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SYNTHESIS AND STRUCTURE–ACTIVITY RELATIONSHIPS OF POTENT CONFORMATIONALLY RESTRICTED RETINOID X RECEPTOR LIGANDS

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Abstract: A series of potent retinoid X receptor (RXR) selective ligands were designed and prepared. The lead compound **6a**, showed good binding (K_d; 3–7 nM) and transactivation (EC_{50}; 19–24 nM) to the RXR subfamily of retinoid receptors. More importantly, a small variation on the aromatic ring moiety led to **6b**, which had less residual RAR agonist activity with RXR binding and potency of 4–5 nM and 5–13 nM, respectively.

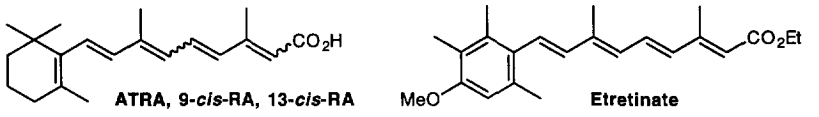
Small, lipid-soluble hormones exert their pleiotropic biological responses via the activation of intracellular receptors (IRs), which comprise the steroid, thyroid, retinoid, as well as, the vitamin D_3 hormone receptor superfamily.[1] Unlike the water-soluble peptide hormones and growth factors that 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 synthesis of mRNA protein and alteration of the biological function of cells. A total of six retinoid receptors have been identified to date that include two distinct families; the retinoic acid receptors ($RAR_{\alpha,\beta,\gamma}$) and the more recently discovered retinoic X receptors ($RXR_{\alpha,\beta,\gamma}$).[2] The RARs are activated by both all-*trans*-retinoic acid (ATRA) and 9-*cis*-retinoic acid (9-*cis*-RA) through a RAR–RXR heterodimer,[2] while the RXRs are activated by 9-*cis*-RA via a RXR–RXR homodimer.[1] Interestingly, RXRs also can heterodimerize with other members of the (IR) superfamily,[1] namely the peroxisome proliferator-activated receptors (PPARs), thyroid hormone receptor (TR), and vitamin D receptor (VDR).

Retinoids regulate many important biological processes such as mediation of cell growth and differentiation in both normal and neoplastic cells,[3] and modulation of programmed cell death also known as apoptosis.[4]

The ability of retinoids, such as all-*trans*-retinoic acid (ATRA), 13-*cis*-retinoic acid (13-*cis*-RA) and etretinate (Chart 1) to modulate cellular growth and differentiation have resulted in the use of these compounds as therapeutics for the treatment of psoriasis and acne.[5] More recently, several retinoids are being studied in clinical trials for potential applications in oncology.[6] 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

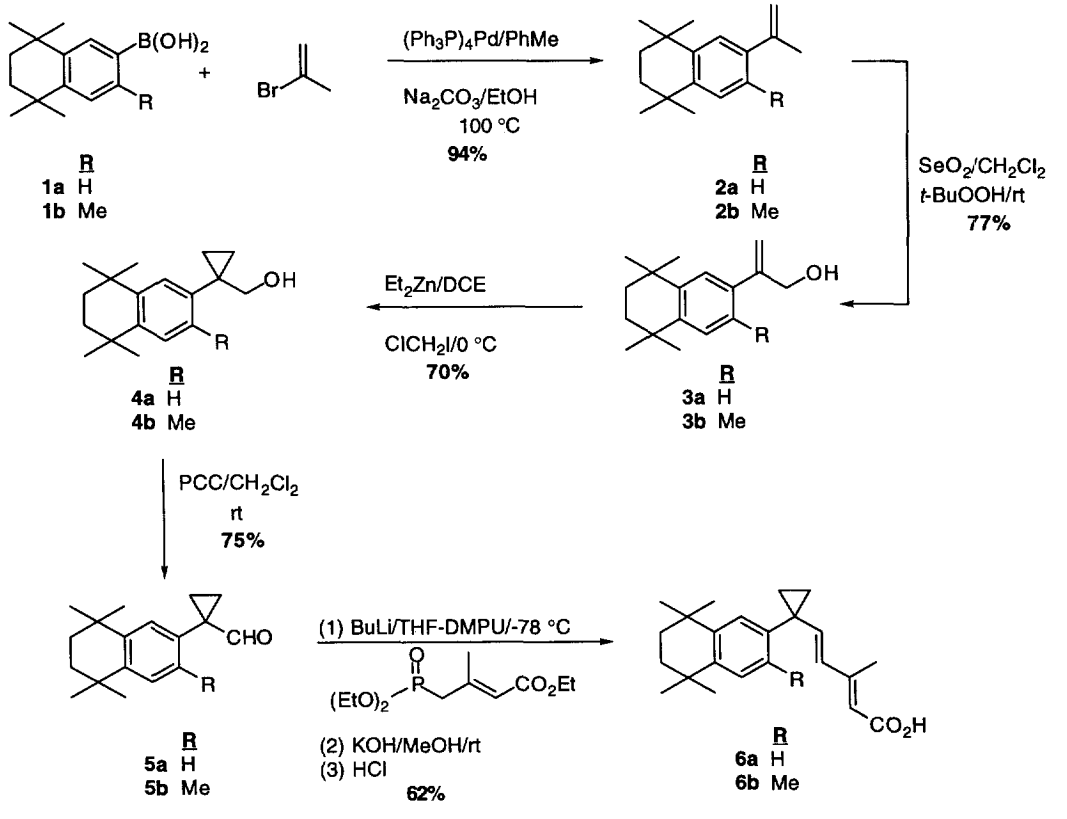
some retinoids. The toxicity of retinoids may be related to their ability to activate multiple retinoid receptors in many target tissues.

Chart 1



Accordingly, the design of potent, novel RXR-selective compounds may afford therapeutic agents with fewer side effects due to a greater specificity of action. The ability of RXRs to form heterodimers with other receptors establishes a central role for these proteins in many endocrine signaling pathways that may have significant applications such as the control of lipid metabolism or treatment of diabetes, both involving a RXR–PPAR heterodimer.[8] A limited number of RXR selective retinoid agonists have been reported in the literature by Dawson,[9] Boehm,[10a,b] and Vuligonda.[11]

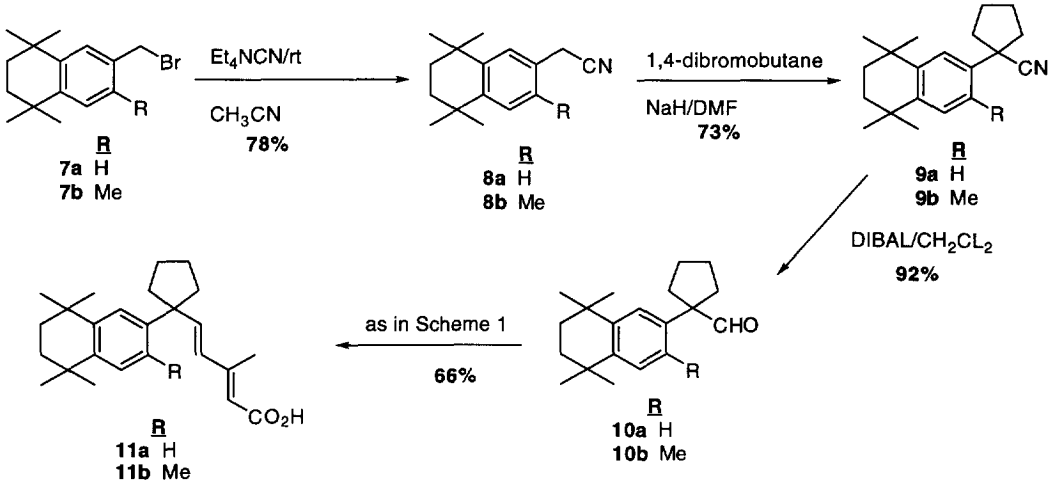
Scheme 1



In our ongoing effort to design more potent RXR agonists with no residual affinity for the RARs, we developed a novel class of RXR selective retinoids based on (2*E*,4*E*)-5-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-cyclopropyl]-3-methyl pentadienoic acid (**6a**). To further expand the structure–activity relationships of compound **6a**, several analogues were made that explored variations on the aromatic ring and the replacement of the cyclopropyl group for a cyclopentyl moiety (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 and 2. Suzuki coupling of boronic acid **1a** with commercially available 2-bromopropene afforded the desired methylstyrene derivative **2a**. Allylic oxidation of **2a** with selenium dioxide and *t*-butylhydroperoxide produced the prerequisite intermediate allylic alcohol **3a**. Cyclopropanation with diethyl zinc and chloroiodomethane[12] gave cyclopropane intermediate **4a**. Oxidation of cyclopropyl alcohol **4a** with PCC gave aldehyde **5a**. Wittig–Emmons–Wadsworth homologation of cyclopropyl aldehyde **5a** with phosphonate ester shown in Scheme 1 provided, after saponification, dienoic acid **6a**.

Compounds **11a** and **11b** were prepared according to Scheme 2. Cyanation of benzyl bromide **7a** with tretraethylammonium bromide in acetonitrile provided benzyl cyanide **8a** which, upon treatment with 1,4-dibromobutane in the presence of sodium hydride in DMF, afforded compound **9a**. DIBAL reduction of the cyanide to the corresponding aldehyde **10a** in dichloromethane gave, after olefination and saponification, the desired dienoic acid **11a**.

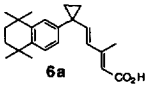
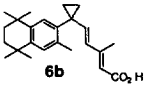
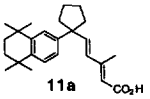
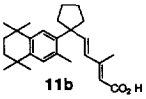
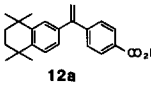
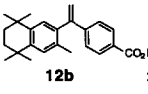
Scheme 2



The retinoids were evaluated in vitro for their ability to bind to the individual RARs and RXRs and induce gene transcription in the cotransfection assay. Cotransfection assays were performed as described,[13]

EC_{50} values being 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 show that (2*E*,4*E*)-5-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-cyclopropyl]-3-methylpentadienoic acid (**6a**) is a potent RXR selective agonist (19–24 nM) with only weak transactivation of the RARs (see Table 1).

Table 1. Cotransfection and Binding Data for Synthetic Retinoids in CV-1 Cells

Structure	EC_{50} (nM) Efficacy (%) K_i(nM)						Structure	EC_{50} (nM) Efficacy (%) K_i(nM)					
	RAR_α	RAR_β	RAR_γ	RXR_α	RXR_β	RXR_γ		RAR_α	RAR_β	RAR_γ	RXR_α	RXR_β	RXR_γ
	NA	287	399	23	19	24		NA	NA	144	5	13	7
	2	21	32	80	100	65		7	5	28	75	133	85
6a	>1000	>1000	>1000	3	7	6	**6b**	>1000	>1000	>1000	4	4	5
	NA	NA	NA	51	127	192		NA	809	1779	2520	2000	2714
	1	3	7	60	145	120		0	0	3	65	51	57
11a	>1000	>1000	>1000	92	124	172	**11b**	>1000	>1000	>1000	>1000	>1000	>1000
	NA	304	266	409	486	404		NA	NA	NA	33	24	25
	16	87	69	98	103	84		6	12	15	83	109	85
12a	944	909	887	150	199	290	**12b**	>1000	>1000	>1000	14	21	29

Efficacy is the maximal observed response normalized relative to that of ATRA. Retinoid cotransfection activity is expressed as potency (EC_{50}) which is the concentration of retinoid required to produce 50% of the maximal observed response. EC_{50} values were determined from full dose response curves ranging from 10^{-12} to 10^{-5} M. NA = Not active.

In contrast, the addition of a methyl group at the 3-position of **6a** to give **6b** resulted in an increase of potency (5–13 nM) for the RXRs and less residual RAR activities. The replacement of the cyclopropane ring of **6a** and **6b** with a cyclopentane moiety afforded compounds **11a** and **11b**, respectively. Analogue **11a** exhibited no RAR activity with lower potencies for the transactivation of the RXRs (51–192 nM) than **6a**.

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 **6b** did not displace [^{3}H]ATRA (>1000 nM) but did displace [^{3}H]-9-*cis*-RA (4–5 nM). In the cotransfection assay, **6b** was an inactive RAR agonist but a potent RXR agonist.

Conformational analysis of compounds was performed using the Tripos forcefield and the random searching algorithm within SYBYL[14] (Tripos Associates St. Louis Mo. USA). The Saunders[15] criteria for conformational search completeness, finding each conformation 6 times, yields a 99.5% chance of having located

all low energy conformations. Shown in Figure 1 are the sets of conformations generated for **12b**, (Targretin®, LGD1069), **6a**, **6b** and **11b**. The RXR specific agonist Targretin® has been reported by Boehm et al.[10b] to be conformationaly limited (into bent conformations) to span four quadrants, as shown with the dihydronaphthalene rings overlapped. From this view the carboxylic acid group spans the circumference of a cone. Examination of the conformations of both cyclopropanes **6a** and **6b** show clearly that they are capable of spanning these RXR active regions. However, when the cyclopropane ring is replaced with a cyclopentyl ring, as for compound **11b**, the molecule adopts more linear diene conformations, instead of the desired bent conformation, which results in a reduction of the RXR activity of this compound. We are consequently able to conclude that the conformational profiles of these compounds correlate very well with their biological profiles.

From the above results, it is probable that the sp^2-like character (ϕ = 118.8°) of the carbon atom of the cyclopropyl ring plays a crucial role in orienting the dienoic side chain in more bent conformations for optimal selectivity and potency for the RXRs than the sp^3 carbon (ϕ = 110.1°) of a cyclopentane ring. Further optimization of the structure–activity relationships for this novel series of compounds, as well as pharmacological evaluation, is currently underway and will be reported in the near future.

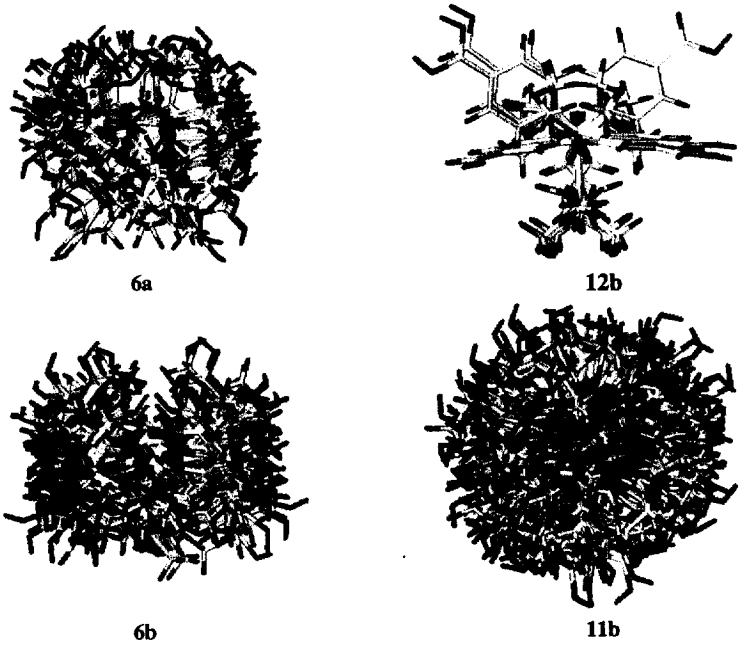


Figure 1. Edge view of low energy conformations of RXR selective retinoids: **6a**, **12b**, **6b**, and **11b**.

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