown efficacy at inhibiting tumor progression in various animal models [70]. Taken together, these data support the potential therapeutic benefit of atypical retinoids used alone or in combination with specific pro-apoptotic ligands (TRAIL or FASL). As the toxic side-effects of 4-HPR are much milder compared with the natural retinoids, this compound has been used in clinical trials for its chemopreventive activity, and showed substantial-to-total regression of various pre-malignant lesions including cutaneous actinic keratoses and oral leukoplakia. Similarly, the 4-carboxy derivative, N-(4-carboxyphenyl)retinamide, promoted regression of cervical displasia in 68% of the patients. Other clinical studies also suggest that 4-HPR could have beneficial preventative effects in both the development of contralateral breast tumor in premenopausal women, and the occurrence of ovarian cancer [25].

Activity of rexinoids

Retinoid X receptors are promiscuous dimerization partners for a large number of nuclear hormone receptors; rexinoid ligands therefore have the potential to affect multiple signal transduction pathways. One of these ligands, the RXR agonist LGD1069 (Targretin; Fig. 5), was shown to prevent the formation and progression of primary and secondary *N*-nitroso-*N*-methylurea (NMU)-induced rat mammary carcinomas. These effects of LGD1069 were also observed in tamoxifen refractory breast tumors of the NMU rat model, and in mammary tumorigenesis of C3(1)-SV40 Tag transgenic mice [71–74]. The chemotherapeutic effect of LGD1069 on breast tumors was associated with a drastic inhibition of tumor cell proliferation and the induction of an 'adipogenesis-like' differentiation program [75]. A phase I–III clinical trial of Targretin for the treatment of refractory advanced-stage cutaneous T-cell lymphoma (CTCL) showed 2% and 43% complete and partial responses, respectively at doses of 300 mg m^{-2} day^{-1}. At doses higher than 300 mg m^{-2} day^{-1}, a response to Targretin was observed in 55% of the patients, including 13% with complete remission [76]. Recent genetic studies also support an active role of RXR in the chemopreventive activity of ATRA in models of skin carcinogenesis [13]. In addition, rexinoids were shown to act synergistically with the protein kinase A pathway to induce the terminal differentiation of APL blasts independently of their sensitivity to ATRA [18]. Although the signaling pathways supporting the chemopreventive and chemotherapeutic activities of rexinoids are still poorly understood, these ligands appear very promising and warrant further investigation into their signal transduction networks. LGD1069 was well tolerated in clinical trials without dose-limiting toxicity up to 500 mg m^{-2} day^{-1}. The most common adverse effects included mild skin toxicity and headaches in up to 50% of the patients, mild to moderate leukopenia and neutropenia in ~30% of the patients, hypercholesterolemia and hypertriglyceridemia in ~50 and ~80% of the patients, respectively, and hypothyroidism in 40% of patients [76,77]. However, because LGD1069 weakly activates RARs at high concentration, the possibility that some of the side-effects observed might be mediated by RAR cannot be ruled out. Importantly, all of the observed side effects were reversible.

The future of selective retinoids in cancer therapy and chemoprevention

Development of the natural vitamin A derivatives, including ATRA, 9cRA and 13cRA, as oral chemotherapeutic agents has been compromised by the severe adverse toxicities they generate through regulation of multiple receptor isotypes. The generation of retinoids and rexinoids with restricted selectivity has opened new avenues for cancer therapy and chemoprevention. However, instead of a broad use, these compounds should be restricted to particular malignancies, such as the use of RARα agonists in patients with APL. In addition, increased therapeutic benefit might be achieved through the combination of retinoid and rexinoid ligands with specific agents of differing modes-of-action, thereby resulting in synergistic anti-tumor activity. Possible candidates for combination treatments include cytotoxic agents such as paclitaxel, or more emerging therapies such as inhibitors of HDACs and/or DNA-, arginine- and lysine- methyltransferase, receptor tyrosine kinases, or pro-apoptotic biological agents such as TRAIL, TNF or FASL. Taken together, these observations suggest further opportunities for cancer therapy and prevention using selective retinoids with tumor suppressor activity and tolerable side effects.

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