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

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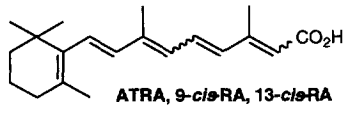
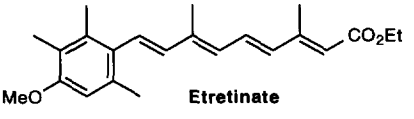
Abstract: A series of potent retinoid X receptor (RXR) selective ligands was designed and prepared. The lead compound **7a** showed good binding (K_i; 20-50 nM) and transactivation (EC_{50}; 40-50 nM) to the RXR subfamily of retinoid receptors. More importantly, small variations in the geometry of the cyclopentane ring moiety led to **9**, one of the most potent RXR agonists to date (K_i: 3-8 nM; EC_{50}: 3-4 nM). © 1997 Elsevier Science Ltd.

A total of six retinoid receptors has been identified to date that include two distinct families: the retinoic acid receptors ($RAR_{\alpha,\beta,\gamma}$) and the more recently discovered retinoid X receptors ($RXR_{\alpha,\beta,\gamma}$).[1] 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] and the RXRs are activated by 9-*cis*-RA through a RXR–RXR homodimer.[2] Retinoids regulate many important biological processes such as mediation of cell growth and differentiation in both normal and neoplastic cells,[3] and the modulation of programmed cell death also known as apoptosis.[4] The ability of retinoids, such as ATRA, 13-*cis* retinoic acid (13-*cis* RA), and etretinate (Chart 1), to modulate cellular growth and differentiation have resulted in their use for the treatment of psoriasis and acne[5] as well as for several forms of cancer.[6] However, the high incidence of undesirable side effects, which include lipid and bone toxicity, teratogenicity and skin irritation,[7] has limited the use of some retinoids. The toxicity of such retinoids may be related to their ability to activate multiple retinoid receptors present in target tissues.

Chart 1



ATRA, 9-*cis*RA, 13-*cis*RA



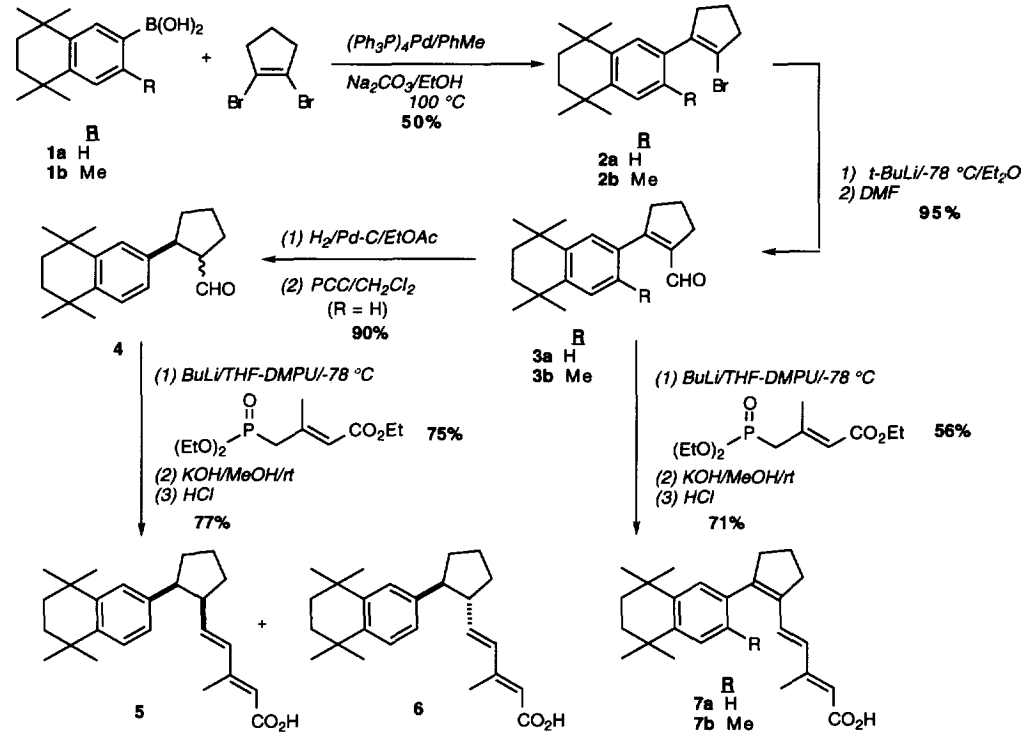
Etretinate

Accordingly, the design of potent, novel RXR-selective compounds may afford therapeutic agents with fewer side effects due to a greater specificity of action. Moreover, RXRs also heterodimerize with other members of the intracellular receptor superfamily,[2] namely the peroxisome proliferator-activated receptors (PPARs), thyroid hormone receptor (TR), and vitamin D receptor (VDR). 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]

In our continuing effort to design more highly potent and selective RXR agonists, we have identified a novel class of RXR selective retinoids that are conformationally constrained compounds related to 9-*cis*-RA. The lead compound of the series is (2E, 4E)-5-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-cyclopentenyl]-3-methylpentadienoic acid **7a**. To further expand the structure–activity relationships of compound **7a**, several analogues were made that explored variations on the 5-membered ring (Table 1). These compounds were evaluated for their ability to bind to the retinoid receptors and to regulate gene expresssion. The synthetic routes to these compounds are shown in Scheme 1, 2, and 3.

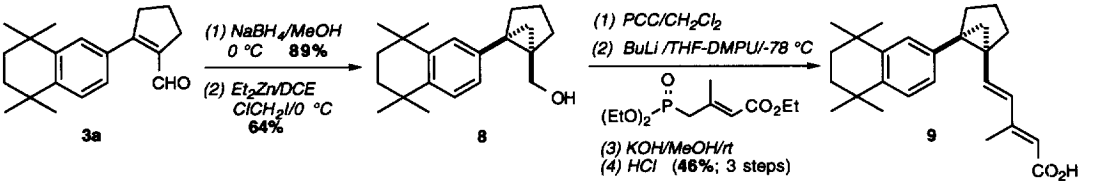
Scheme 1



Suzuki coupling of boronic acid **1a** with commercially available 1,2-dibromocyclopentene afforded the desired vinyl bromide **2a**. Trapping of the vinyl anion of **2a** with DMF produced the prerequisite intermediate aldehyde **3a**. Wittig–Emmons–Wadsworth homologation of cyclopentene aldehyde **3a** with phosphonate ester shown in Scheme 1 provided, after saponification, trienoic acid **7a**. Hydrogenation of α,β-unsaturated aldehyde **3a** afforded a diastereomeric mixture of alcohols which was oxidized with PCC to give **4** as a mixture of

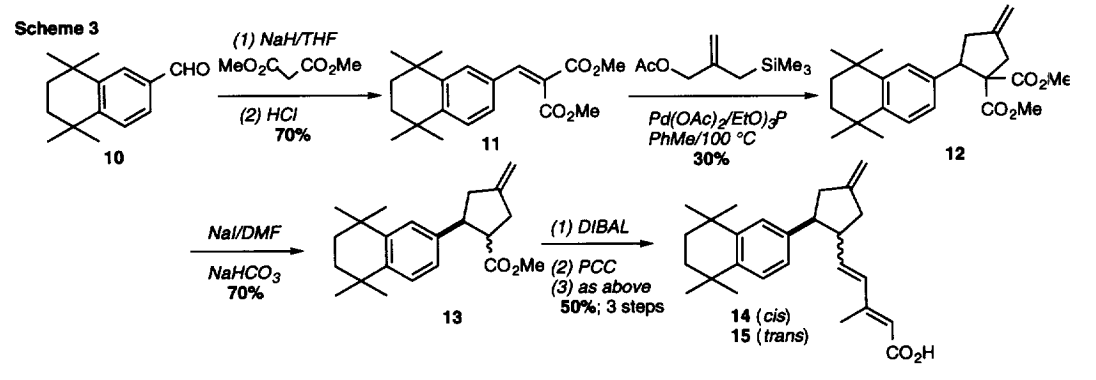
diastereoisomers. Olefination of the mixture with phosphonate ester gave, after saponification, the dienoic acids **5** and **6** in a 8:1 ratio (*Z*:*E*). The relative stereochemistries were confirmed by NOE experiments. The syntheses of the bicyclo[3.1.0]hexane **9** is delineated in Scheme 2. Reduction of aldehyde **3a** with sodium borohydride in methanol, followed by cyclopropanation with diethyl zinc and chloroiodomethane[12] gave cyclopropane intermediate **8**.

Scheme 2



Oxidation of alcohol **8** with PCC and olefination-saponification steps went uneventfully to produce dienoic acid **9**. Compounds **14** and **15** were prepared according to Scheme 3 using the well-precedented Trost palladium-catalyzed [3+2] cycloaddition reaction[13] as the key step. Knoevenagel condensation of aldehyde **10** with dimethyl malonate afforded the diester **11**, an activated Michael acceptor. Cycloaddition of diester **11** and 2-[(trimethylsilyl)methyl]-2-propen-1-yl acetate in the presence of palladium acetate and triethyl phosphite in toluene at 100 °C gave the desired 3-methylidenecyclopentane diester precursor **12**. Decarboxylation with sodium iodide and sodium carbonate in DMF provided the ester **13**. Diisobutylaluminium hydride reduction followed by oxidation afforded a diastereomeric mixture of aldehydes, which upon subjection to an olefination-saponification protocol, produced diastereoisomeric dienes **14** and **15**.

Scheme 3



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 assay. Cotransfection assays were performed as described,[14] with EC_{50} values 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_i values are reported in nM. Cotransfection data show that (2*E*,4*E*)-5-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-cyclopentenyl]-3-methylpentadienoic acid **7a** is a potent RXR selective agonist (40–53 nM) with only weak transactivation of the RARs (see Table 1).

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

Structure	EC_{50} (nM) / Efficacy (%) / K_i (nm)	RAR_α	RAR_β	RAR_γ	RXR_α	RXR_β	RXR_γ
7a	EC_{50} (nM)	NA	339	1146	53	42	40
	Efficacy (%)	9	49	33	74	70	62
	K_i (nm)	>1000	>1000	>1000	20	36	58
7b	EC_{50} (nM)	NA	NA	NA	95	191	128
	Efficacy (%)	2	8	2	40	44	42
	K_i (nm)	>1000	>1000	>1000	5	25	22
5	EC_{50} (nM)	NA	NA	NA	31	29	25
	Efficacy (%)	1	7	3	96	101	58
	K_i (nm)	>1000	>1000	>1000	15	11	26
6	EC_{50} (nM)	NA	637	1291	8	10	6
	Efficacy (%)	2	21	22	116	110	70
	K_i (nm)	>1000	>1000	>1000	3	6	5
9	EC_{50} (nM)	NA	NA	209	4	3	3
	Efficacy (%)	2	2	16	77	92	77
	K_i (nm)	>1000	>1000	>1000	3	10	5
16	EC_{50} (nM)	NA	NA	NA	62	62	44
	Efficacy (%)	1	1	3	52	50	38
	K_i (nm)	>1000	>1000	>1000	17	29	26
17	EC_{50} (nM)	NA	809	1779	7	14	16
	Efficacy (%)	3	23	23	94	110	72
	K_i (nm)	>1000	>1000	>1000	4	10	7
14	EC_{50} (nM)	NA	NA	NA	169	220	204
	Efficacy (%)	1	4	6	100	369	90
	K_i (nm)	>1000	>1000	>1000	66	556	161
15	EC_{50} (nM)	NA	559	355	189	222	212
	Efficacy (%)	1	24	31	110	169	99
	K_i (nm)	>1000	>1000	>1000	38	252	54
Targretin™	EC_{50} (nM)	NA	NA	NA	28	25	20
	Efficacy (%)	1	1	1	83	109	85
	K_i (nm)	>1000	>1000	>1000	36	21	29

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 9-*cis*-RA and is expressed as potency (EC_{50}), which is the concentration of retinoid required to produce 50% of the maximal observed response. NA = Not Active

In contrast, the addition of a methyl group at the 3-position of **7a** to give **7b** resulted in an increase of selectivity for the RXRs. Analogue **7b** exhibited no RAR activity with lower potencies for the transactivation of the RXRs (95-191 nM) than **7a**. Saturation of the cyclopentene double bond of **7a** led to a slight increase in RXR agonist potency in the cotransfection assay. The *trans*-disubstituted cyclopentane **6** displayed comparable biological activities for the RARs, and more importantly, a fourfold increase in potency (6–10 nM) and highier efficacy (70-116%) than **7a** for RXRs. The *cis*-cyclopentane **5** was a slightly less potent RXR agonist than its *trans* isomer **6**, but demonstrated no transactivation for the RAR receptors in the cotransfection assay (see Table 1). The addition of a methyl group on cyclopentane analogues **5** and **6** afforded compounds **16** and **17** respectively. Both exhibited comparable biological activities and the same RAR/RXR profile (see Table 1). When an *exo* methylene was incorporated into *cis* and *trans* cyclopentanes **5** and **6**, to give compounds **14**

and **15**, a decrease of an order of magnitude or more in potency for the RXRs was observed. An interesting boost in potency was obtained by the replacement of the cyclopentane moiety with a bicyclo [3.1.0] hexane as in analogue **9**. Indeed, the addition of a bridgehead methylene on *cis* cyclopentane **5** resulted in a tenfold increase in potency for RXRs, with no significant increase in RAR activity. Compound **9** exhibited nanomolar potency (3–4 nM) for all three RXR isoforms in the cotransfection assay, making this compound one of the most potent RXR selective agonists reported to date.

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 the analogues correlated fairly well with the data from the cotransfection assay (Table 1). As an example, compound **5** did not displace [^{3}H]ATRA (>1000 nM), but displaced [^{3}H]-9-*cis*-RA (11- 26 nM), and in the cotransfection assay, it was inactive at RARs but potent at RXRs. In contrast, **7b** selectively competed with [^{3}H]-9-*cis*-RA at the RXR, but transactivation at the RXRs was 10- to 20-fold weaker.

Conformational analysis on this class of compounds was performed using the Tripos forcefield in SYBYL[15] (Tripos Associates St. Louis Mo. USA). Shown in Figure 1 are the sets of conformations generated by random searching algorithm within SYBYL for Targretin™ (LGD1069), **7b**, **5**, and **9**. The criteria for completeness was finding each conformation six times, which yields a 99.5% chance of having located all conformations.[15] Targretin™ has been reported by Boehm et al.[10b] and was shown to be conformationaly limited to span four quadrants as viewed with the dihydronaphthalene rings overlapped. The conformations of compounds **7b**, **5**, and **9** showed a strong similarity to Targretin™, and are consistent with the previously reported RXR conformational profile.[10b] We are consequently able to conclude that the conformational profiles of these compounds correlate very well with their biological activities.

From the above results, it is evident that the conformation of the 5-membered ring plays a crucial role in orienting the pentadienoic side chain with respect to the tetrahydrotetramethylaphthalene moiety for optimal selectivity and potency for the RXRs. 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.

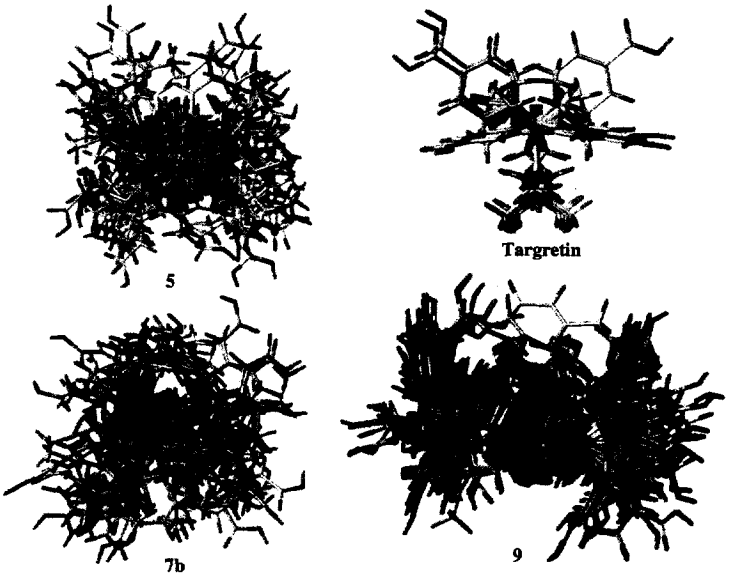


Figure 1. Front views of low energy conformations of RXR selective retinoids: **Targretin**, **5**, **7b**, and **9**.

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