Synthesis and Characterization of a Highly Potent and Effective Antagonist of Retinoic Acid Receptors

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Retinoids are small molecule hormones that directly regulate gene transcription as a result of binding and activation of nuclear receptors.[1] There are two families of receptors, the retinoic acid receptors (RARs)[2] and the retinoid X receptors (RXRs),[3] each having α, β, and γ subtypes. *all-trans*-Retinoic acid (RA) (Chart 1) is the physiological ligand for the RARs and activates the RAR hormonal pathway through RAR-RXR heterodimers.[4] The putative hormone for the RXRs, 9-*cis*-retinoic acid (9-*cis*-RA),[5] activates RXR hormonal pathways through RXR–RXR homodimers.[6] In addition, 9-*cis*-RA also binds and activates the RARs. Endogenous retinoids are believed to play many fundamental physiological roles including those in development, maintenance of normal patterns of differentiation and proliferation, and immune function.[7] As a result, pharmacologically applied retinoids exhibit profound antiproliferative, anti-inflammatory, and differentiation inducing effects in a variety of disease models.[8] Because of these pharmacological effects, RA and synthetic RAR agonists are of considerable therapeutic value in the treatment of several human diseases such as psoriasis,[9] acne,[10] and cancer.[11]

Although many structural classes of RAR agonists are known,[12] antagonists of RAR function have been described only recently.[13] Compounds of the type **1** and **2** (Chart 2) are relatively low-affinity antagonists and inhibit RA-induced activity only when used in 100–1000-fold excess. Compound **3** was recently shown to be a potent antagonist of RA-induced differentiation in human promyelocytic leukemia (HL-60) cells and also to compete with RA binding to HL-60 nuclear extracts.[13d] In this communication we describe the synthesis and biological activity of AGN 193109 (**4**), *a very high affinity antagonist of RA-induced function at all three RAR subtypes*. Compound **4** will be a powerful tool in defining the precise functions of the RAR hormonal pathways in development and in the adult animal and also in elucidating the molecular mechanisms of RAR-mediated transcriptional regulation. In addition, **4** is an invaluable lead compound for determining potential pharmacological applications for RAR antagonists in disease models. Parenthetically, it should be noted that antagonists of the other members of the steroid–retinoid nuclear receptor superfamily, such as tamoxifen[14] and RU 486,[15] have found wide applications in clinical medicine.

Compound **4** is based upon the diarylacetylene class of retinoids[16] and is readily available from tetralone **5**[17] (Scheme 1). Bromination of **5** in the presence of $AlCl_3$ provided **6** in 80% yield.[18] Diarylacetylene **7** was prepared using sequential palladium-catalyzed coupling reactions. Thus, reaction of **6** with an excess of TMS-acetylene in the presence of catalytic Pd(II) and Cu(I) afforded the desired acetylene substitution product. The coresponding terminal acetylene, obtained by removal of the TMS group using K_2CO_3 in MeOH at room temperature, was subjected to a second palladium-catalyzed coupling reaction with ethyl 4-iodobenzoate to give keto ester **7** in 48% yield from tetralone **6**. Conversion of **7** to vinyl triflate **8**[19] followed by Pd(0)-catalyzed coupling of this product with the organozinc reagent prepared from 4-bromotoluene provided ester **9** in 72% yield.[20] Ester hydrolysis, using aqueous LiOH in THF at room temperature, afforded **4** as a colorless solid.

The biological activity of **4** was evaluated in RAR binding and transactivation assays. Binding constants (K_d values) were determined for these compounds using baculovirus-expressed retinoic acid receptors (RARα, -β, and -γ) and retinoid-X receptors (RXRα, -β and -γ) as described.[5a,21] Compound **4** binds with very high affinity (K_d = 2–3 nM) to all three RAR subtypes (Table 1) and has approximately 4–6-fold higher affinity for the

Chart 1

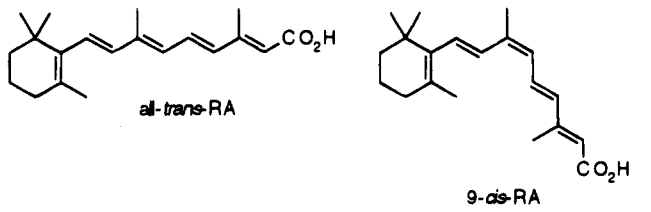

Chart 2

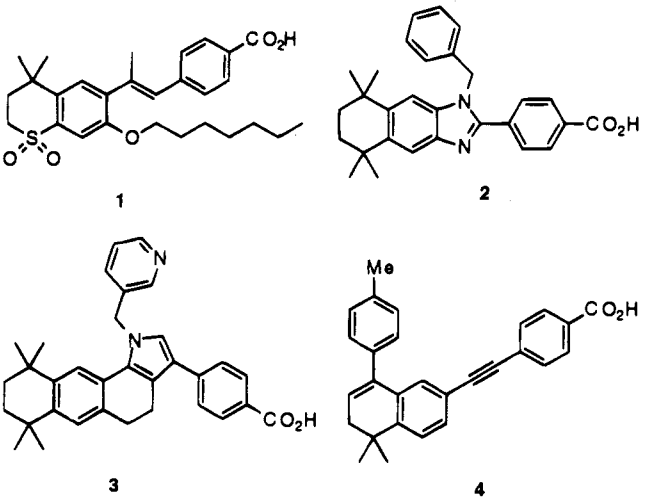

Table 1. EC_{50} and K_d Values (nM) for RA and Antagonist **4**

entry		RAR α	RAR β	RAR γ
RA	EC_{50}[a]	7	1	0.7
	K_d[b]	9	12	19
4	EC_{50}[a]	NA[c]	NA[c]	NA[c]
	K_d[b]	2	2	3

[a] Transactivation assays were performed in CV-1 cells cotransfected with the luciferase reporter plasmid ΔMTV-TREp-LUC and an expression vector of the indicated retinoid receptor. [b] K_d values 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.[21b] [c] NA = not active.

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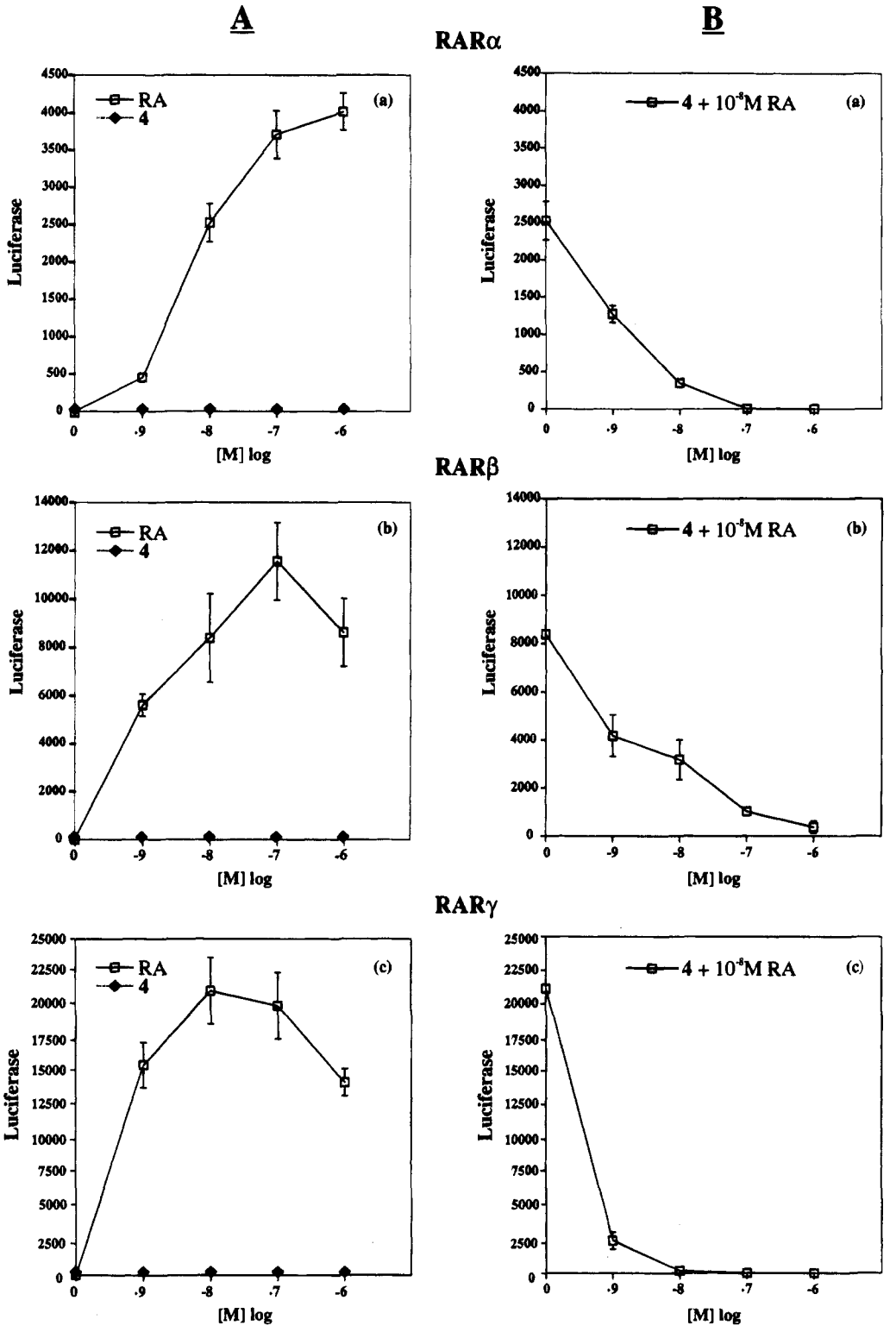
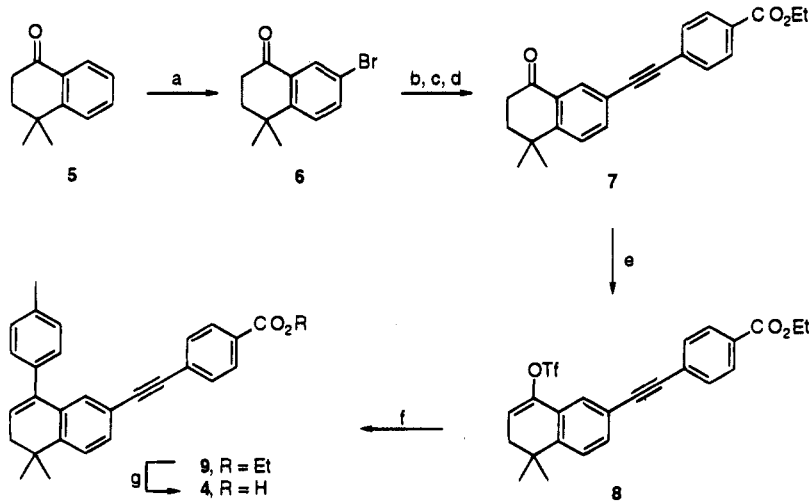


Figure 1. (A) Dose-response curves for RA and **4** in CV-1 cells transfected with RAR holoreceptors and a TRE_{pal}-luciferase reporter gene at (a) RARα, (b) RARβ, and (c) RARγ. (B) Effect of **4** on transactivation activity induced by RA (10^{-8} M) at (a) RARα, (b) RARβ, and (c) RARγ. In each figure the vertical scale is in relative light units, and the horizontal scale is the log molar concentration of the retinoid.

receptors relative to the natural hormone, RA. The functional activity of **4** was evaluated in transactivation assays using CV-1 cells transfected with RAR holoreceptors and a TRE_{pal}-luciferase reporter gene.[22] In this assay, RA activates transcription effectively through each of the RAR subtypes (Figure 1A). Very interestingly, however, **4** has absolutely no activity in these transactivation assays for all three RARs even at receptor-saturating concentrations (Figure 1A). Also, compound **4** is completely RAR specific, since it does not bind to or transactivate through any of the RXRs (data not shown).

Given that the binding assays are carried out in cell-free systems and as such measure the affinity of ligand to monomeric RAR, it is possible that the lack of transcriptional activity observed for **4** is due to its inability to bind RAR–RXR heterodimers. In order to determine whether **4** could actually bind to RAR–RXR

Scheme 1[a]



[a] (a) $AlCl_3/Br_2/CH_2Cl_2$/70 °C (80%); (b) TMS-acetylene/$(PPh_3)_2PdCl_2$/CuI/Et_2NH/60 °C; (c) K_2CO_3/MeOH (76% for b and c); (d) 4-$IC_6H_4CO_2Et$/$(PPh_3)_2PdCl_2$/CuI/Et_2NH/room temperature (64%); (e) $NaN(SiMe_3)_2$/THF/−78 °C/*N*-(5-chloro-2-pyridyl)triflimide/0 °C to room temperature (77%); (f) 4-BrC_6H_4Me/*t*-BuLi/THF/−78 °C/$ZnCl_2$, to room temperature/$Pd(PPh_3)_4$/5 °C (72%); (g) LiOH/THF/H_2O (97%).

heterodimers and antagonize RAR agonist function, we examined the ability of **4** to block RA-induced gene transcription in the CV-1 assay. At a fixed concentration of RA which induced significant transcriptional activity (Figure 1A), coadministration of **4** resulted in a decrease of activity in a dose dependent manner (Figure 1B). *At equimolar concentrations, 4 inhibited RA activity by approximately 85%, 62%, and 100% at RARα*, RARβ, and RARγ, respectively. Further, a 10-fold excess of **4** completely abrogated the transactivation activity of RA at all three RARs. We have observed identical activity for **4** in a variety of other RAR-based assays (data not shown), such as dose dependent inhibition of RA activity in a chimaeric estrogen receptor–RAR transactivation assay and suppresion of transglutaminase induction by RAR agonists in 3T3 cells stably transfected with RARβ and RARγ. These data clearly demonstrate that compound **4** is a potent and effective antagonist of RA function at the RARs.

In summary, **4** is representative of a novel class of RAR antagonists which effectively block hormone-induced activation and is the most potent and effective antagonist of all three RAR subtypes known to date. Upon binding the RARs, **4** must induce different conformational changes in the receptors relative to those resulting from binding of an agonist. Thus, **4** should be a very useful tool in the study of the molecular mechanisms of trascriptional activation, such as the role of coactivator proteins in transducing signal from the activated receptor to the transcriptional machinery. Compound **4** will also be invaluable in elucidating the physiological role of RA both as a morphogen during development and as a hormone in the adult. Finally, RAR antagonists such as **4** will almost certainly find therapeutic applications, the most obvious of which is the prevention and treatment of the side effects associated with systemic retinoid therapy, such as mucocutaneous toxicity.[23]

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