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Expedited Articles

Identification of Highly Potent Retinoic Acid Receptor α-Selective Antagonists

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The syntheses and full retinoid receptor characterization of a novel series of retinoic acid receptor α (RARα) antagonists, **1**−**5**, are described. These compounds bind with high affinity to RARα but were completely inactive in gene transactivation. They were also potent and effective antagonists of retinoic acid (RA) induced gene transcription at RARα. Compounds **1**−**5** exhibited varying degrees of selectivity for RARα relative to RAR*β*/*γ*, with compound **5** being the most selective in both binding and functional antagonism assays. These compounds will be invaluable tools in delineating the physiological roles of RARα in development and in the adult animal and may themselves be useful therapeutic agents in human diseases associated with RARα.

Introduction

Retinoids are small-molecule hormones that affect a variety of fundamental biological process such as cell differentiation and proliferation and apoptosis.[1] Retinoids elicit their biological effects by regulating gene transcription through a series of nuclear receptors which are ligand-inducible transcription factors belonging to the steroid receptor superfamily.[2] The retinoid receptors are classified into two families, the retinoic acid receptors (RARs)[3] and the retinoid X receptors (RXRs),[4] each consisting of three distinct subtypes (α, *β*, and *γ*). The RARs function *in vivo* as RAR−RXR heterodimers.[5] *all-trans*-Retinoic acid (RA) (Chart 1) is the physiological hormone for the RARs.[2] RA binds with approximately equal affinity to all three RAR subtypes and effectively activates gene transcription through all RAR−RXR heterodimers.

Retinoids are extensively used in dermatology[6] and have shown therapeutic promise in other disease areas including oncology.[7] However, the clinical use of nonselective retinoids, compounds that activate the full range of RAR-mediated pathways, is invariably accompanied by a broad spectrum of toxic side effects.[8] Since the individual RAR subtypes have distinct tissue distribution patterns[9] and appear to regulate different subsets of genes,[10] compounds that are selective for these subtypes will have more restricted pharmacological effects and better therapeutic indices in specific disease applications. In addition, such selective retinoids, both agonists and antagonists, will be invaluable tools in understanding the physiology associated with each RAR subtype and in identifying newer disease applications.

Although several classes of RAR antagonists have been reported in the literature,[11] truly potent RAR subtype selective antagonists remain elusive targets. Ro

Chart 1

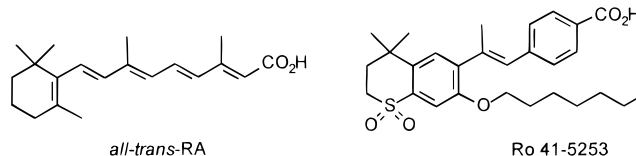


Chart 2

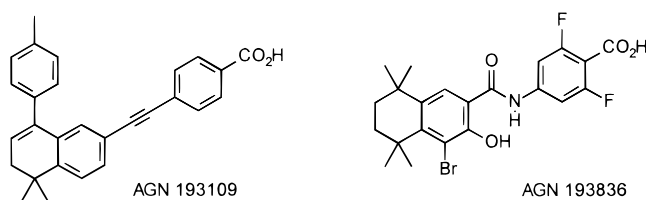
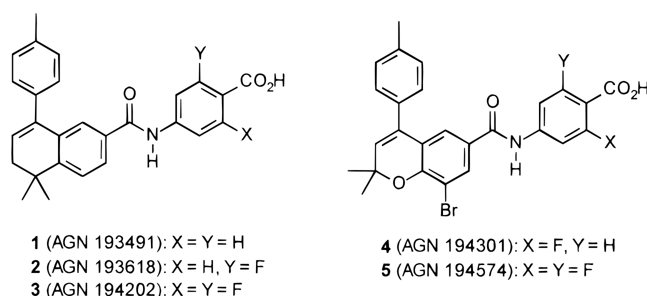


Chart 3



41-5253 (Chart 1) is an RARα selective antagonist, but must be present in approximately 1000-fold molar excess to completely suppress the activity induced by RA.[12] We have previously reported the discovery of AGN 193109 (Chart 2), a potent RAR antagonist.[11a] More recently, we identified the RARα specific agonist AGN 193836 (Chart 2).[13] In this paper we present the results of our studies which combined the structural features responsible for RAR antagonism with those affording RARα selectivity to produce the highly potent and selective antagonists **1**−**5** (Chart 3) of RARα function. Of particular interest are compounds **4** and **5** which completely abrogate RA-induced gene transcrip-

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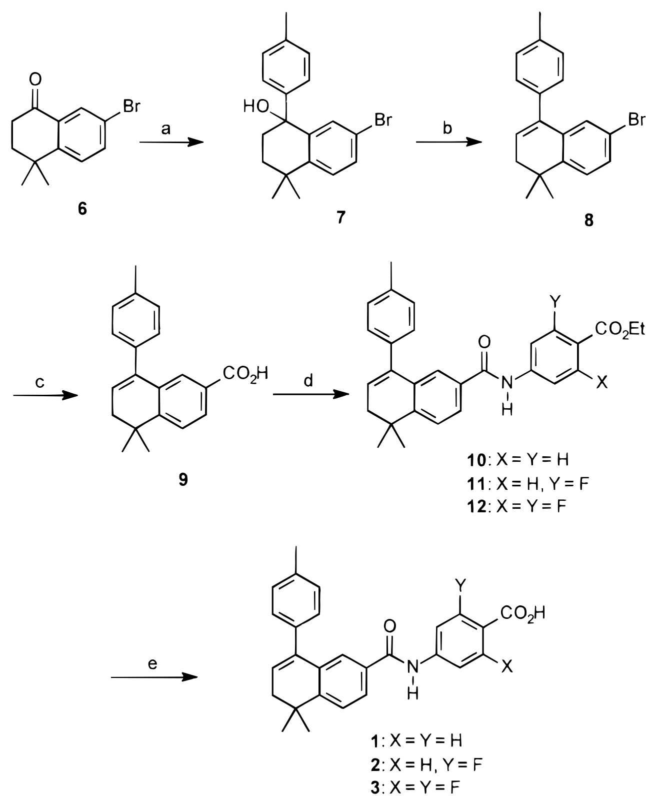
Scheme 1[a]



[a] (a) Mg^0/4-bromotoluene/THF/1 h (62%); (b) *p*-TsOH·H_2O/benzene/80 °C/2 h (81%); (c) *t*-BuLi/THF/−78 °C/CO_2, then HCl/−78 °C to room temperature (75%); (d) EDC/DMAP/DMF/4-$H_2NC_6H_4CO_2Et$ (**10**, 74%), or 2-F-4-$H_2NC_6H_3CO_2Et$ (**11**, 50%), or 2,6-F_2-4-$H_2NC_6H_2CO_2Et$ (**12**, 19%); (e) NaOH/MeOH/H_2O (**1**, 73%; **2**, 72%; **3**, 70%).

tion through RARα at 10-fold lower and equimolar concentrations, respectively.

Chemistry

The synthesis of compounds **1**−**3** are described in Scheme 1. Grignard reaction of *p*-tolylmagnesium bromide and bromotetralone **6**[11a] gave the corresponding alcohol **7**. Dehydration of **7** using catalytic *p*-TsOH afforded dihydronaphthalene **8**. Compound **8** was subjected to lithium−halogen exchange followed by a CO_2 quench to afford the carboxylic acid **9** in good yield. Coupling of **9** with the appropriate aminobenzoates afforded the amides **10**−**12**, which after hydrolysis under basic conditions gave the corresponding acids **1**−**3**.

Compounds **4** and **5** were prepared according to Scheme 2. Dihydrocoumarin (**13**) was exposed to excess methylmagnesium chloride and the resultant tertiary alcohol cyclized using aqueous H_2SO_4 to give chroman **14**. Acylation of **14** under Friedel−Crafts conditions afforded methyl ketone **15**. Oxidation of **15** using NaOCl followed by Fisher esterification provided ethyl ester **16** in 92% overall yield. Bromination of **16** in acetic acid afforded **17** which was then oxidized using CrO_3 in HOAc to yield **18**. Addition of *p*-tolylmagnesium bromide to ketone **18**, followed by *p*-TsOH-catalyzed dehydration, gave **19**. Hydrolysis of the ethyl ester followed by coupling of the resulting acid **20** with the appropriate 4-aminobenzoate[13] gave **21** and **22**. A second hydrolysis of the terminal ethyl ester group with base afforded compounds **4** and **5**.

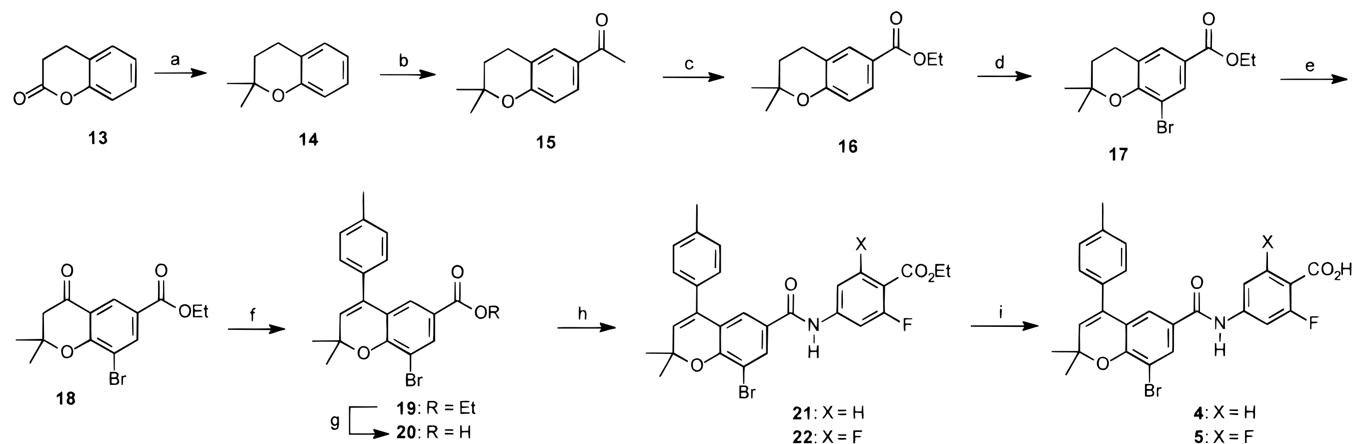
Results and Discussion

The binding affinities of compounds **1**−**5** were measured using baculovirus-expressed RARs and RXRs.[14] None of the compounds showed any affinity to the RXRs (data not shown). All of the compounds bound with high affinity to RARα, having values comparable to or higher than RA (Table 1). In contrast, compounds **1**−**5** displayed much lower affinity to RARβ and RARγ, and with varying degrees of selectivity. In the dihydronaphthalene series, compound **1** showed approximately 40−100-fold higher affinity to RARα relative to RARβ and RARγ. Introduction of a fluorine atom *ortho* to the carboxylic acid as in **2** increased the affinity to RARα and displayed greater α/β selectivity. The difluoro analog **3** was found to be even more RARα selective, having an α/β-selectivity of approximately 100-fold, and a preference for RARα relative to RARγ approaching 1000-fold. In the dimethylchromene series, the monofluoro analog **4** exhibited very high affinity and selectivity for RARα. The corresponding difluoro analog **5** was the most interesting in the series. In addition to an approximately 20-fold higher affinity for RARα than the natural hormone RA, compound **5** also exhibited true pharmacological specificity for RARα by binding to RARα with approximate 1000-fold and 10000-fold higher affinity than to RARβ and RARγ, respectively.

The ability of retinoids **1**−**5** to induce gene transcription was determined using CV-1 cells transfected with individual RAR holoreceptors and an MTV-4(R5G)-luciferase reporter.[15] While RA effectively transactivated all three RAR subtypes, compounds **1**−**5** had absolutely no activity at any of the RARs (Figure 1). These data, when viewed with the receptor binding data (Table 1), suggested that compounds **1**−**5** would act as effective RARα-selective antagonists. As a result, functional antagonism assays were performed using the transactivation assays described above and a constant dose (10^{-8} M) of RA as the test agonist (Figure 2). Compounds **1** and **2** were effective antagonists of RA function at RARα, inhibiting RA-induced gene transcription by approximately 75% at equimolar concentrations and completely abrogating RA activity at a 10-fold molar excess. However, at higher concentrations, compounds **1** and **2** also inhibited RA-induced activity at RARβ and RARγ by approximately 35%. The data for compound **3** reveal a somewhat less potent RA antagonist at RARα, requiring ∼100-fold molar excess to completely suppress RA activity. In the dimethylchromene series, **4** and **5** were very potent antagonists at RARα in accord with their high binding affinities to this receptor subtype. Compound **4** was the most potent antagonist at RARα, showing complete inhibition at a concentration 10-fold lower than the test agonist. At RARβ and RARγ, inhibition was observed only at higher concentrations. Consistent with K_d values, compound **5** was also found to be a potent antagonist at RARα by completely inhibiting RA activity at approximately equimolar concentrations. It is also a very selective antagonist since even at 1 μM concentration (100-fold molar excess) it had no effect on RA activity at RARγ and only partially blocked activity at RARβ.

In summary, we have identified a series of high-affinity RARα ligands which are also highly selective antagonists of RA-induced gene transcriptional activity at RARα. These compounds, together with the previ-

Scheme 2[a]



[a] (a) MeMgCl/THF/then H_2SO_4/H_2O/100 °C (100%); (b) $CH_3COCl/AlCl_3/CH_2Cl$ (54%); (c) NaOCl/NaOH/dioxane/65 °C then EtOH/H_2SO_4/Δ (92%); (d) Br_2/HOAc (100%); (e) $CrO_3/HOAc/Ac_2O$ (76%); (f) *p*-tolyl-MgBr/THF then *p*-TsOH·H_2O/toluene/Δ (25%); (g) NaOH/EtOH/H_2O (98%); (h) EDC/DMAP/DMF/2-F-4-$H_2NC_6H_3CO_2Et$ (**21**, 63%), or 2,6-difluoro-4-$H_2NC_6H_2CO_2Et$ (**22**, 33%); (i) NaOH/EtOH/H_2O (**4**, 86%; **5**, 70%).

Table 1. Binding Affinity (K_d) of Retinoids to RARα/β/γ[a]

entry	RAR α	RAR β	RAR γ
retinoic acid	15 ± 1.7	13 ± 2.5	18 ± 1
1	27 ± 15	1020 ± 316	3121 ± 1457
2	5.7 ± 0.9	622 ± 185	863 ± 138
3	32 ± 15	2256 ± 1257	>30000
4	2.8 ± 1.1	320 ± 87	7258 ± 2648
5	1.5 ± 0.8	898 ± 362	10618 ± 3688

[a] K_d values (mean ± SEM of triplicate determinations) were determined *via* competition of [^{3}H]-(*all-E*)-retinoic acid (5 nM) binding with unlabeled test retinoid at baculovirus expressed RARs and application of the equation of Cheng and Prussof.[14c]

ously identified RARα-specific agonists,[13a] will be very powerful tools in elucidating the physiological roles of RARα in development and in the adult animal. Compounds of this type could also be effective therapeutic agents in diseases where the inappropriate activation of RARα is pathogenic.

Experimental Section

General Methods. ^{1}H NMR spectra were recorded using a Varian Gemini 300 spectrometer (300 MHz) in $CDCl_3$ unless otherwise indicated. Mass spectra were recorded using VG-analytical 7070E organic mass spectrometer. High-resolution mass spectra were recorded using VG autospec 3000 mass spectrometer. 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).

7-Bromo-4,4-dimethyl-1-hydroxy-1-(4-methylphenyl)-1,2,3,4-tetrahydronaphthalene (7). To magnesium turnings (648.0 mg, 27.0 mmol) in THF (25 mL) was added a solution of 4-bromotoluene (5.40 g, 31.8 mmol) in THF (10 mL) in two portions. The reaction was initiated by the addition of 2 mL of the solution, followed by slow addition of the remaining solution *via* an addition funnel. The mixture was stirred at room temperature for 1 h and then transferred to a second flask through a canula. To this Grignard reagent was added a solution of 3,4-dihydro-4,4-dimethyl-7-bromo-1(2*H*)-naphthalenone (**6**) (4.0 g, 15.9 mmol) in THF (15 mL). The resulting solution was heated at reflux for 12 h, cooled to room temperature, and quenched by slow addition of ice-cold 10% aqueous HCl. The reaction mixture was extracted with Et_2O, and the combined organic layers were washed with H_2O and brine and dried over $MgSO_4$. Concentration of the dry solution under reduced pressure provided an oil which after column chromatography (hexane: EtOAc, 96:4) afforded 3.4 g (62%) of the desired product as a colorless solid: ^{1}H NMR δ 7.36 (1H, dd, *J* = 2.1, 7.6 Hz), 7.26 (3H, m), 7.12 (3H, s), 2.34 (3H, s), 2.24−2.04 (2H, m), 1.81 (1H, m), 1.55 (1H, m), 1.35 (3H, s), 1.30 (3H, s).

7-Bromo-3,4-dihydro-4,4-dimethyl-1-(4-methylphenyl)-naphthalene (8). To a solution of compound **7** (3.4 g, 9.85 mmol) in benzene (40 mL) in a flask equipped with a Dean−Stark trap was added catalytic amount of *p*-toluenesulfonic acid. The resulting solution was heated at reflux for 2 h. The resulting solution was cooled to room temperature, extracted with Et_2O, dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography (hexanes) afforded 2.63 g (81%) of the desired compound as a colorless solid: ^{1}H NMR δ 7.32 (1H, dd, *J* = 2.1, 8.2 Hz), 7.21 (5H, m), 7.15 (1H, d, *J* = 2.1 Hz), 5.98 (1H, t, *J* = 4.7 Hz), 2.40 (3H, s), 2.32 (2H, d, *J* = 4.7 Hz), 1.30 (6H, s). Anal. ($C_{19}H_{19}Br$) C, H.

5,6-Dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenecarboxylic Acid (9). To a solution of **8** (250.0 mg, 0.764 mmol) in THF (2.0 mL) at −78 °C was added *tert*-butyllithium (1.0 mL, 1.68 mmol, 1.7 M solution in pentane). After 1 h of stirring at −78 °C, gaseous CO_2 was bubbled through the reaction for 1 h. The reaction mixture was warmed to room temperature and quenched by the addition of 10% aqueous HCl. Extraction with EtOAc, followed by drying (Na_2SO_4) of the combined organic layers, and concentration under reduced pressure afforded 167 mg (75%) of the desired compound as a colorless solid: ^{1}H NMR δ 7.94 (1H, dd, *J* = 1.8, 8.1 Hz), 7.76 (1H, d, *J* = 1.8 Hz), 7.45 (1H, d, *J* = 8.1 Hz), 7.24 (4H, m), 6.01 (1H, t, *J* = 4.7 Hz), 2.40 (3H, s), 2.36 (2H, d, *J* = 4.7 Hz), 1.35 (6H, s). Anal. ($C_{20}H_{20}O_2$) C, H.

Ethyl 4-[[[5,6-Dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl]carbonyl]amino]benzoate (10). A solution of **9** (170.0 mg, 0.58 mmol), ethyl 4-aminobenzoate (115.0 mg, 0.70 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (145.0 mg, 0.76 mmol) and 4-(dimethylamino)pyridine (92.4 mg, 0.76 mmol) in DMF (6.0 mL) was stirred at room temperature for12 h. The solution was diluted with EtOAc, washed with H_2O, dried ($MgSO_4$), and concentrated to give an oil. Column chromatography (hexanes: EtOAc, 85:15) afforded 187 mg (74%) of the desired product as a colorless solid: ^{1}H NMR δ 8.02 (2H, d, *J* = 8.7 Hz), 7.72 (2H, m), 7.65 (2H, d, *J* = 8.7 Hz), 7.52 (1H, d, *J* = 1.8 Hz), 7.48 (1H, d, *J* = 8.0 Hz), 7.25 (4H, m), 6.15 (1H, t, *J* = 4.9 Hz), 4.36 (2H, q, *J* = 7.1 Hz), 2.40 (3H, s), 2.38 (2H, d, *J* = 4.9 Hz), 1.39 (3H, t, *J* = 7.1 Hz), 1.37 (6H, s); ^{13}C NMR δ 166.1, 165.8, 149.8, 142.0, 138.6, 137.2, 134.7, 132.2, 130.8, 129.2, 128.4, 127.2, 126.2, 126.0, 124.4, 124.3, 119.0, 77.4, 77.2, 77.0, 76.8, 76.6, 60.9, 38.6, 34.0, 28.0, 21.2, 14.3; MS (EI) *m*/*e* 439, 275 (base peak), 232.

Ethyl 2-Fluoro-4-[[[5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl]carbonyl]amino]benzoate (11).

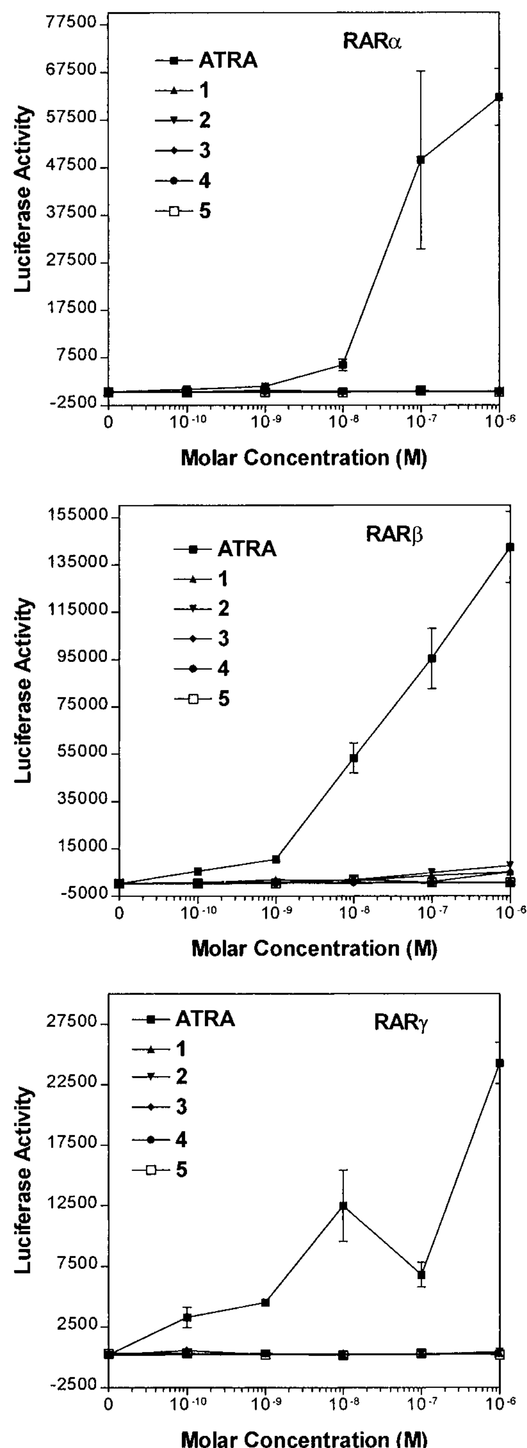


Figure 1. Dose–response curves for RA and retinoids **1**–**5** in CV-1 cells transfected with RAR holoreceptors and an MTV-4(R5G)-luciferase reporter plasmid at each RAR. ER-RARα, ER-RARβ, and ER-RARγ-mediated luciferase activity was measured for RA and compounds **1**–**5**. The vertical scale is the luciferase activity (mean ± SEM of triplicate determinations). The horizontal scale is the molar concentration of the retinoids.

Using the same procedure as for the synthesis of compound **10,** compound **11** was obtained in 50% yield as a colorless solid: ^{1}H NMR δ 7.91 (1H , t, *J* = 8.4 Hz), 7.80 (1H, bs), 7.70 (2H, m), 7.51 (1H, d, *J* = 2.0 Hz), 7.48 (1H, d, *J* = 8.1 Hz), 7.22 (m, 5H), 6.06 (1H, t, *J* = 4.6 Hz), 4.37 (2H, q, *J* = 7.1 Hz), 2.40 (3H, s), 2.38 (2H, d, *J* = 4.6 Hz), 1.39 (3H, t, *J* = 7.1 Hz), 1.37 (6H, s); ^{13}C NMR δ 165.8, 164.4, 164.0, 160.9, 150.1, 143.5, 143.3, 138.6, 137.2, 134.8, 132.8, 131.8, 129.3, 128.4, 127.3, 126.1, 124.5, 124.4, 114.5, 114.2, 114.0, 108.0, 107.6, 61.1, 38.5, 34.0, 28.0, 21.2, 14.3; MS (EI) *m/e* 457, 443, 275 (base peak).

Ethyl 2,6-Difluoro-4-[[[5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl]carbonyl]amino]-benzoate (12). Using the same procedure as for the synthesis of compound **10,** compound **12** was obtained in 19% yield as a colorless solid: ^{1}H NMR δ 7.82 (b, 1H), 7.68 (1H, dd, *J* = 2.1, 7.1 Hz), 7.50 (1H, d, *J* = 2.1 Hz), 7.47 (1H, d, *J* = 7.1 Hz), 7.28 (2H, d, *J* = 9.8 Hz), 7.23 (4H, m), 6.06 (1H, t, *J* = 4.8 Hz), 4.39 (2H, q, *J* = 4.1 Hz), 2.40 (3H, s), 2.37 (2H, d, *J* = 4.8 Hz), 1.38 (3H, t, *J* = 4.1 Hz), 1.36 (6H, s).

4-[[[5,6-Dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl]carbonyl]amino]benzoic Acid (1). To a solution of **10** (26.5 mg, 0.06 mmol) in EtOH (3.0 mL) and THF (4.0 mL) was added NaOH (240.1 mg, 6.00 mmol, 3.0 mL of 2 M aqueous solution). After 72 h of stirring at room temperature, the reaction was quenched by the addition of 10% aqueous HCl. Extraction (EtOAc), drying ($MgSO_4$), and concentration under reduced pressure provided a solid. Recrystallization from CH_3CN afforded 18 mg (73%) of the title compound as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 10.4 (1H, s), 7.91–7.81 (5H, m), 7.54 (1H, d, *J* = 8.1 Hz), 7.45 (1H, d, *J* = 1.7 Hz), 7.23 (4H, s), 6.04 (1H, t, *J* = 4.7 Hz), 2.35 (5H, s), 1.33 (6H, s); ^{13}C NMR (DMSO-d_6) δ 166.9, 166.0, 148.7, 143.2, 138.1, 136.9, 136.6, 133.5, 130.1, 129.1, 128.2, 126.7, 125.3, 125.0, 123.9, 119.2, 33.5, 27.7, 20.8; MS (EI) *m/e* 411, 275 (base peak). Anal. ($C_{27}H_{25}NO_3$) C, H, N.

2-Fluoro-4-[[[5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl]carbonyl]amino]benzoic Acid (2). Using the same procedure as for the synthesis of **1**, compound **2** was obtained as a solid in 72% yield: ^{1}H NMR (acetone-d_6) δ 9.84 (1H, b), 7.91 (1H, t, *J* = 8.6 Hz), 7.85 (2H, m), 7.64 (1H, d, *J* = 1.9 Hz), 7.52 (2H, m), 7.23 (4H, s), 6.04 (1H, t, *J* = 4.7 Hz), 2.37 (2H, d, *J* = 4.7 Hz), 2.36 (3H, s), 1.35 (6H, s); ^{13}C NMR (acetone-d_6) δ 166.9, 165.0, 164.8, 164.7, 161.6, 150.3, 146.1, 145.9, 139.7, 138.2, 137.7, 135.1, 133.7, 133.2, 130.0, 129.2, 127.6, 127.3, 126.2, 124.9, 115.7, 115.6, 114.1, 113.9, 108.4, 108.0, 39.1, 34.5, 28.2, 21.1; MS (EI) *m/e* 429, 369 (base peak), 275, 231; HRMS found 429.1734 (M), calcd for $C_{27}H_{24}FNO_3$ 429.1740 (M). Anal. ($C_{27}H_{24}FNO_3 \cdot 0.6H_2O$) C, H, N.

2,6-Difluoro-4-[[[5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl]carbonyl]amino]benzoic Acid (3). Using the same procedure as for the synthesis of **1**, compound **3** was obtained as a solid in 70% yield. ^{1}H NMR δ 7.88 (1H, bs), 7.67 (1H, dd, *J* = 2.1, 7.1 Hz), 7.50 (1H, d, *J* = 2.1 Hz), 7.47 (1H, d, *J* = 7.1 Hz), 7.30 (2H, d, *J* = 10.3 Hz), 7.21 (4H, m), 6.05 (1H, t, *J* = 4.8 Hz), 2.39 (3H, s), 2.37 (2H, d, *J* = 4.8 Hz), 1.36 (6H, s); MS (EI) *m/e* 447, 403, 275; HRMS found 447.1604 (M), calcd for $C_{27}H_{23}F_2NO_3$ 447.1646 (M). Anal. ($C_{27}H_{23}F_2NO_3 \cdot 1.6H_2O$) C, H, N.

2,2-Dimethylchroman (14). To a solution of dihydrocoumarin (**13**) (5.0 g, 33.78 mmol) in dry THF (50 mL) at 0 °C was added dropwise methylmagnesium chloride (33.7 mL, 3M solution in THF, 0.1 mol). The reaction mixture was slowly warmed up to room temperature and stirred overnight. Extraction (Et_2O), drying (Na_2SO_4), and concentration under reduced pressure afforded the diol (6.25 g) as a colorless solid. The diol was combined with 15% aqueous H_2SO_4 (50 mL) in benzene (15 mL) and the resulting mixture heated at 105 °C for 2 h. Upon being cooled to room temperature, the mixture was extracted with Et_2O, dried (Na_2SO_4), and concentrated under reduced pressure to give 6.0 g (100%) of the title compound as a light yellow liquid: ^{1}H NMR δ 7.08 (2H, m), 6.80 (2H, m), 2.79 (2H, t, *J* = 6.8 Hz), 1.81 (2H, t, *J* = 6.8 Hz), 1.35 (6H, s).

6-Acetyl-2,2-dimethylchroman (15). To a solution of **14** (3.15 g, 19.4 mmol) in dry CH_2Cl_2 (100 mL) was added acetyl chloride (1.52 mL, 21.3 mmol) followed by $AlCl_3$ (2.84 g, 21.3 mmol). The reaction mixture was stirred at room temperature for 30 min and then poured into ice water. Extraction (CH_2Cl_2), drying (Na_2SO_4), and concentration under reduced pressure afforded a yellow oil. Column chromatography (EtOAc: hexanes, 1:9) yielded 2.15 g (54%) of **13** as a yellow solid: ^{1}H NMR δ 7.73 (2H, m), 6.80 (1H, d, *J* = 8.3 Hz), 2.83 (2H, t, *J* =

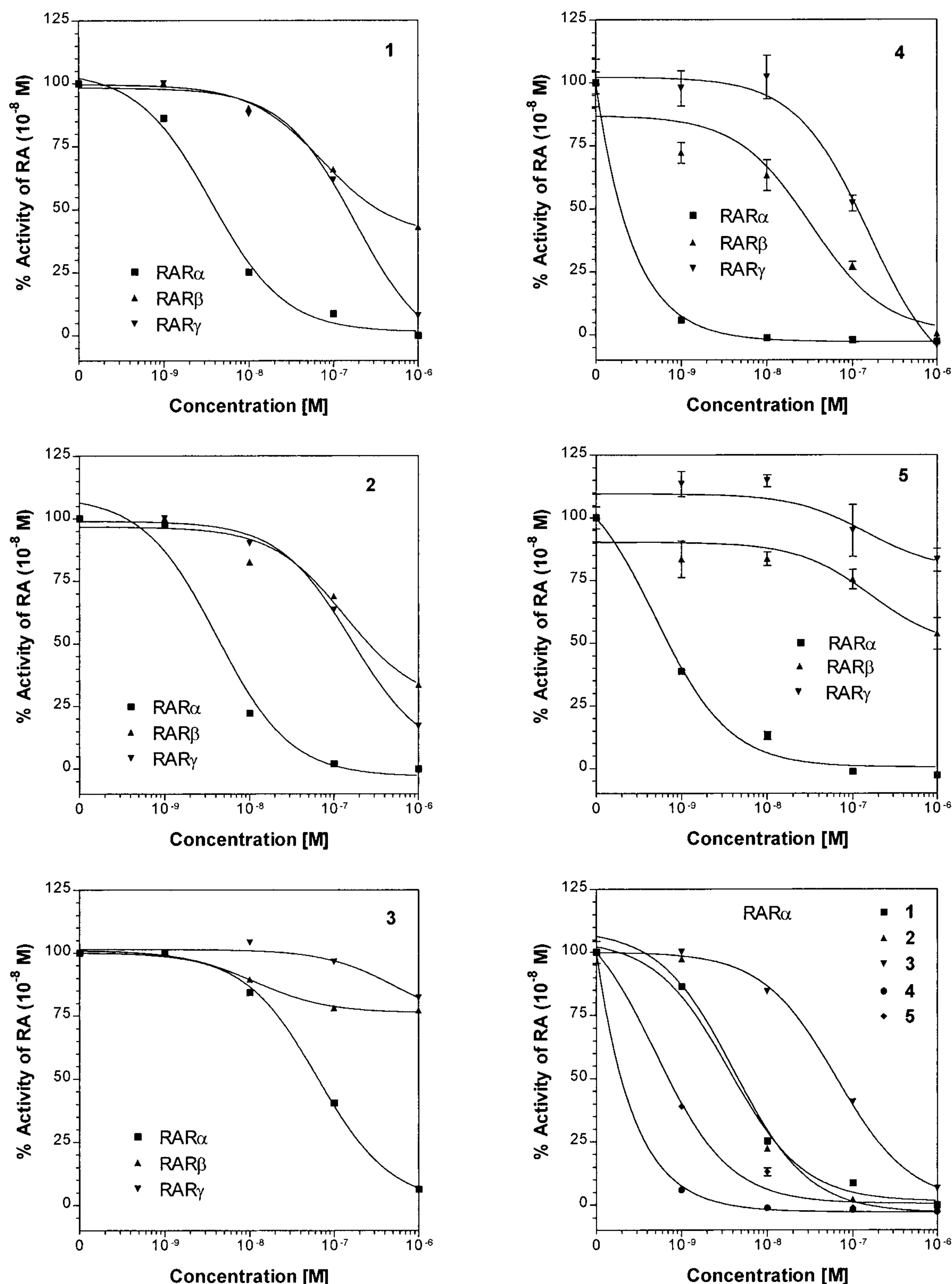


Figure 2. The antagonist effect of compounds **1**−**5** on transactivation activity induced by RA (10^{-8} M) at each RAR and a comparison of **1**−**5** at RARα. In each panel, the vertical scale is the percentage transactivation activity of RA (10^{-8} M) in the presence of the antagonist. The horizontal scale is the molar concentration of the antagonist.

6.9 Hz), 2.54 (3H, s), 1.84 (2H, t, J = 6.9 Hz), 1.36 (6H, s). Anal. ($C_{13}H_{16}O_2$) C, H.

Ethyl 2,2-Dimethylchroman-6-carboxylate (16). A solution of **15** (2.15 g, 10.5 mmol) and NaOH (2.0 g, 50 mmol) in dioxane (50 mL) and bleach (50 mL, 5.25% NaOCl) was heated at 65 °C for 4 days. Upon cooling to room temperature Na_2SO_3 was added until an aliquot of this solution remained colorless color when treated with of one drop of a solution of I_2 in CCl_4. The solution was acidified with H_2SO_4 (pH 4) and extracted with ether. The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (EtOAc:hexanes, 1:3) afforded 1.98 g (92%) of 2,2-dimethylchroman-6-carboxylic acid as a light tan solid: ^{1}H NMR δ 7.78 (2H, m), 6.82 (1H, d, J = 8.3 Hz), 2.83 (2H, t, J = 6.7, Hz), 1.85 (2H, t, J = 6.7 Hz), 1.37 (6H, s).

To a solution of this solid (1.64 g, 7.95 mmol) in EtOH (50 mL) was added H_2SO_4 (0.5 mL). The mixture was heated overnight at 95 °C, cooled to room temperature and concentrated under reduced pressure. Column chromatography (EtOAc:hexanes, 1:5) afforded 1.91 g (100%) of **16** as a colorless solid: ^{1}H NMR δ 7.79 (2H, m), 6.79 (2H, d, J = 8.3 Hz), 4.34 (2H, q, J = 7.2 Hz), 2.82 (2H, t, J = 6.7 Hz), 1.84 (2H, t, J =

6.7 Hz), 1.38 (3H, t, J = 7.2 Hz), 1.36 (6H, s). Anal. ($C_{14}H_{18}O_3{\cdot}0.25H_2O$) C, H.

Ethyl 8-Bromo-2,2-dimethylchroman-6-carboxylate (17). To a solution of **16** (500 mg, 2.14 mmol) in HOAc (4 mL) was added Br_2 (0.11 mL, 2.14 mmol). The reaction mixture was stirred at room temperature overnight, and then a stream of air was passed through the reaction mixture to remove the excess Br_2. Removal of the solvent under reduced pressure and column chromatography using the residue (EtOAc:hexanes, 1:9) afforded 741 mg (100%) of **17** as an oil: ^{1}H NMR δ 8.05 (1H, d, J = 2.2 Hz), 7.73 (1H, d, J = 2.2 Hz), 4.34 (2H, q, J = 7.1 Hz), 2.84 (2H, t, J = 6.7 Hz), 1.85 (2H, t, J = 6.7 Hz), 1.40 (6H, s), 1.38 (3H, t, J = 7.2 Hz). Anal ($C_{14}H_{17}BrO_3{\cdot}0.2H_2O$) C, H.

Ethyl 8-Bromo-2,2-dimethyl-4-oxochroman-6-carboxylate (18). To a solution of HOAc (15 mL) and Ac_2O (7.5 mL) at 0 °C was added CrO_3 (1.18 g, 12 mmol) in small portions. This solution was stirred for 15 min and diluted with benzene (10 mL), and a solution of **17** (741 mg, 2.38 mmol) in benzene (5 mL) was slowly added over several minutes. After 4 h stirring at 0 °C, the reaction mixture was poured over ice and extracted with EtOAc, and the combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to give an oil. Column chromatography (EtOAc:hexanes, 1:9) yielded 589 mg (76%) of **18** as a colorless solid: ^{1}H NMR δ 8.5 (1H, d, J = 2.1 Hz), 8.40 (1H, d, J = 2.1 Hz), 4.37 (2H, q, J =7.1 Hz), 2.80 (2H, s), 1.54 (6H, s), 1.40 (3H, t, J = 7.1 Hz). Anal. ($C_{14}H_{15}BrO_4$) C, H, N.

Ethyl 8-Bromo-2,2-dimethyl-4-(4-methylphenyl)-4(2*H*)-chroman-6-carboxylate (19). To a solution of **18** (589 mg, 1.81 mmol) in THF (20 mL) under Ar at −78 °C was added *p*-tolylmagnesium bromide (2.16 mL, 2.72 mmol, 1 M solution in ether). The reaction mixture was slowly warmed to room temperature and stirred overnight. Saturated NH_4Cl solution was added to the reaction at 0 °C and the resulting mixture extracted with ether. The combined organic layers were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. Column chromatography (EtOAc:hexanes, 1:3) afforded 297 mg of a mixture of **19** and the intermediate tertiary alcohol as an oil. This mixture was combined with *p*-TsOH (20 mg) in dry toluene (15 mL) and heated at 110 °C for 30 min. Upon being cooled to room temperature, the solvent was removed under reduced pressure and the product 182 mg (25%) isolated by column chromatography (EtOAc:hexane, 1:3): ^{1}H NMR δ 8.1 (1H, d, J = 1.7 Hz), 7.68 (1H, d, J = 1.7 Hz), 7.22 (4H, s), 5.67 (1H, s), 4.29 (2H, q, J = 7.1 Hz), 2.41(3H, s), 1.56 (6H, s), 1.33 (3H, t, J = 7.1 Hz); MS (EI) *m*/*e* 402, 400, 387, 385, 359, 357. Anal. ($C_{21}H_{21}BrO_3{\cdot}0.45H_2O$) C, H.

8-Bromo-2,2-dimethyl-4-(4-methylphenyl)-4(2*H*)-chroman-6-carboxylic Acid (20). To a solution of **19** (189 mg, 0.51 mmol) in EtOH (15 mL) and THF (10 mL) was added 20% aqueous NaOH (2 mL). The reaction mixture was warmed to 45 °C for 2 h, cooled to room temperature, and acidified to pH 4 with 10% aqueous HCl. Extraction (EtOAc), drying ($MgSO_4$), and concentration under reduced pressure afforded **18** (171 mg) (98%) as a colorless solid: ^{1}H NMR δ 8.15 (d, J = 2.0 Hz, 1H), 7.71 (d, J = 2.0 Hz, 1H), 7.21 (m, 4H), 5.69 (s, 1H), 2.42(s, 3H), 1.58 (s, 6H).

Ethyl 2-Fluoro-4-[[[8-bromo-2,2-dimethyl-4-(4-methylphenyl)-6-chromanyl]carbonyl]amino]benzoate (21). To a solution of **20** (100 mg, 0.29 mmol) in CH_2Cl_2 (8 mL) was added DMAP (87 mg, 0.69 mmol), ethyl 4-amino-2-fluorobenzoate (53 mg, 0.29 mmol), and EDC (72 mg, 0.37 mmol). The reaction mixture was stirred at room temperature overnight and then concentrated to dryness. Column chromatography (EtOAc:hexanes, 1:3) afforded 98 mg (63%) of **21** as a colorless solid: ^{1}H NMR δ 7.99 (1H, bs), 7.89 (1H, t, J = 7.5 Hz), 7.88 (1H, d, J = 2.1 Hz), 7.65 (1H, dd, J = 12.8, 2.1 Hz), 7.25 (1H, dd, J = 7.5 Hz, 2.1 Hz), 7.19 (4H, s), 5.70 (1H, s), 4.36 (2H, q, J = 7.2 Hz), 2.38 (3H, s), 1.56 (6H, s), 1.39 (3H, t, J = 7.2 Hz); MS (EI) *m*/*e* 537, 537, 524, 526, 357, 355, 312. Anal ($C_{28}H_{25}BrFNO_4$) C, H, N.

2-Fluoro-4-[[[8-bromo-2,2-dimethyl-4-(4-methylphenyl)-6-chromanyl]carbonyl]amino]benzoic Acid (4). To a solution of **21** (135 mg, 0.25 mmol) in EtOH (5 mL) was added 20% aqueous NaOH (2 mL). The reaction mixture was stirred at room temperature overnight and acidified to pH 4 with 10% aqueous HCl. The EtOH was removed under reduced pressure, and ethyl acetate and water were added to the residue. The organic layer was separated, washed with saturated $NaHCO_3$ and saturated aqueous NaCl, and dried over $MgSO_4$. Concentration under reduced pressure afforded 110 mg (86%) of **4** as a colorless solid: ^{1}H NMR δ (acetone-d_6) δ 8.09 (1H, d, J = 2.1 Hz), 7.91 (1H, t, J = 7.5 Hz), 7.68 (1H, d, J = 2.1 Hz), 7.83 (1H, dd, J = 12.8, 2.1 Hz), 7.50 (1H, dd, J = 7.5, 2.1 Hz), 7.27 (4H, s), 5.87 (1H, s), 2.37 (3H, s), 1.56 (6H, s); MS (EI) *m*/*e* 511, 509, 496, 494 , 452, 450, 357, 355, 312. Anal. ($C_{26}H_{21}BrFNO_4{\cdot}0.6H_2O$) C, H, N.

Ethyl 2,6-Difluoro-4-[[[8-bromo-2,2-dimethyl-4-(4-methylphenyl)-6-chromanyl]carbonyl]amino]benzoate (22). Using the same procedure as for the synthesis of compound **21**, compound **20** (100 mg, 0.29 mmol) afforded 53 mg (33%) of **22** as a colorless oil: ^{1}H NMR δ 7.87 (1H, d, J = 2.1 Hz), 7.47 (1H, d, J = 2.1 Hz), 7.28 (2H, d, J = 8.7 Hz), 7.19 (4H, m), 5.70 (1H, s), 4.38 (2H, q, J = 7.2 Hz), 2.41 (3H, s), 1.57 (6H, s), 1.38 (3H, t, J = 7.2 Hz); MS (EI) *m*/*e* 557, 555, 542, 540, 448, 446. Anal. ($C_{28}H_{24}BrF_2NO_4$) C, H, N.

2,6-Difluoro-4-[[[8-bromo-2,2-dimethyl-4-(4-methylphenyl)-6-chromanyl)carbonyl]amino]benzoic acid (5). Using the same procedure as for the synthesis of compound **4**, compound **22** (53 mg, 0.1 mmol) afforded 35 mg (70%) of **5** as a colorless solid: ^{1}H NMR δ (acetone-d_6) 9.93 (1H, bs), 8.07 (1H, d, J = 2.1 Hz), 7.66 (1H, d, J = 2.1 Hz), 7.54 (2H, d, J = 9.9 Hz), 7.27 (4H, s), 5.88 (1H, s), 2.38 (3H, s), 1.56 (6H, s). MS (EI) *m*/*e* 514, 512, 485, 483, 470, 468. Anal. ($C_{26}H_{20}BrF_2NO_4$) C, H, N.

Binding Assay. Each receptor subtype was expressed in baculovirus. Stock solutions of all compounds were prepared as 10 mM ethanol solutions and serial dilutions carried out into 1:1 DMSO:glycerol, 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 μL and contained 10-40 μg of extract protein along with 5 nM of [^{3}H]-all-*trans*-retinoic acid and varying concentrations of competing ligand that ranged from 0 to 10^{-5} M. The assays were run using a Biomek formatted for a 96-well minitube system. Incubations were carried out at 4 °C until equilibrium was achieved. Nonspecific binding was defined as that binding remaining in the presence of 1000 nM of unlabeled RA. At the end of the incubation period, 50 μL of 6.25% hydroxyapitite was added in a wash buffer which consisted of 100 nM KCl, 10 mM Tris, and 0.5% Triton X-100. The mixture was vortexed and incubated for 10 min at 4 °C and centrifuged and the supernatant removed. The hydroxyapitite 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, 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−Prussof equation[14c] to the IC_{50} values, the labeled ligand concentration, and the K_d of the labeled ligand.

Transfections and DNA Constructs. Transfections were carried out as previously described.[15] Briefly, 4×10^5 CV-1 cells were transiently transfected *via* calcium phosphate precipitation[16] with 0.7 μg of the reporter plasmid MTV-4(R5G)-Luc, 0.1 μg of the the β-galactosidase expression plasmid pCH110 (Pharmacia), 0.01 μg of the plasmid pRS-hRXRα,[4a] and 0.05 μg of either pRS-RARα-P-GR, pcDNA3-RARβ-P-GR, or pcDNA3-RARγ-P-GR. Eighteen hours after introduction of the DNA precipitants, the cells were rinsed with phosphate-buffered saline (PBS) and fed with D-MEM (Gibco-BRL) containing 10% activated charcoal extracted fetal bovine serum (Gemini Bio-Products). Cells were treated for 18 h with 10 nM ATRA in conjunction with the compounds indicated in the figure (antagonism studies). After being rinsed with PBS, cells were lysed and luciferase activity was measured as previously described.[17] Luciferase values represent the mean ± SEM of triplicate determinations normalized to β-galactosidase activity.

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