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Retinoic Acid Receptor Ligands Based on the 6-Cyclopropyl-2,4-hexadienoic Acid

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Abstract—A series of novel cyclopropanyl methyl hexadienoic acid retinoids was designed and prepared. These compounds exhibited either selective activity as RXR agonists or pan-agonists on one or more of each of the RAR and RXR isoforms. The most potent pan-agonist **5a** (RAR's $EC_{50} = 17–59$ nM; RXR's $EC_{50} = 6–14$ nM) showed good antiproliferative properties in the in vitro cancer cell lines, ME 180 and RPMI 8226.

Small lipid-soluble molecule hormones exert their pleiotropic biological responses via the activation of intracellular receptors (IRs) which comprise the steroid, thyroid, retinoid, as well as, the vitamin D_3 hormone receptor superfamilies.[1] Unlike water-soluble peptide hormones and growth factors that bind to cell surface receptors, lipophilic hormones elicit their effects by entering the cell and binding to their cognate receptors. The ligand-induced transcription factors undergo an allosteric change which enables the complex to bind with high affinity, as a dimer, to specific DNA sequences known as the hormone response elements (HREs). Binding induces gene transcription which results in the synthesis of mRNA protein and alteration of the biological function of cells. The vitamin A metabolite, retinoic acid, has long been recognized to induce a broad spectrum of biological effects. In addition, a variety of structural analogues of retinoic acid (RA) have been synthesized that have been found to be bioactive. These retinoids are known to be key players in the regulation of many important biological processes such as mediation of cell growth and differentiation in both normal and neoplastic cells,[2] and modulation of programmed cell death also known as apoptosis.[3a,b] Some, such as all-*trans* retinoic acid (ATRA), 13-*cis* retinoic acid (13-*cis* RA) and etretinate (Chart 1) have shown to modulate cellular growth and differentiation which have found utility as therapeutic agents for the treatment of psoriasis and acne.[4]

It is now known that retinoids regulates the activity of two distinct families; the retinoic acid receptors ($RAR_{\alpha,\beta,\gamma}$) and the more recently discovered retinoic X receptors ($RXR_{\alpha,\beta,\gamma}$).[5] The RARs are activated by both ATRA and 9-*cis* retinoic acid (9-*cis* RA) through a RAR-RXR heterodimer,[5] whereas the RXRs are activated by 9-*cis*RA via an RXR-RXR homodimer.[1] Several retinoids were also studied for potential applications in oncology.[6a,b] However, the high incidence of undesirable side effects which include lipid and bone toxicity, teratogenicity and skin irritation[7] have been associated with the use of some retinoids. The toxicity of retinoids may be related to their ability to activate multiple retinoid receptors in many target tissues. In view of the related, but clearly distinct, nature of these receptors, retinoids which are more selective for the RAR subfamily or the RXR subfamily would be of great value for selectively controlling processes mediated by one or more of the RAR or RXR isoforms. This would provide the capacity for independent control of the physiologic processes mediated by the RARs or RXRs. For example, the ability of RXRs to form heterodimers with other receptors establishes a central role for these proteins in many endocrine signaling pathways

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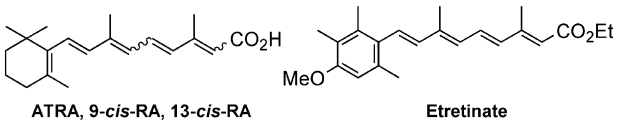


Chart 1.

that may have significant applications such as the control of lipid metabolism or treatment of diabetes; both involving an RXR-PPAR heterodimer.[8a,b] In addition, pan-agonist retinoids that activate one or more isoforms of both the RARs and RXRs could also be valuable as antiproliferative agents that might be less teratogenic. Finally, these compounds may be of greater stability and devoid of the isomerically labile 9-*cis* double bond present in many RXR selective and pan-agonist analogues.

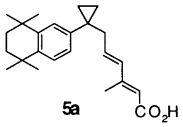
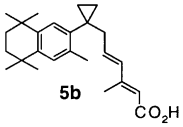
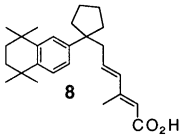
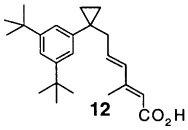
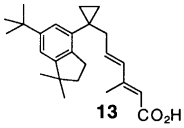
Accordingly, the design of potent and novel RXR-selective and pan-agonist compounds bearing the cyclopropanyl methyl functionality as a bioisostere for the 9-*cis* olefin may provide or lead to therapeutic agents producing fewer side effects. The cyclopropylmethyl moiety should allow the new compounds to adopt both conformations of ATRA and 9-*cis*-RA.A limited number of RXR selective retinoid agonists have been reported in the literature by Dawson,[9] Boehm,[10a,b] and Vuligonda and Chandraratna.[11]

In our efforts to design more potent retinoids, we have developed a novel class of RXR selective and pan-agonists series based on (2*E*,4*E*)-6-[1-(tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) cyclopropanyl]-3-methyl hexadienoic acid (**5a**) scaffold. To further expand on the structure–activity relationships of this series, several analogues have been made exploring variations on the aromatic ring fragments and replacement of the cyclopropyl group by a cyclopentyl moiety within the hexadienoic acid side chain (Table 1). These compounds were evaluated for their ability to bind to the retinoid receptors and to regulate gene expression. The synthetic routes to these compounds are shown in Schemes 1–3. Suzuki coupling of boronic acid **1a** with commercially available 3-bromo-3-butene-1-ol afforded the desired homoallylic alcohol **2a**. Cyclopropanation with diethyl zinc and chloroiodomethane[12] provided the cyclopropane intermediate **3a**. Oxidation of the cyclopropyl alcohol **3a** with PCC afforded aldehyde **4a**. Wittig–Emmons–Wadsworth homologation of aldehyde **4a** with the corresponding phosphonate ester and subsequent saponification (Scheme 1) yielded dienoic acid **5a**.

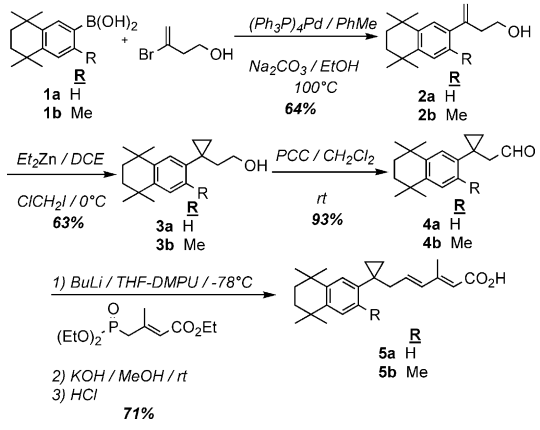
Compound **8** was prepared as shown in Scheme 2. One carbon homologation of **6**[13] with (methoxymethyl) triphenylphosphonium bromide and sodium amide in toluene under reflux conditions, followed by hydrolysis gave aldehyde **7**.

Olefination of **7** followed by saponification of the ester furnished dienoic acid **8**. Compound **12** was prepared as described in Scheme 1 starting from 1-bromo-3,5-di-*t*-butyl benzene.

Table 1. Cotransfection data for synthetic retinoids in CV-1 cells[a]

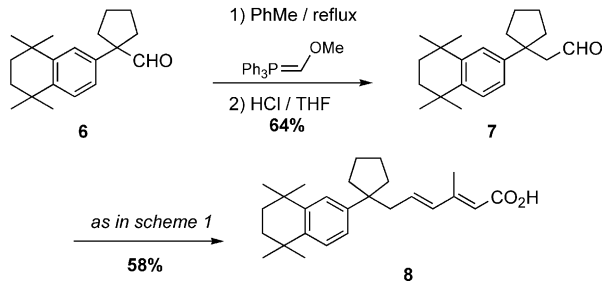
	EC_{50} (nM) / K_d (nM)					
	RAR_α	RAR_β	RAR_γ	RXR_α	RXR_β	RXR_γ
 **5a**	59 **644**	17 **463**	24 **552**	13 **2**	6 **6**	14 **8**
 **5b**	NA **>1000**	NA **>1000**	NA **>1000**	31 **16**	9 **18**	36 **153**
 **8**	NA **>1000**	NA **>1000**	NA **>1000**	63 **41**	19 **7**	24 **6**
 **12**	733 **>1000**	139 **>1000**	391 **>1000**	30 **3**	19 **8**	23 **16**
 **13**	NA **>1000**	NA **>1000**	NA **>1000**	34 **6**	22 **15**	20 **22**
ATRA	436 **15**	78 **17**	19 **17**	1015 **53**	1211 **306**	961 **306**
9-*cis*-RA	220 **93**	29 **97**	50 **148**	195 **8**	128 **15**	124 **14**

NA, not active.
[a]EC_{50} values were determined from full dose response curves ranging from 10^{-12} to 10^{-15} M. Retinoid activity was normalized relative to that of ATRA and is expressed as potency (EC_{50}), which is the concentration of retinoid required to produce 50% of the maximal observed response.

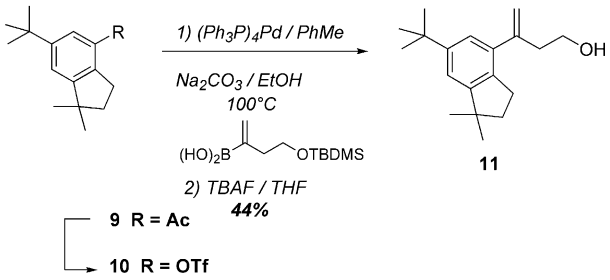


Scheme 1.

Finally, indanyl based compound **13** was prepared through intermediate **11** (Scheme 3) and precursor **9** as the starting material. Bayer–Villager oxidation of **9** followed by the formation of triflate **10** and subsequent



Scheme 2.



Scheme 3.

Suzuki coupling with a vinylboronic acid afforded the desired homoallylic alcohol **11**.

The above retinoids were evaluated in vitro for their ability to bind to the individual RARs and RXRs and induce gene transcription in the cotransfection assays. Cotransfection assays were performed as described by Berger et al.,[14] EC_{50} values are reported in nM. Binding assays for both receptor isoforms were performed in a similar manner as described in Boehm et al.[10b] using [^{3}H]-9-*cis*-RA as the radioligand for the RXRs and [^{3}H]ATRA for the RARs. K_d values are reported in nM. Cotransfection data indicate that (2*E*,4*E*)-6-[1-(tetrahydro tetramethyl-2-naphthalenyl)-cyclopropanyl]-3-methyl hexadienoic acid 5a is a potent pan-agonist (6–59 nM) for both the RARs and the RXRs (Table 1).

In contrast, the addition of a methyl group at the 3-position of **5a** giving **5b** resulted in a potent RXR selective agonist (9–36 nM) with complete disappearance of binding and transactivation of the RARs. The replacement of the cyclopropane ring of **5a** with a cyclopentane moiety afforded **8**. Interestingly, this compound was devoid of RAR activity but had good potency to transactivate the RXRs comparable to that of **5b**. Replacement of the tetrahydrotetramethyl naphthaleneyl group with a 3,5-di-*t*-butyl benzene (**12**) or an indanyl moiety (**13**) resulted in a moderate pan-agonist and a potent RXR selective agonist respectively.

These compounds were further evaluated in a competitive binding assay using [^{3}H]ATRA and [^{3}H]-9-*cis*-RA as radioligands for RARs and RXRs, respectively. The binding activity of these analogues correlated fairly well with the data from the cotransfection assay (Table 1). As an example, compound **8** did not displace [^{3}H]ATRA (>1000 nM) but did displace [^{3}H]-9-*cis*-RA (6–41 nM). In the cotransfection assay, **8** was an inactive RAR agonist but a potent RXR agonist.

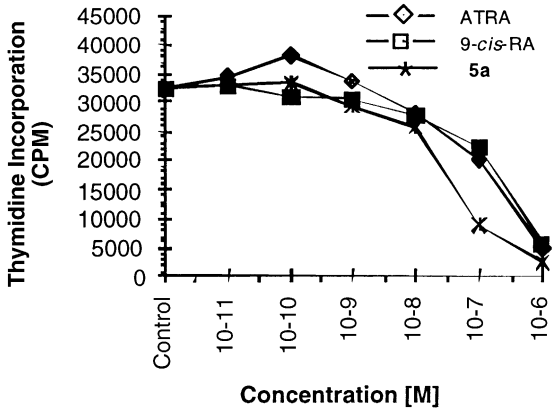


Figure 1. Proliferation of ME 180 cells.

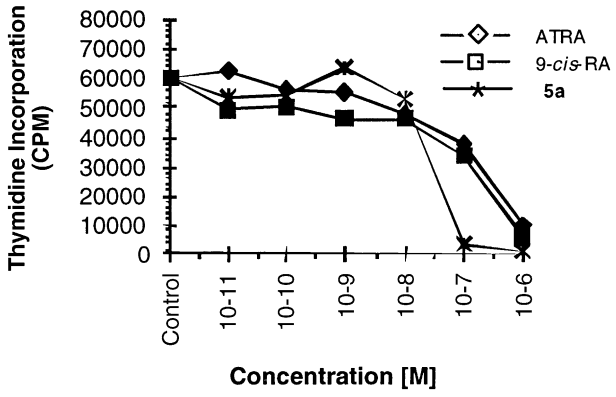


Figure 2. Proliferation of RPMI 8226 cells.

The potent pan-agonist **5a** was also evaluated in two in vitro antiproliferative assays using the RPMI 8226 and ME 1800 cell lines. RPMI 8226 is a human hematopoietic cell line obtained from the peripheral blood of a patient with multiple myeloma, which is a recognized model for multiple myelomas.[15] The second one, ME 180, is a human epidermoid carcinoma cell line derived from the cervix and is also a recognized model for squamous cell carcinoma and related malignancies.[16] Measurement of levels of radiolabeled thymidine incorporated into the aforementioned cell lines provided a direct measurement of the antiproliferative properties of the compounds. Dose–response curves for ATRA, 9-*cis*-RA and **5a** are shown below in Figures 1 and 2.

The above data demonstrate that compound **5a** exhibited good inhibition of proliferation in these cell lines, comparable or better to that of ATRA and 9-*cis*-RA. In conclusion, the cyclopropylmethyl was shown to be a good replacement for the 9-*cis*-olefin and add a new scaffold for novel retinoids.

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