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STRUCTURAL BASIS FOR THE DIFFERENTIAL RXR & RAR ACTIVITY OF STILBENE RETINOID ANALOGS

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Abstract. Retinoids elicit their biological actions by activating nuclear receptors that regulate gene transcription. There are six known retinoid receptors which belong to the retinoic acid receptor (RAR) and retinoid X receptor (RXR) families. We report RXR-active stilbene retinoid analogs and discuss the structural features that impart RAR and RXR activation properties to compounds of this class.

Retinoids are natural and synthetic analogs of vitamin A (retinol). Naturally occurring retinoids are implicated in a wide variety of biological effects including embryogenesis, cell growth and differentiation, epithelial homeostasis, and immunocompetence.[1] Clinically, retinoids are used for the treatment of several skin diseases including acne, psoriasis, and photoaging.[2] However, the currently used retinoids are of limited use as drugs for other disorders because they possess a number of detrimental side effects such as bone and lipid toxicity[3] and teratogenicity.[4] Synthetic retinoids displaying fewer side effects may be useful in a variety of other areas, including oncology,[1a,5] ophthalmology,[6] immunology[1a,7] and cardiovascular disease.[8]

Retinoids induce cellular responses by binding to and activating a number of nuclear receptors that regulate target gene transcription by binding to enhancer regions known as retinoic acid response elements (RAREs).[9] There are six known retinoid receptors: three retinoic acid receptors (RARα, -β, and -γ)[10] and three retinoid X receptors (RXRα, -β, and -γ).[11] The physiological hormones for the RARs and RXRs are proposed to be all-*trans*-Retinoic Acid (RA)[9] and 9-*cis*-Retinoic Acid (9-*cis* RA),[12] respectively. However, 9-*cis* RA can bind to and transcriptionally activate the RARs as well. In order for RARs to bind to RAREs and induce gene transcription effectively, they must form heterodimers with RXRs.[13] However, in the presence of 9-*cis* RA or RXR-specific ligands, RXR_α can form homodimers that bind and activate specific genes.[14] Since the retinoid receptors have distinct tissue distribution patterns (e.g., RARγ is the predominant RAR in skin),[15] and because the target gene specificity's of the receptors are different,[16] it is clear that independent response pathways can be elicited by retinoid analogs of differing receptor specificity. It would be expected that receptor specific retinoids (RSRs) would elicit more restricted responses than their non-specific counterparts. Thus, an RSR could have efficacy in a particular disease accompanied by only limited toxic side effects and hence be of much greater therapeutic value than non-specific retinoids.

As part of our ongoing retinoid program, we had observed that retinoids induce tissue transglutaminase (Tgase) activity by different mechanisms in mouse macrophages and HL-60 cdm-1 cells.[17] In particular, we discovered that potent RAR agonists, such as (*E*)-4-[2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthaleneyl)propen-1-yl]benzoic acid (TTNPB, **1**),[18] were very effective in inducing Tgase activity in

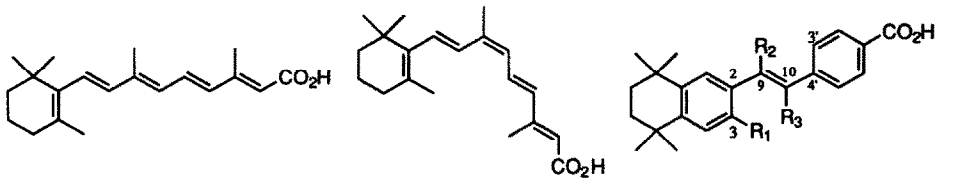
macrophages but were completely inactive in HL-60 cells. On the other hand, RA and 3-methyl-TTNPB (**2**, a weak RAR agonist) were both effective inducers of Tgase in HL-60 cells. These facts and other evidence led us to hypothesize the existence of a non-RAR mediated mechanism of retinoid action in HL-60 cells. We have subsequently shown that induction of Tgase activity in these cells is a RXR_α mediated response.[16]

In keeping with our previous observations, we report here that 3-Me TTNPB is an effective activator of RXR_α while TTNPB is essentially inactive at this receptor.[19] In this report, we discuss the structure activity relationships associated with RAR or RXR activity of stilbene analogs such as TTNPB and 3-Me TTNPB and use energy minimized molecular models to rationalize the observed relationships. These findings provide insight that will facilitate the development of ligands that are specific for RAR and RXR driven gene transcription.

We determined the transactivation properties of retinoid analogs by measuring their ability to induce transcription in cells transiently cotransfected with a receptor gene construct and a reporter gene. Since retinoid receptors are members of the steroid receptor family of nuclear receptors that are characterized by homologous functional domains, we used hybrid receptors that contain the amino terminus and DNA-binding domain of the estrogen receptor (ER) and the hormone-binding domain of the retinoid receptors. These ER-RAR (or ER-RXR) chimaeric receptors bind to and activate transcription from promoter sequences recognized by the ER (estrogen response element-ERE), but do so in response to a retinoid ligand.[20] With these constructs, we could use an ER-responsive reporter gene that cannot be activated by endogenous retinoid receptors, which are present in most all mammalian cells. Previous studies have shown that the activation characteristics of hybrid receptors are determined by their ligand binding domain.[21] Thus, this is a useful system for comparing retinoid activities at each receptor subtype. We determined the transactivational potencies of analogs (see Table 1) at each of the RAR subtypes (α, β and γ) and at RXR_α.

In considering TTNPB and 3-Me TTNPB, it was very interesting to us that a structural modification as minimal as replacing the C-3 hydrogen with a methyl group, resulted in such a remarkable reversal in receptor activity. These results may be explained in one of two ways: (a) the benzylic methyl group in 3-Me TTNPB undergoes oxidative metabolic transformation to produce an entirely different compound that is the authentic RXR selective agent or, (b) the more sterically demanding methyl group causes a conformational change in the molecule that allows it to interact more favorably with the RXR and less favorably with the RARs. In order to delineate which of these two processes is operative, we prepared **3** and **4**,[17] which are expected to be metabolically inert but would be conformationally similar to 3-Me TTNPB. The receptor data for these analogs (Table 1, entries v and vi) shows that they have receptor activation profiles very similar to 3-Me TTNPB. Thus, we conclude that the 'α-methyl effect' is primarily steric in nature. In order to increase our understanding of the substituent requirements for these analogs to exhibit RXR activity, we evaluated several other known analogs of 3-Me TTNPB.[17] The receptor data is summarized in Table 1. Entry vii illustrates that the analogs require substituents at both C3 and C9 for RXR inducing activity. However, neither substituent is required for RAR activity (entry viii). Methyl, chloro, and bromo substituents at C3 are ideal for RXR agonist activity and smaller (TTNPB) or larger (entry x) groups diminish activity. The (*E*)-olefin geometry is necessary for activity at either receptor (entry xi).

Table 1. Transcriptional activation assay data for analogs of 3-Me TTNPB.



All-*trans*-retinoic acid (RA) 9-*cis*-retinoic acid (9-cis-RA) substituted stilbenes

		stilbene substitution			EC50 (nM)			
entry	number	R_1	R_2	R_3	RAR_α	RAR_β	RAR_γ	RXR_α
i	RA				5.0	1.5	0.5	NA
ii	9-cis-RA				102	3.3	6.0	13.0
iii	**1**	H	Me	H	21.0	4.0	2.4	NA
iv	**2-(E)**	Me	Me	H	4580	74.0	152	385
v	**3**	Cl	Me	H	> 1000	21.0	77.0	275
vi	**4**	Br	Me	H	989	21.0	91.0	298
vii	**5**	Me	H	H	15	0.4	1.4	NA
viii	**6**	H	H	H	11.0	0.4	0.4	NA
ix	**7**	H	H	Me	24.0	0.5	0.4	NA
x	**8**	Et	Me	H	NA	961	195	2220
xi	**2-(Z)**	Me	Me	H	NA	NA	NA	> 6400

NA indicates Not Active (i.e. $EC_{50} > 10^4$ nmol)

With regards to conformational differences between TTNPB and 3-Me TTNPB, an unfavorable steric interaction between the C-3 methyl substituent and the C-10 hydrogen is present in 3-Me TTNPB and not TTNPB. Thus, the differences in receptor selectivity between TTNPB and 3-Me TTNPB may be attributed to differences in dihedral angles θ_1 and θ_2 about the C2-C9 and C10-C4' single bonds, respectively[22]. In order to examine this, we used a computer-assisted molecular modeling program[23] to examine conformational differences in the energy minimized structures of TTNPB and 3-Me TTNPB. The tortional angles for TTNPB are $\theta_1 = -38.7°$ and $\theta_2 = 48.7°$, and for 3-Me TTNPB are $\theta_1 = 71.7°$ and $\theta_2 = 52.6°$. These calculations confirm that relative to TTNPB, the C3 methyl substituent in 3-Me TTNPB causes a pronounced twist of θ_1, but had only a minor effect on θ_2. It is our hypothesis that this difference in the dihedral angle about the C2-C9 bond is responsible for the observed effect on receptor selectivity.

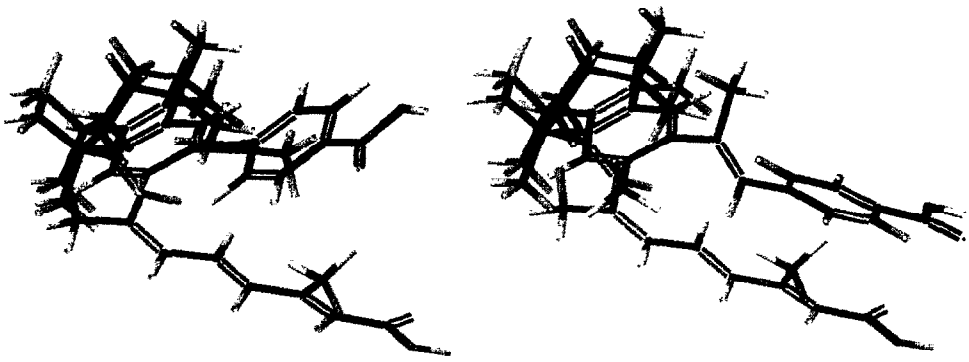


Figure 1. Energy minimized structures[20] of 9-*cis* RA and TTNPB (left), and 9-*cis* RA and 3-Me TTNPB (right). The cyclohexenyl ring of 9-*cis* RA (shaded) has been superimposed on the saturated ring of the tetrahydronaphthalene moiety of TTNPB and 3-Me TTNPB. The interatomic distance between the carbonyl groups of 9-*cis* RA and TTNPB is 7.1 Å. For 9-*cis* RA and 3-Me TTNPB it is 4.5 Å.

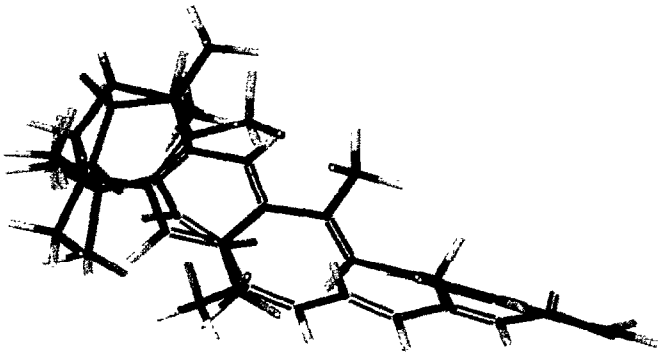


Figure 2. Overlapped energy minimized structures[20] of 9-*cis* RA (shaded) and 3-Me TTNPB.

In considering the structural characteristics that may account for the observed RXR activity of these compounds we have compared energy-minimized structures of 3-Me TTNPB and TTNPB to those of 9-*cis* RA.In Figure 1, we have overlapped the cyclohexenyl rings of the energy minimized structures of 9-*cis* RA and TTNPB, and of 9-*cis* RA and 3-Me TTNPB, to compare the spatial orientation of the polar carboxy termini of these compounds. Although the overlap is not exceptional in these restricted structures, it is illustrative that the carboxyl moiety of 3-Me TTNPB is about 2.6 Å closer than that of TTNPB to the polar carboxy terminus of 9-

cis RA. In addition, the plane of the C13-C14 allylic group of 9-*cis* RA lies in approximately the same plane as the benzoate ring of 3-Me TTNPB while the plane of the benzoate ring of TTNPB is nearly orthogonal. Indeed, excellent overlap between 3-Me TTNPB and 9-*cis* RA results if the cyclohexenyl ring of 9-*cis* RA is not strictly superimposed (Figure 2). Perhaps what is most important is that the C3 methyl group of 3-Me TTNPB overlaps well with the C9 olefinic carbon of 9-*cis* RA; without this lipophilic substituent, the pocket of the receptor that houses the allylic 9-*cis* double bond is vacant. We must note however that these are not the only low energy conformations available to TTNPB, and that there is an energy difference of only about one kilocalorie per mole between this conformation of TTNPB and one in which the dihedral angles θ_1 and θ_2 are the same as they are for 3-Me TTNPB. In addition, there are certainly other factors that could affect receptor binding and activity that are unaccounted for by these molecular models. Nevertheless, we feel the above arguments provide insight into the structural characteristics required in this series of compounds to induce RXR activity.

In summary, we have shown that simple modifications (substitution at C-3) of the stilbene skeleton of TTNPB can lead to retinoid analogs of significant potency at the RXR receptor. We ascribe this RXR selective activity primarily to a conformational effect resulting from the steric interaction between the C-3 substituent and the C-10 hydrogen. These insights into the structural requirements for RAR and RXR activity of stilbene analogs should facilitate the development of new classes of RXR selective analogs. Such RXR specific analogs will be very useful pharmacological tools in elucidating the biology associated with the RXR family of receptors.

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23 Conformational analyses and structures were generated by a Cache MOPAC application by Tektronix, Irvine, CA.

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