

# A New Class of RAR Subtype Selective Retinoids: Correlation of Pharmacological Effects with Receptor Activity

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**Abstract**—The synthesis and biological activity of a series of structurally related retinoids with different RAR subtype selectivities are described. These retinoids bind to all three RAR subtypes but in functional transactivation assays, they show RAR $\beta$  or RAR $\beta,\gamma$  selectivity with weak RAR $\alpha$  activity. The subtype selectivity of these retinoids was found to correlate with their efficacy (ODC inhibition) and toxicity (topical irritation and teratogenicity) profiles. The degree of RAR $\gamma$  transactivation activity correlates with their topical toxicity and teratogenicity as measured by the inhibition of chondrogenesis. Of the RAR $\beta$  selective retinoids reported here, retinoid **12** is the most promising, as it is completely devoid of two common retinoid related toxicities, namely topical irritation and teratogenesis. © 1999 Elsevier Science Ltd. All rights reserved.

## Introduction

Natural retinoids, which are derivatives of retinol (vitamin A), regulate a variety of physiological processes including embryogenesis, reproduction, cell proliferation and differentiation, vision, and immune function.<sup>1</sup> The mechanism of action of retinoids has come under intense study due to their therapeutic potential in several areas including cancer,<sup>2</sup> skin disorders,<sup>3</sup> metabolic disease,<sup>4</sup> and HIV-induced lymphopenia.<sup>5</sup> The biological actions of retinoids result from the regulation of gene transcription through the intermediacy of nuclear receptors.<sup>6</sup> There are two families of retinoid receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs), each with three subtypes ( $\alpha$ ,  $\beta$ , and  $\gamma$ ). The RARs and RXRs are ligand-dependent transcription factors which belong to the superfamily of steroid nuclear receptors. Retinoic acid (RA) (Fig. 1), the physiological hormone for the RARs, binds with similar affinity to all the three RAR subtypes, and does not bind to the RXRs. In contrast, the putative hormone for the RXRs, 9-*cis* retinoic acid (9-*cis* RA) (Fig. 1), binds with similar affinities to the RXRs and RARs.<sup>6c</sup>

The utility of nonselective retinoids in the treatment of human disease has been limited by the toxicity associated with these compounds. The possibility that certain retinoid toxicities may be associated with specific RAR subtypes is supported by the implication of RAR $\gamma$ , the major RAR subtype found in the skin,<sup>7a</sup> with retinoid induced topical irritation.<sup>7d</sup> The fact that the distribution of various receptor subtypes is relatively tissue specific<sup>7</sup> suggests that receptor-selective retinoids would be more specific in their biological action. As a consequence, receptor-selective retinoids could be associated with fewer toxicities than their nonselective counterparts and hence would be preferred therapeutic agents. Several classes of RAR<sup>8</sup> and RXR<sup>9</sup> specific ligands have been described, and recently RAR subtype selectivity<sup>10</sup> and even specificity<sup>11</sup> has been achieved. As part of an ongoing program to design more selective retinoids, we had previously investigated dihydronaphthalenic retinoids substituted at the C-1 sp<sup>2</sup> carbon (**1**, Figure 1) and obtained significant receptor subtype and functional selectivity.<sup>11b,12</sup> Here we report on the structure-activity relationship (SAR) of optically pure tetrahydronaphthalenic retinoids substituted at the C-1 sp<sup>3</sup> carbon (**2**, Figure 1), and demonstrate distinct RAR subtype selectivities which are dependent upon the relative stereochemistry of the functional group at the C-1 position. We also correlate the RAR activation profiles of these compounds with activity in other pharmacological and toxicological assays.

Key words: RARs; subtype selective retinoids; ODC; retinoid toxicity; teratogenicity.

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## Results and Discussion

### Synthesis

The retinoids described in this paper were prepared starting from the known ketone **3**.<sup>12</sup> Enantioselective reduction of **3** gave the *R* (**4**) and *S* (**5**) alcohols (Scheme 1). The C-1 stereochemistry of the alcohols was assigned on the basis of results using three different chiral reducing agents and comparison with literature reports on reduction of related ketones.<sup>13</sup> The enantiomeric alcohols **4** and **5** were prepared on multigram scale by NaBH<sub>4</sub> reduction of **3** and separation of the racemic mixture by chiral HPLC. The optically pure alcohols **4** and **5** were each reacted with dihydropyran (DHP) in the presence of a catalytic amount of *p*-toluene sulfonic acid (*p*-TSA) and the pairs of diastereomeric tetrahydropyran (THP) ethers **6/7** and **8/9** were separated by normal phase HPLC (Scheme 2). Although the absolute stereochemistries at the anomeric carbon in the THP ring were not defined, the diastereomeric pairs **6/8** and **7/9** were shown to be related as enantiomers on the basis of their specific rotations. The ethyl esters **4–9**

were hydrolyzed to the corresponding carboxylic acids **10–15** (Scheme 3) without loss of optical purity. The optical integrity of the final acids was established by obtaining constant specific rotation by repeat crystallization.

### Biological evaluation

Binding affinities of the retinoid analogues were determined using baculovirus expressed receptors and competition with [<sup>3</sup>H]-RA for the RARs and [<sup>3</sup>H]-9-*cis*-RA for the RXRs.<sup>14</sup> The functional gene transcriptional activities of the retinoids were measured in transactivation assays in CV-1 cells transiently cotransfected with an individual receptor gene construct and a reporter gene.<sup>15</sup> Several retinoids were also tested in an in vivo assay of efficacy, namely that of inhibition of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced ornithine decarboxylase (ODC) activity in hairless mouse skin. ODC is a critical enzyme in the biosynthetic pathway to polyamines, and is elevated in cells prior to a hyperproliferative response.<sup>16</sup> Thus, inhibition of ODC activity could be predictive of the potential clinical utility of

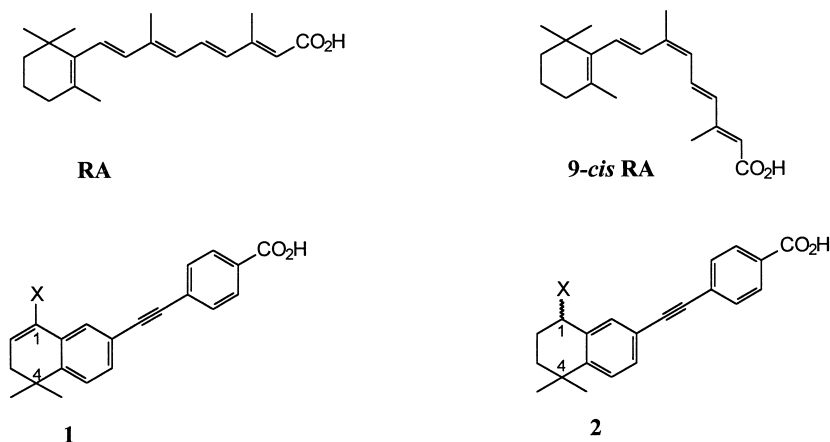
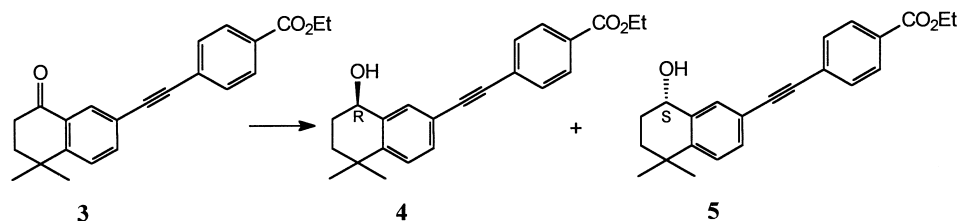


Figure 1.



	$[\alpha]_D^{21}$	-80.0°	+81.0°
	RT <sup>a</sup>	9.5	6.6
Chiral reducing agent		Ratio of <i>R</i> ( <b>4</b> ): <i>S</i> ( <b>5</b> ) <sup>b</sup>	
R (+) BINAL-H		6/1	
(-) DIP Chloride		1/4	
OAB-BH <sub>3</sub>		7/1	

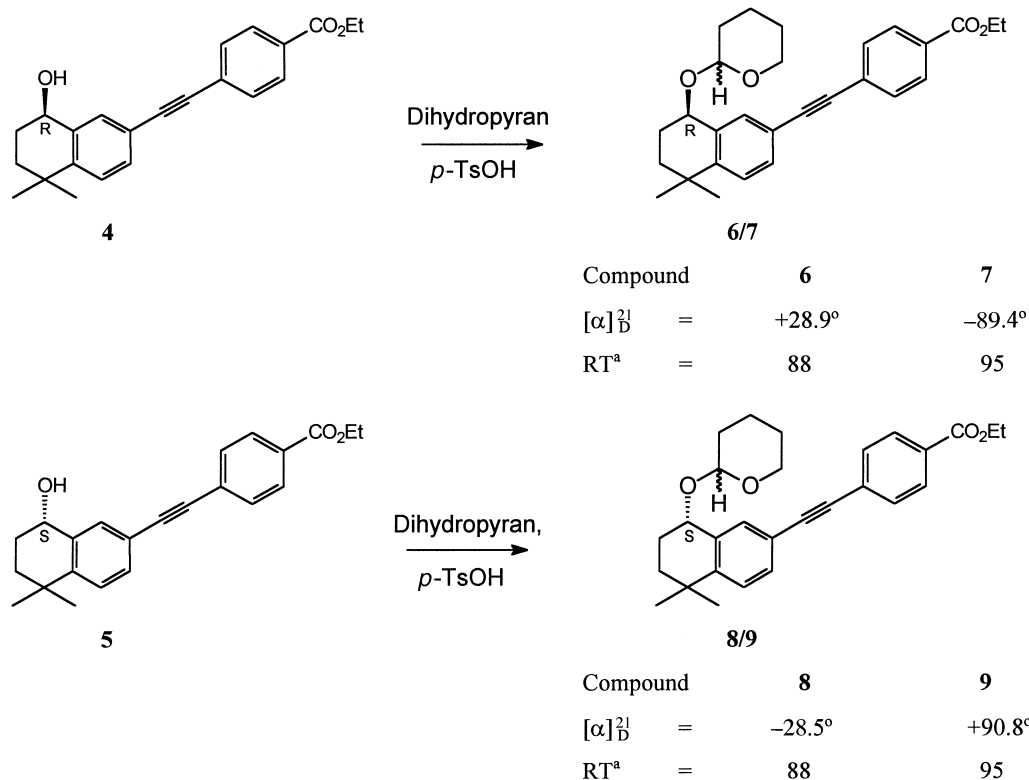
<sup>a</sup>RT is retention time in minutes on a chiral HPLC column (Chiralcel OD-H; 9:1 hexane:isopropanol). <sup>b</sup>The ratios were determined by integration of areas under peaks in the chiral HPLC chromatograms.

Scheme 1.

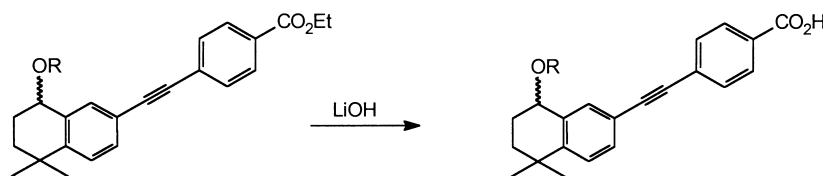
retinoids in the treatment of hyperproliferative skin diseases such as psoriasis. The toxicities of these retinoids were determined using an in vivo topical irritation assay in hairless mice<sup>17</sup> and an in vitro inhibition of chondrogenesis assay in mouse embryo limb bud mesenchymal cells.<sup>18</sup> The latter assay is predictive of the in vivo teratogenic potential of retinoids.<sup>19</sup>

The free carboxylic acids **10–15** were evaluated in the receptor binding assay and were found to bind RARs with different affinities (Table 1). However, none of the compounds had any affinity for the RXRs (data not

shown). The enantiomeric alcohols **10** and **11** had relatively low binding affinities to the RARs. This effect may be due to unfavorable interactions of the hydroxyl group with a hydrophobic region of the receptor. Interestingly, the *R* alcohol (**10**) had a 2.5- to 6-fold lower affinity to the RARs than the corresponding *S* alcohol (**11**), indicating that the introduction of a hydroxyl group in the former spatial orientation is specially disfavored. Conversion of the C-1 hydroxyl into lipophilic moieties as in the THP ethers (**12–15**) resulted in increased affinity for the RARs. These compounds with increased hydrophobicity at C-1 (**12–15**) are quite similar in their



**Scheme 2.** THP = tetrahydropyranyl. Note that the absolute stereochemistry at the anomeric carbon is not defined. (a) RT is retention time in minutes on a normal-phase HPLC column (Partisil-10 PAC column; hexane:ethylacetate, 19:1).



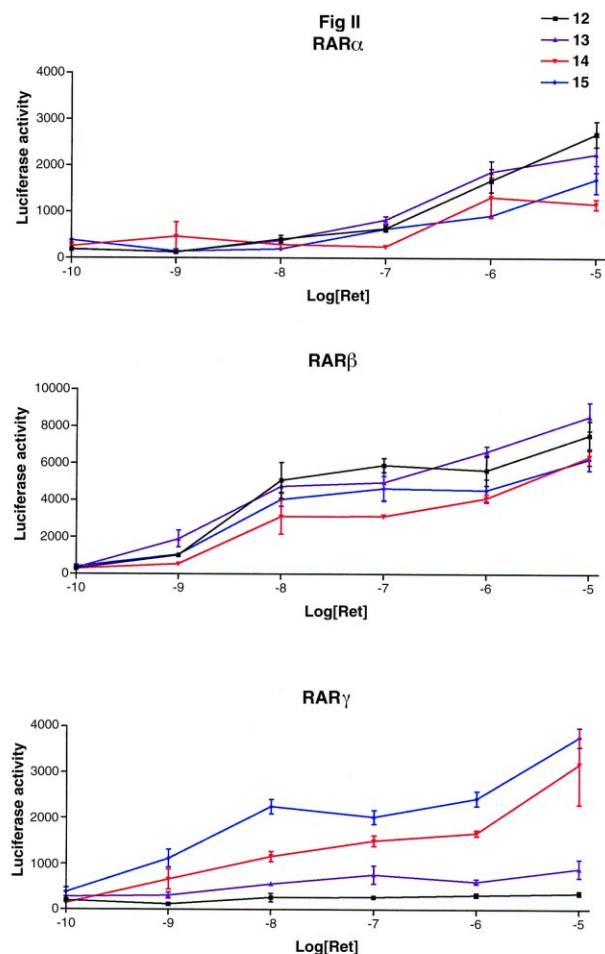
Ester	C-1 stereochemistry	Optical rotation $[\alpha]_D^{25}$	PRODUCT	
			R	Carboxylic acid #
<b>4</b>	<i>R</i>	-86.4°	H	<b>10</b>
<b>5</b>	<i>S</i>	+83.9°	H	<b>11</b>
<b>6</b>	<i>R</i>	+21.0°	THP	<b>12</b>
<b>7</b>	<i>R</i>	-80.0°	THP	<b>13</b>
<b>8</b>	<i>S</i>	-20.9°	THP	<b>14</b>
<b>9</b>	<i>S</i>	+79.6°	THP	<b>15</b>

**Scheme 3.**

relative binding affinities to the three RAR subtypes. In addition, these compounds are somewhat RAR $\beta$  selective, binding with 5- to 20-fold higher affinity to RAR $\beta$  compared to RAR $\alpha$  and RAR $\gamma$ . Although compounds **12–15** are quite similar in their relative binding affinities to the three RAR subtypes, they are quite distinct in their abilities to activate gene transcription. Thus, the THP ether compounds **14** and **15**, which have *S* stereochemistry at C-1, are quite potent at RAR $\beta$  and RAR $\gamma$  and activate RAR $\alpha$  only at higher concentrations in the transactivation assays (Fig. 2). In contrast, compounds **12** and **13**, which have *R* stereochemistry at C-1, are

**Table 1.** Competitive binding assay data for retinoid acids **10–15**

Retinoid	$K_d$ (nM) RAR		
	$\alpha$	$\beta$	$\gamma$
RA	9	12	19
<b>10</b>	3516	1329	2607
<b>11</b>	580	549	571
<b>12</b>	282	48	282
<b>13</b>	116	20	188
<b>14</b>	182	31	171
<b>15</b>	325	39	208



**Figure 2.** Dose–response curves at each RAR for retinoids **12–15** in CV-1 cells transfected with RAR holoreceptors and a MTV-4 (R5G)-luciferase reporter plasmid.

quite RAR $\beta$  selective, again activating RAR $\alpha$  only at higher concentrations and having little or no activity at RAR $\gamma$ . Interestingly, changes in the relative stereochemistry at the anomeric carbon of the THP ether can also lead to subtle changes in transactivation. Thus, compound **12** has no detectable activity at RAR $\gamma$ , while compound **13** has a very low but detectable level of activity at RAR $\gamma$ . Also, compound **15** appears to be more potent at RAR $\gamma$  than the related compound **14**. The transactivation data for these closely related compounds illustrate the phenomenon of functional selectivity where compounds that bind to a receptor with similar affinity elicit quite different functional effects because they cause subtly different conformational changes in the receptor. It should however be noted that in some cases, as in compounds **14** and **15**, there is a lack of exact correlation between binding affinities and potencies in the transactivation assays. This may be because the binding assays are performed with baculovirus expressed RARs where the receptors presumably are in a monomeric form while the transactivation assays utilize transfected cells where the RARs can form heterodimers. For comparison purposes, RA had transactivation potencies in the range 1–10 nM (data not shown) and binding affinities in the range 10–20 nM (Table 1) at the three RARs.

The ODC inhibition<sup>16</sup> and topical irritation assays in hairless mouse were carried out using the diastereomeric ethyl ester derivatives (**6–9**) of compounds **12–15** (Table 2). It is expected that the ethyl esters act as pro-drugs and are converted in vivo to the active free acid forms during the course of the assays.<sup>20a</sup> Zorac,<sup>TM</sup> a currently marketed retinoid for the topical treatment of psoriasis, is an ethyl ester derivative of the acetylenic class of retinoids, similar to the retinoids of the present discussion. Extensive pharmacokinetic studies on Zorac<sup>TM</sup> and related acetylenic retinoids have established that the esters are readily hydrolyzed to the active free acid forms in vivo.<sup>20</sup> We<sup>20c</sup> and others<sup>20d</sup> have employed synthetic retinoid esters in the ODC inhibition assay and reached similar conclusions that benzoate ester retinoids and their corresponding free acids are equally active in this assay. In addition, the ethyl esters of these acetylenic retinoids are less irritating and have better topical therapeutic indices than the corresponding free acids in the topical irritation assay making them ideal pro-drugs for the study. Compounds **8** and **9**, the esters of the RAR $\beta,\gamma$  active retinoids **14** and **15**, are potent

**Table 2.** ODC inhibition and irritation assay data for retinoid esters **6–9**

Retinoid	ODC Inhibition		Cutaneous toxicity <sup>a</sup>	
	Dose (nmol/25 g)	% Inhibition of ODC	Dose (nmol/25 g)	Toxicity score
<b>6</b>	30	61	2000	0
<b>7</b>	3	70	100	3
<b>8</b>	0.3	75	110	11
<b>9</b>	0.3	79	42	11

<sup>a</sup>Cutaneous toxicity is a composite measure of flaking and abrasion of the skin on hairless mice caused by the test retinoid with a maximum possible score of 17.<sup>17</sup>

inhibitors of ODC, giving 75 and 79% inhibition, respectively, at a dose of 0.3 nmol/25 g (Table 2). Compound **7**, the free acid (**13**) of which is potent at RAR $\beta$  and only weakly active at RAR $\gamma$ , is about tenfold less potent than **8** or **9**. Compound **6**, the acid (**12**) of which is transcriptionally inactive at RAR $\gamma$ , is about 100-fold less potent than **8** or **9** since it gives only 61% inhibition at a dose of 30 nmol/25 g. Thus, in this series of compounds, the ability to activate RAR $\gamma$  correlates well with ODC inhibitory activity suggesting that RAR $\gamma$  plays a major role in the inhibition of ODC activity in hairless mouse skin. Note also that **12** (active form of **6**) is weakly active at RAR $\alpha$ , suggesting that RAR $\alpha$  does not play a significant role in ODC inhibitory activity. It is also interesting that compound **6**, the active form of which (compound **12**) has absolutely no RAR $\gamma$  transcriptional activity, still has ODC inhibitory activity although of reduced potency. Since **12** still binds to RAR $\gamma$ , it is possible that with this compound the transactivation and ODC inhibitory activities through RAR $\gamma$  have been separated similar to the previously described separation of transactivation and anti-AP1 activities.<sup>21</sup> These data suggest that the mechanism of retinoid inhibition of ODC involves antagonism of the activity of a transcription factor such as AP-1 or NF-IL6<sup>22</sup> and not direct transcriptional activation of retinoid responsive genes.

The ability of these retinoid esters to induce topical irritation in hairless mice was measured using a cutaneous toxicity score which is an aggregate measure of the skin flaking and abrasion that is induced by applied retinoid.<sup>17</sup> Compounds **8** and **9**, the esters of the RAR $\beta$ , $\gamma$  active retinoids are quite irritating, giving a toxicity score of 11 at doses of 110 and 42 nmol/25 g, respectively (Table 2). Compound **7** has an intermediate topical irritation potential and compound **6** causes absolutely no irritation even at a 20-fold higher dose (Table 2). Clearly, the topical irritation potential of these THP ether retinoids tracks with their RAR $\gamma$  transactivation activity. These data suggest that RAR $\gamma$  transactivation is associated with topical irritation and are in agreement with a recent report.<sup>7d</sup>

The activities of compounds **12–15** in the chondrogenesis inhibition assay are summarized in Table 3. Compounds **14** and **15**, the RAR $\beta$ , $\gamma$  active retinoids, are highly potent in this assay having IC<sub>50</sub> values of 0.15 and 0.045 ng/mL, respectively. In comparison, compound **13**, which has only weak RAR $\gamma$  activity, is about 100-fold less potent than **14** or **15**. The RAR $\beta$ -selective analogue **12**, which has no RAR $\gamma$  transcriptional activity and only weak RAR $\alpha$  activity, is essentially inactive

in this assay having an IC<sub>50</sub> value of >10,000 ng/mL. These data which suggest that RAR $\gamma$  plays a major role in mediating the toxic effects of retinoids are consistent with previous studies using receptor knockout animals.<sup>7e</sup> These results establish for the first time the correlation of ligand mediated RAR $\gamma$  transactivation with chondrogenesis inhibition. Our data also suggest that RAR $\beta$ -specific retinoids may be devoid of teratogenic potential, at least in those aspects of teratogenicity which are predicted by the chondrogenesis inhibition assay.

## Conclusion

In conclusion, we have shown that the absolute stereochemistry at the C-1 position of acetylenic retinoids imparts unique transactivation profiles to this class of retinoids. In addition, subtle changes in the stereochemistry at the C-1 asymmetric carbon can lead to dramatic changes in the receptor transactivation profiles of structurally similar retinoids. Our data show that RAR $\gamma$  transactivation correlates with ODC inhibition and topical irritation. Further, we show for the first time a correlation of RAR $\gamma$  transactivation with chondrogenesis inhibition. Finally, in compound **12**, we have synthesized an RAR $\beta$  subtype selective transactivator with complete lack of transactivation through RAR $\gamma$  and with weak RAR $\alpha$  activity, which retains ODC inhibitory activity and hence potentially anti-proliferative activity. Compound **12** is greatly reduced in topical irritation and in teratogenic potential and could be a retinoid with superior therapeutic index in hyperproliferative diseases such as psoriasis. These findings demonstrate that it is possible to selectively and favorably modulate the biological activities (efficacy and toxicity) of retinoid analogues by changing their receptor activation profiles and this augurs well for the future of retinoid therapeutics.

## Experimental

Melting points were determined using a Thomas–Hoover melting point apparatus and are uncorrected. <sup>1</sup>H NMR spectra were recorded using a Varian Gemini 300 spectrometer (300 MHz) and <sup>13</sup>C NMR spectra using a Varian XL 300 spectrometer (75 MHz) in the solvent indicated. Enantiomeric ratio was determined using Chiralcel OD-H column, with hexane:EtOAc (9:1) as mobile phase. Optical rotations were recorded on Perkin–Elmer Model 241 polarimeter. Elemental analyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ.

Thin-layer chromatography (TLC) was carried out using Whattman silica gel 60 A plates (0.25 mm). Flash chromatography was performed using E. Merck silica gel 60 (230–400 mesh). All reactions were carried out under a positive pressure of argon using reagent grade or anhydrous solvents as received. The phrase ‘dried and evaporated’ indicates drying over MgSO<sub>4</sub> followed by evaporation of the solvents under house vacuum.

**Table 3.** Chondrogenesis inhibition assay<sup>18</sup> data for retinoid acids **12–15**

Retinoid	IC <sub>50</sub> (ng/mL)
<b>12</b>	> 10,000
<b>13</b>	15
<b>14</b>	0.15
<b>15</b>	0.045

**Enantioselective reduction of ketone 3 with (–) DIP chloride<sup>TM</sup>.** To a cold (–25°C) solution of (–) DIP chloride<sup>TM</sup> in THF was added ketone 3 (71 mg, 0.2 mmol) in THF (1 mL). The mixture was stirred for 72 h at –25°C. The solvent was removed under reduced pressure, the residue was dissolved in ether (5 mL) and diethanolamine (75 mg, 0.7 mmol) was added via syringe. The mixture was stirred for 2 h at ambient temperature. The resultant solid was filtered, washed with ether, the combined organic layer was dried and evaporated. Column chromatography afforded 48 mg (68%) of the desired alcohol: mp 120–121°C. The enantiomeric ratio was determined by chiral HPLC. *R*: alcohol 4; rt, 9.5 min (20%).  $[\alpha]_D^{22}$  –80.0° (*c* 0.0035, CH<sub>2</sub>Cl<sub>2</sub>). Anal. calcd for C<sub>23</sub>H<sub>24</sub>O<sub>3</sub>; C, 79.31; H, 6.90; found C, 78.92, H, 7.07. *S*: alcohol 5; rt, 6.6 min (80%).  $[\alpha]_D^{22}$  +81.0° (*c* 0.003, CH<sub>2</sub>Cl<sub>2</sub>). Anal. calcd for C<sub>23</sub>H<sub>24</sub>O<sub>3</sub>; C, 79.31; H, 6.90. Found: C, 79.11; H, 7.09. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.27 (s, 3H), 1.34 (s, 3H), 1.41 (t, 3H, *J* = 7.1 Hz), 1.63 (m, 1H), 1.87 (m, 2H), 2.10 (m, 1H), 4.39 (q, 2H, *J* = 7.1 Hz), 4.73 (brs, 1H), 7.33 (d, 1H, *J* = 8.1 Hz), 7.42 (dd, 1H, *J* = 1.8, 8.1 Hz), 7.56 (d, 2H, *J* = 8.4 Hz), 7.65 (d, 1H, *J* = 1.8 Hz), 8.03 (d, 2H, *J* = 8.4 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 14.3, 28.8, 31.2, 31.3, 34.1, 34.3, 61.1, 68.6, 88.2, 92.2, 120.2, 126.8, 128.0, 129.4, 129.6, 130.9, 131.4, 131.6, 138.2, 146.7, 166.1.

**Enantioselective reduction of ketone 3 with (R)-(+)-BINAL-H.** To a solution of LAH in THF (2 mL, 2 mmol) was added anhydrous EtOH (92 mg, 2 mmol) in THF (2 mL) dropwise (10 min). To this solution (R)-(+)-1,1'-bi-2-naphthol (310 mg, 1.1 mmol) in THF (2 mL) was added dropwise (15 min). The mixture was stirred for additional 30 min and cooled to –78°C and ketone 3 (68 mg, 0.2 mmol) in THF (1 mL) was added. The mixture was stirred at –50°C for 16 h. Reaction was quenched with EtOH (1 mL), and diluted with ether (50 mL) and EtOAc (15 mL). The mixture was washed with water (5 mL), 3 N NaOH (3×5 mL), water (5 mL) and brine (5 mL). The organic layer was dried and evaporated. Column chromatography afforded 62 mg of the desired alcohol. *R*: alcohol 4, 85%. *S*: alcohol 5, 15%.

**Enantioselective reduction of ketone 3 with (S)-(–)-BINAL-H.** Ketone 3 was reduced using the same procedure as used with the (R)-(+)-BINAL-H reagent, instead (S)-(–)-BINAL-H was used as the reducing agent. *R*: alcohol 4, 17%. *S*: alcohol 5, 83%.

**Enantioselective reduction of ketone 3 with (S)-(–)-OAB-BH<sub>3</sub>.** To a cold (–20°C) solution of ketone 3 (300 mg, 0.9 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was added 2-propanol (104 mg, 1.7 mmol), (S)-(–) B-methyloxazoborolidine (288 mg, 1.04 mmol) and borane-THF (1 M solution in THF) (1 mL) sequentially. The reaction was stirred for 4 h at –20°C and 16 h at ambient temperature. To the reaction water (3 mL) was added and extracted with EtOAc (2×20 mL). The combined organic layer was washed with water (5 mL), brine (5 mL) dried and evaporated. The crude material was chromatographed (hexane:EtOAc, 9:1) to afford the alcohol as a white solid 267 mg (89%). *R*: alcohol 4, 88%. *S*: alcohol 5, 12%.

**Preparation of tetrahydropyranyl ethers 6 and 7.** To a cold (0°C) solution of *R* alcohol 4 (272 mg, 0.78 mmol), DHP (1 mL) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added *p*-toluenesulfonic acid (*p*-TsOH) (10 mg) and the mixture was stirred for 8 h at rt. The reaction was quenched with aqueous NaHCO<sub>3</sub> (10 mL) extracted with EtOAc (2×25 mL). The organic layer was washed with brine, dried, and evaporated. The crude material was purified by chromatography (hexane:EtOAc, 19:1). The two diastereomers were separated by normal-phase HPLC (hexane:EtOAc, 19:1) to afford 6 (138 mg) and 7 (135 mg).

**Diastereomer 6:** HPLC (rt), 88 min  $[\alpha]_D^{22}$  +28.9° (*c* 0.0063, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.25 (s, 3H), 1.32 (s, 3H), 1.40 (t, 3H, *J* = 7.1 Hz), 1.50–2.15 (m, 10H), 3.55–3.65 (m, 1H), 3.95–4.05 (m, 1H), 4.38 (q, 2H, *J* = 7.1 Hz), 4.64 (t, 1H, *J* = 4.9 Hz), 4.89 (t, 1H, *J* = 4.9 Hz), 7.32 (d, 1H, *J* = 7.3 Hz), 7.41 (dd, 1H, *J* = 1.8, 7.3 Hz), 7.47 (d, 1H, *J* = 1.8 Hz), 7.57 (d, 2H, *J* = 8.3 Hz), 8.01 (d, 2H, *J* = 8.3 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 14.2, 19.7, 25.5, 27.4, 31.0, 31.4, 31.5, 34.0, 34.6, 61.0, 62.6, 74.4, 87.9, 92.6, 99.1, 119.6, 127.0, 128.1, 129.4, 129.6, 130.9, 131.3, 131.9, 136.1, 147.2, 166.0. Anal. calcd for C<sub>28</sub>H<sub>32</sub>O<sub>4</sub>; C, 77.78; H, 7.41. Found: C, 78.06; H, 7.51.

**Diastereomer 7:** HPLC (rt), 95 min  $[\alpha]_D^{22}$  –89.4° (*c* 0.008, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.27 (s, 3H), 1.33 (s, 3H), 1.41 (t, 3H, *J* = 7.2 Hz), 1.50–1.70 (m, 5H), 1.72–2.10 (m, 5H), 3.55–3.67 (m, 1H), 4.02–4.10 (m, 1H), 4.39 (q, 2H, *J* = 7.2 Hz), 4.79 (t, 1H, *J* = 5.9 Hz), 4.83–4.94 (m, 1H), 7.31 (d, 1H, *J* = 8.1 Hz), 7.41 (dd, 1H, *J* = 1.8, 8.1 Hz), 7.58 (d, 2H, *J* = 8.3 Hz), 7.69 (d, 1H, *J* = 1.8 Hz), 8.02 (d, 2H, *J* = 8.3 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 14.2, 19.9, 24.1, 25.4, 31.1, 31.2, 31.3, 34.0, 34.3, 61.0, 63.0, 71.1, 87.8, 92.8, 95.7, 119.9, 126.6, 128.2, 129.3, 129.5, 130.7, 131.4, 132.2, 136.5, 147.0, 166.0. Anal. calcd for C<sub>28</sub>H<sub>32</sub>O<sub>4</sub>; C, 77.78; H, 7.41. Found: C, 77.44; H, 7.42.

**Preparation of tetrahydropyranyl ethers 8 and 9.** Employing the procedure used for the preparation of 6 and 7, compounds 8 and 9 were prepared from alcohol 5.

**Diastereomer 8:** HPLC (rt), 88 min.  $[\alpha]_D^{22}$  –28.5° (*c* 0.0066, CH<sub>2</sub>Cl<sub>2</sub>). Anal. calcd for C<sub>28</sub>H<sub>32</sub>O<sub>4</sub>; calcd C, 77.78; H, 7.41. Found: C, 77.53, H, 7.39. It is identical to compound 6 by <sup>1</sup>H and <sup>13</sup>C NMR.

**Diastereomer 9:** HPLC (rt), 95 min  $[\alpha]_D^{22}$  +90.8° (*c* 0.0057, CH<sub>2</sub>Cl<sub>2</sub>). Anal. (C<sub>28</sub>H<sub>32</sub>O<sub>4</sub>): C, 77.78; H, 7.41. Found: C, 77.53, H, 7.48. It is identical to compound 7 by <sup>1</sup>H and <sup>13</sup>C NMR.

**Compound 10.** (Standard hydrolysis procedure): To a solution of ester 4 (46 mg, 0.11 mmol) in THF (1 mL), MeOH (0.5 mL), was added LiOH in water (0.5 M, 1 mL). The mixture was stirred for 16 h at rt. The mixture was diluted with EtOAc (30 mL), acidified to pH 5 using ice-cold 10% HCl. The mixture was washed with brine (2×5 mL), dried, and evaporated. Recrystallization from hexane:ether gave 10 as a white solid (35 mg). **Compound 10:**  $[\alpha]_D^{22}$  –86.4° (*c* 0.0025, acetone). <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>): δ 1.28 (s, 3H), 1.30 (s, 3H), 1.60–1.70 (m, 1H), 1.75–1.98 (m, 2H), 2.00–2.10 (m, 1H), 4.65 (t, *J*

= 4.8 Hz, 1H), 7.40 (brs, 2H), 7.66 (d,  $J$  = 8.2 Hz, 3H), 8.05 (d,  $J$  = 8.2 Hz, 2H);  $^{13}\text{C}$  NMR (acetone- $d_6$ ):  $\delta$  31.46, 34.82, 35.72, 68.35, 88.39, 93.26, 120.35, 127.64, 128.79, 130.64, 131.00, 132.26, 132.37, 132.63, 140.82, 147.57, 166.92. Anal. calcd for  $\text{C}_{21}\text{H}_{20}\text{O}_3$ : C, 78.75; H, 6.25. Found: C, 78.50; H, 5.99.

**Compound 11.** Employing the standard hydrolysis procedure, acid **11** was prepared from ester **5**. Compound **11**:  $[\alpha]_D^{22} + 83.9^\circ$  ( $c$  0.0023, acetone). It is identical to compound **10**, by  $^1\text{H}$  and  $^{13}\text{C}$  NMR. Anal. calcd for  $\text{C}_{21}\text{H}_{20}\text{O}_3$ : calcd C, 78.75; H, 6.25. Found: C, 78.50 H, 6.50.

**Compound 12.** Employing the standard hydrolysis procedure, acid **12** was prepared from ester **6**. Compound **12**:  $[\alpha]_D^{22} + 21.0^\circ$  ( $c$  0.0019,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (acetone- $d_6$ ):  $\delta$  1.25 (s, 3H), 1.31 (s, 3H), 1.55–2.10 (m, 10H), 3.50 (3.61 (m, 1H), 3.86–3.96 (m, 1H), 4.65 (t, 1H,  $J$  = 4.5 Hz), 4.91 (t, 1H,  $J$  = 3.1 Hz), 7.45 (d, 2H,  $J$  = 1.1 Hz), 7.50 (brs, 1H), 7.65 (dd, 2H,  $J$  = 1.9, 8.4 Hz), 8.05 (dd, 2H,  $J$  = 8.4 Hz);  $^{13}\text{C}$  NMR (acetone- $d_6$ ):  $\delta$  20.4, 26.3, 28.0, 29.1, 30.6, 31.7, 34.6, 35.2, 62.8, 74.9, 88.5, 93.1, 99.6, 120.3, 128.1, 128.7, 130.1, 130.9, 131.7, 132.3, 133.0, 137.5, 148.2, 166.9. Anal. calcd for  $\text{C}_{26}\text{H}_{28}\text{O}_4$ : calcd C, 77.22; H, 6.93. Found: C, 76.42; H, 6.94.

**Compound 13.** Employing the standard hydrolysis procedure, acid **13** was prepared from ester **7**. Compound **13**:  $[\alpha]_D^{22} - 80.0^\circ$  ( $c$  0.0015,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (acetone- $d_6$ ):  $\delta$  1.26 (s, 3H), 1.31 (s, 3H), 1.55–1.65 (m, 4H), 1.65 (2.10 (m, 6H), 3.51–3.60 (m, 1H), 3.92–4.02 (m, 1H), 4.74 (t, 1H,  $J$  = 4.4 Hz), 4.88 (brs, 1H), 7.43 (brs, 2H), 7.65 (s, 1H), 7.65 (d, 2H,  $J$  = 8.4 Hz), 8.05 (d, 2H,  $J$  = 8.4 Hz);  $^{13}\text{C}$  NMR (acetone- $d_6$ ):  $\delta$  21.0, 25.2, 26.4, 31.4, 31.6, 32.1, 34.7, 35.2, 63.7, 72.3, 88.5, 93.3, 97.2, 120.5, 127.7, 128.8, 130.6, 130.9, 131.5, 132.3, 133.4, 138.1, 148.2, 166.9. Anal. calcd for  $\text{C}_{26}\text{H}_{28}\text{O}_4$ : C, 77.22; H, 6.93. Found: C, 76.64; H, 7.00.

**Compound 14.** Employing the standard hydrolysis procedure, acid **14** was prepared from ester **8**. Compound **14**:  $[\alpha]_D^{22} - 20.9^\circ$  ( $c$  0.0033,  $\text{CH}_2\text{Cl}_2$ ). It is identical to compound **12**, by  $^1\text{H}$  and  $^{13}\text{C}$  NMR. Anal. calcd for  $\text{C}_{26}\text{H}_{28}\text{O}_4$ : C, 77.22; H, 6.93. Found: C, 76.59; H, 7.00.

**Compound 15.** Employing the standard hydrolysis procedure, carboxylic acid **15** was prepared from ester **9**. Compound **15**:  $[\alpha]_D^{22} + 79.6^\circ$  ( $c$  0.0022,  $\text{CH}_2\text{Cl}_2$ ). It is identical to compound **13**, by  $^1\text{H}$  and  $^{13}\text{C}$  NMR. Anal. calcd for  $\text{C}_{26}\text{H}_{28}\text{O}_4$ : C, 77.22; H, 6.93. Found: C, 76.73; H, 7.04.

#### RAR cotransfection transactivation assay

Eukaryotic expression vectors pRShRAR- $\alpha$ , pRShRAR- $\beta$  and pRShRAR- $\gamma$  were cotransfected with the d-MTV-Luc reporter plasmid containing two copies of the TRE-palindromic response element into green monkey CV-1 cells using calcium phosphate precipitation. After 18 h the cells were rinsed with phosphate buffered media and refed with growth media. After 18 h of hormone

treatment cells were harvested in 0.1 M  $\text{K}_3\text{PO}_4$  (pH 7.8), 1.0% Triton X-100, 1.0 mM DTT, 2 mM EDTA. Luciferase activity was measured using firefly luciferin (Analytical Luminescence Laboratory) and an EG&G Berthold 96-well plate luminometer. Luciferase values represent the mean  $\pm$  SEM of triplicate determinations.

#### Binding assay

Each receptor subtype (RAR  $\alpha, \beta, \gamma$ ) was expressed in Baculovirus. Stock solutions of all compounds were prepared as 10 mM ethanol solutions and serial dilutions carried out into DMSO:glycerol (1:1), 120 mM KCl, 8 mM Tris, 5 mM CHAPS, 4 mM DTT, and 0.24 mM PMSF at pH 7.4 at room temperature.

The final assay volume was 250  $\mu\text{L}$  and contained 10–40  $\mu\text{g}$  of extract protein along with 5 nM of  $[\text{H}]$  all *trans* retinoic acid and varying concentrations of competing ligand at a range from 0– $10^{-5}$  M. The assays were run using a Biomek formatted for a 96-well minitube system. Incubations were carried out at 4  $^\circ\text{C}$  until equilibrium was achieved. Nonspecific binding was defined as that binding remaining in the presence of 1000 nM of unlabelled RA. At the end of the incubation period, 50  $\mu\text{L}$  of 6.25% hydroxyapatite was added in a wash buffer which consisted of 100 mM KCl, 10 mM Tris, and 0.5% Triton X-100. The mixture was vortexed and incubated for 10 min at 4  $^\circ\text{C}$ , centrifuged and supernatant removed. The hydroxyapatite was washed three more times with the buffer and the amount of receptor–ligand complex determined by liquid scintillation counting of the pellet. After correcting for nonspecific binding,  $\text{IC}_{50}$  values were determined graphically from a log–logit plot of the data. The  $K_d$  values were determined by application of the Cheng–Prusoff equation to the  $\text{IC}_{50}$  values, the labelled ligand concentration, and the  $K_d$  of the labeled ligand.

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