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SYNTHESIS OF HIGHLY POTENT RXR-SPECIFIC RETINOIDS: THE USE OF A CYCLOPROPYL GROUP AS A DOUBLE BOND ISOSTERE

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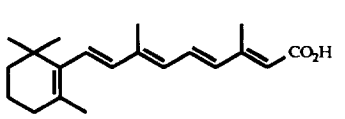
Abstract: Retinoids act through two distinct hormonal pathways activated by RAR and RXR ligands. We describe the synthesis of C9-C10 locked retinoid analogs, including the most potent RXR - specific agonist known to date (Compound **5**), and discuss the structural features that lead to this specificity.

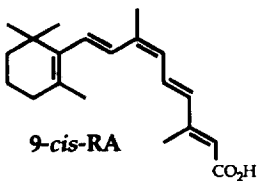
Retinoids are small molecule hormones that elicit pleiotropic biological responses by activating two families of nuclear receptors that are structurally and evolutionarilly related to the steroid/thyroid hormone receptor superfamily.[1] The two families are the retinoic acid receptors (RARs)[2] and the retinoid X receptors (RXRs)[3] and each family consists of three subtypes (α, β, and γ) which are encoded by distinct genes. Physiologically, retinoid hormones regulate a variety of very basic biological functions both in development and in the adult.[4] Disruption of the normal pathways of retinoid homeostasis either by vitamin A deficiency[5] or by alteration of retinoid receptors[6] can lead to disease conditions. Consistent with their broad physiological effects, retinoids are of potential clinical use in a variety of areas including dermatology,[7] oncology,[8] opthalmology[9] and cardiovascular disease.[10] However, the currently available retinoids are widely used only for the treatment of skin diseases because of dose limiting toxicities associated with their use in other indications. The full clinical potential of this important class of compounds will be realized only with the availability of pharmacologically selective analogs that are efficacious in a given disease with the accompaniment of an acceptable range of side effects. A clear understanding of the biological roles of the retinoid receptor families would greatly facilitate the design of analogs that are targeted for specific diseases.

The physiological hormone for the RARs is all-*trans*-retinoic acid (RA)[1] and that for the RXRs is its geometric isomer, 9-*cis*-retinoic acid (9-*cis*-RA).[11] While RA binds selectively to the RARs, 9-*cis*-RA binds with equal avidity to both RXRs and RARs. The situation is further complicated by the fact that while RXR hormonal pathways are mediated by RXR homodimers,[12] the RAR pathways require RAR-RXR heterodimers.[13] In additon, RA and 9-*cis*-RA can readily be interconverted under biological assay conditions. Thus, these polyolefinic hormones are of only limited use in elucidating the precise biological roles of each receptor family. Synthetic ligands that specifically activate only the RXR or RAR hormonal pathway and which cannot be converted into forms that activate the other pathway would be of much greater use in this regard. We and others have previously described structural modifications around stilbene[14] and benzophenone[15] structures

that lead to RXR-selective analogs. In this communication, we describe the use of the cyclopropyl ring as an isostere for the C9-C10 double bond to obtain locked 9-*cis* and 9-*trans* retinoid analogs. The 9-*cis*-locked analog **5** is the most potent RXR analog described to date. Because of its intrinsic pharmacologic selectivity and because it cannot be converted to an RAR active form, compound **5** would be a very useful tool in elucidating the biology associated with specific activation of RXRs *in vivo*.

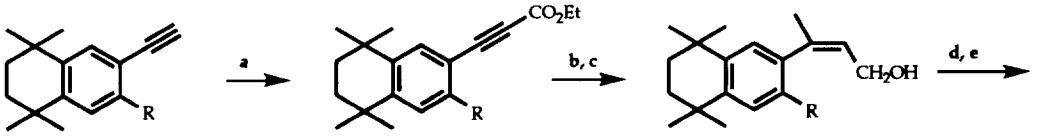


RA



9-*cis*-RA

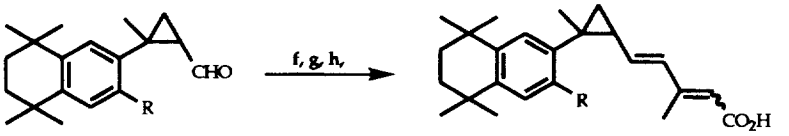
Scheme 1



R = H; 1
R = Me; 1a

R = H; 2 (52%)
R = Me; 2a (82%)

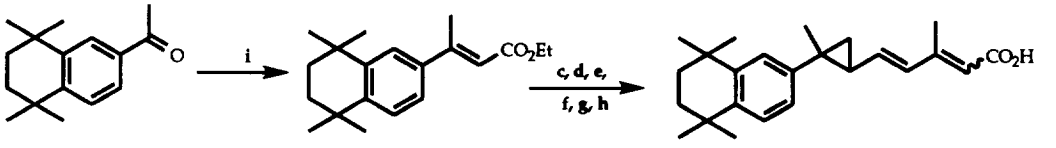
R = H; 3 (90%)
R = Me; 3a (65%)



R = H; 4 (38%)
R = Me; 4a (35%)

R = H, 13E; 5 (65%)
R = Me, 13E; 6 (55%)
R = Me, 13Z; 7 (8%)

Scheme 2



8

9 (65%)

13E; 10 (23% from 9)
13Z; 11 (4% from 9)

a. n-BuLi, $ClCO_2Et$. b. Me_2CuLi, -78 °C c. DiBAl-H d. Sm, CH_2I_2 e. $(COCl)_2$, DMSO, Et_3N f. Diethyl (E)-3-ethoxy-carbonyl-2-methylallylphosphonate, n-BuLi g. HPLC separation on Whatman Partisil 10 column using 2% ethyl acetate in hexane h. LiOH i. Triethylphosphonoacetate, NaH

The syntheses of the *cis*-cyclopropyl analogs, **5-7** are illustrated in Scheme 1. The terminal alkynes **1**[16] were converted to the propargyl esters **2** and then reacted with dimethyl cuprate[17a] followed by DiBAl-H reduction to afford the Z-allylic alcohols **3**,[17b] exclusively. Samarium promoted cyclopropanation[18] of **3** followed by Swern oxidation[19] gave the cyclopropyl aldehydes **4**. Identical sequences of Horner-Emmons reaction of **4** with diethyl (E)-3-ethoxycarbonyl-2-methylallylphosphonate,[20] HPLC separation of the retinoid ester products followed by base hydrolysis afforded the 13-E isomer **5** as the sole isolable product in the desmethyl series and the 13-E isomer **6** and 13-Z isomer **7** (minor) in the α-methyl series. An identical synthetic sequence starting from the ester **9**[21] gave the *trans*-cyclopropyl analogs **10** and **11** (Scheme 2).

The biological activities of these analogs were determined in both receptor binding and functional transactivation assays at each of the RAR and RXR subtypes (Table I). Binding affinities (K_d) were determined using baculovirus expressed RARs and RXRs.[22] The functional activities of the analogs were determined in a series of transactivation assays in CV-1 cells separately transfected with each of the RAR or RXR holoreceptors

Table I Receptor binding and transcriptional activation data for retinoids.

		RAR			RXR		
Number		α	β	γ	α	β	γ
RA	K_d[a]	15	13	18	$>10^3$	$>10^3$	350
	EC_{50}[b]	7	1	0.7	900	1400	1100
9-cis-RA	K_d	11	7	22	9	11	16
	EC_{50}	191	50	45	250	200	140
5	K_d	9530	20472	15942	1.5	2.5	1.8
	EC_{50}	$>10^3$	$>10^3$	$>10^3$	1.5	2.0	1.0
6	K_d	$>10^3$	$>10^3$	$>10^3$	71	56	42
	EC_{50}	$>10^3$	$>10^3$	$>10^3$	130	100	71
7	K_d	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$
	EC_{50}	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$
10	K_d	556	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$
	EC_{50}	$>10^3$	340	200	$>10^3$	$>10^3$	$>10^3$
11	K_d	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$
	EC_{50}	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$

a. K_d values are given in nmolar concentration and were determined by competition of 5nM [^{3}H]-RA (for RARs) or 5nM [^{3}H]-9-cis-RA (for RXRs) with unlabelled test retinoid for baculovirus expressed receptors.
b. Transactivation assays were performed in CV-1 cells transfected with an expression vector for the indicated retinoid receptor and a luciferase reporter plasmid. A ΔMTV-TREp-Luc reporter was used for the RARs, a CRABPII-tk-Luc reporter was used for RXRα and RXRγ, and a CPRE3-tk-Luc reporter was used for RXRβ. EC_{50} values are given in nmolar concentration and are calculated as the concentrations giving 50% of the maximal activity (at 10^{-5}M). Compounds showing less than 20% of the maximal activity of RA were considered inactive.

and luciferase reporter genes under the control of appropriate RAR responsive elements (RAREs) or RXR responsive elements (RXREs).[23,14c] Interestingly, the 9-*cis*-locked analog **5** is a highly potent and specific RXR agonist. Compound **5** binds with approximately 4 to 10 - fold higher affinity to the RXRs than 9-*cis*-RA, and it transactivates the RXRs with approximately 100 fold higher potency. Thus, **5** is a very good mimic of 9-*cis* -RA at the RXRs. Very surprisingly however, **5** does not bind to or effectively transactivate any of the RARs even at very high concentrations. This indicates that the conformational restrictions imposed on **5** by the aromatic and cyclopropyl rings prevent it from adopting the conformation in which 9-*cis*-RA interacts with RARs. Consequently, these data also imply that 9-*cis*-RA interacts with the RARs and RXRs in distinct conformations, since we have succeeded in selecting out its RXR activity with relatively conservative structural changes. The α-methyl substituted analog **6** is also RXR - specific although about 50 fold less potent than **5**. This trend is opposite to that observed in the stilbene[14] and benzophenone[15] series where α-methyl substitution significantly increases activity at the RXRs. The 9-*trans*-locked cylcopropyl analog **10** is RAR specific but is, unexpectedly, about 100 fold less potent than RA. Thus, while the cyclopropyl ring serves as an appropriate isostere for the C9-C10 double bond in terms of the RXR activity of 9-*cis*-RA, it is a poor isostere for the C9-C10 bond in terms of retaining the RAR activity of both 9-*cis*-RA and RA. The 13-*cis* isomers, **7** and **11** are completely inactive indicating a preference for 13-*trans* geometry for both RXR and RAR activities. It should be noted that all of the cyclopropyl analogs were tested as racemic mixtures. It would be interesting to determine which of the biological activities observed reside with each of the enantiomers.

In summary, we demonstrate that a *cis*-cyclopropyl group can be used as an effective isostere for the 9-*cis* double bond of 9-*cis*-RA in terms of its activity at the RXRs but not at the RARs. Thus, the *cis* locked analogs **5** and **6** are potent and effective activators of RXRs but not of RARs. Surprisingly, the *trans*-cyclopropyl analog **10** is only a weak activator of RARs. Compound **5** is the highest affinity and most potent RXR agonist described to date and would be a very useful tool in defining the biology associated with the RXR hormonal pathways.

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