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DIFFERENTIAL RXR & RAR ACTIVITY OF STILBENE RETINOID ANALOGS BEARING THIAZOLE AND IMIDAZOLE CARBOXYLIC ACIDS

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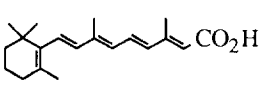
Abstract. Retinoids are hormones which activate two distinct families of nuclear receptors: the retinoic acid receptors ($RAR_{\alpha,\beta,\gamma}$) and the retinoid X receptors ($RXR_{\alpha,\beta,\gamma}$). We report thiazole and imidazole substituted stilbene analogs that have RXR specific or pan-agonist activity and discuss the structural features that impart these differential activation properties to compounds of this class.

Several biological responses are controlled by retinoids, which are natural and synthetic derivatives of vitamin A.[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 cause a number of detrimental side effects such as bone and lipid toxicity[3] and teratogenicity[4] at the doses required for efficacy. Synthetic retinoids displaying increased efficacy and fewer side effects may be useful in a variety of other areas, including oncology,[5] ophthalmology,[6] immunology[7] and cardiovascular disease.[8]

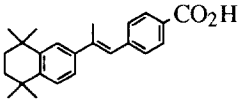
The naturally occurring retinoids are hormones that act through a series of nuclear receptors that belong to the steroid/thyroid hormone receptor superfamily. The six known retinoid receptors are divided into two subfamilies: the retinoic acid receptors (RARα, β, and γ) and the retinoid X receptors (RXRα, β, and γ).[9] The physiological hormone for the RARs is all-*trans*-retinoic acid (RA), which can only bind to the RARs.[10] In contrast, 9-*cis*-retinoic acid (9-*cis* RA), the putative hormone for the RXRs, binds to and transactivates both RXRs and RARs.[11] These nuclear receptor proteins bind to specific DNA sequences known as RA response elements (RAREs) as dimers and regulate gene transcription upon activation by ligand binding. Under physiological conditions, RARs form heterodimers with RXRs to effectively bind RAREs and induce gene transcription.[12] In the presence of RXR ligands, RXRs form homodimers which bind to distinct RAREs and induce gene transcription.[13] RXRs also form functional heterodimers with other nuclear receptors, including the vitamin D_3 and thyroid hormone receptors.[9, 12] We have previously reported the design and synthesis of stilbene retinoid analogs with potent RXR-selective and pan-agonist (activate both RXRs and RARs) activities, and used these compounds to demonstrate differences in the biological activities associated with the RAR and RXR receptor pathways, suggesting that RAR agonists and RXR agonists may have different therapeutic applications. Moreover, RXR agonists are significantly reduced in teratogenic potency relative to RAR agonists and may therefore have significant advantages in clinical practice. Recent findings also suggest that retinoids with pan-agonist profiles may have better therapeutic efficacy in certain indications than RAR specific ligands.[14] Thus, it is important to develop analogs with selectivity for the RARs and RXRs as well as those with mixed activity in order to determine the full therapeutic potential of retinoids.

Stilbene retinoid analogs of RA, such as (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-1-yl]benzoic acid (TTNPB, **1**), are potent RAR-specific agonists. Recently, we[15] and others[16] reported that simple modifications of the stilbene skeleton of TTNPB can lead to retinoid analogs of significant potency at the RXR receptor. Thus, 3-methyl-TTNPB (**2**) is an effective activator of RXR_{α} while

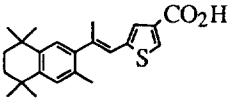
TTNPB is essentially inactive at this receptor. In addition, we have found that substitution of the benzoate moiety of 3-methyl-TTNPB with heteroaromatic carboxylic acids results in analogs with greater RXR selectivity as well as those with pan-agonist activities.[17] We used conformational analyses[15b] to show that the activity and selectivity of these analogs is dependent on the relative orientations of the polar caboxylic acid group and the lipophilic naphthalene ring. Thus, the 2,4-substituted thiophene **3** specifically activates only the RXRs whereas the 2,5-substituted thiophene **4** is a pan-agonist. Continuing with this series, we now report that RXR potency and selectivity may be further increased by substitution of the thiophene moiety of **4** with thiazole and imidazole carboxylates, examine structural and electronic factors that are associated with RXR activity and show that, with appropriate substitutions, it is possible to obtain RXR active compounds with different degrees of RAR activity.



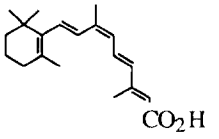
RA



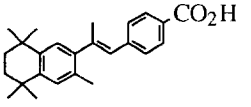
TTNPB (1)



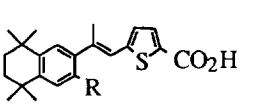
3



9-*cis*-RA



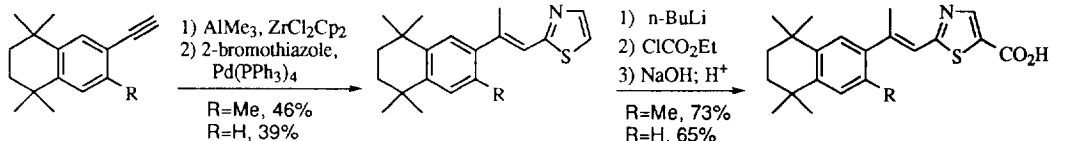
3-Methyl-TTNPB (2)



4, R = Me
5, R = H

We determined the transactivation properties of retinoid analogs by measuring their ability to induce transcription in CV-1 cells transiently cotransfected with a receptor gene construct and a reporter gene.[18] The receptor expression vectors pRS-hRAR$_\alpha$, pRS-hRAR$_\beta$, pRS-hRAR$_\gamma$, pRS-hRXR$_\alpha$, pRS-hRXR$_\beta$ and pRS-hRXR$_\gamma$ used in the cotransfection assay have been described previously.[10b,19] These expression plasmids contain RAR and RXR cDNAs under the control of the Rous Sarcoma Virus long terminal repeat. The reporter plasmid ΔMTV-TREp-LUC,[20] containing a firefly luciferase reporter gene[21] under the control of a basal mouse mammary tumor virus promoter containing two copies of the TRE-palindromic response element, was used for transfections with RAR expression plasmids. The TREpal element has been used extensively [20,22] in RAR transactivation analyses. For RXR$_\alpha$ and RXR$_\gamma$ transfections the reporter plasmid TK-CRBPII-LUC, containing two copies of the RXR-responsive element from the cytosol retinol binding protein II gene,[23] was used. The reporter plasmid TK-CPRE3-LUC, containing the chicken ovalbumin promoter COUP-TF response element,[24] was used for RXR$_\beta$ transfections.

Scheme 1. Synthesis of thiazole substituted stilbenes.



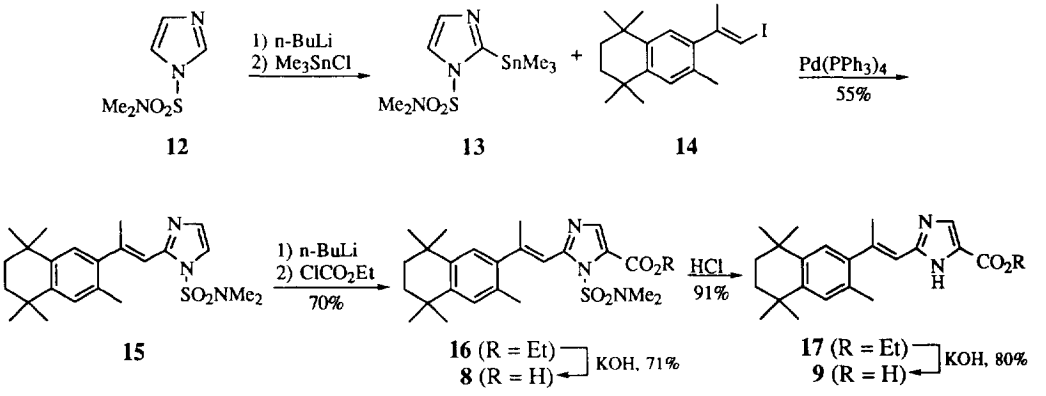
10a, R = Me
10b, R = H

11a, R = Me
11b, R = H

6, R = Me
7, R = H

The analogs used in this study were obtained as follows.[25] RA was purchased from Sigma Chemical Co. 9-*cis* RA, TTNPB (**1**), 3-Me TTNPB (**2**) and thiophene analogs **3**, **4** and **5** were prepared as described in the literature.[26] Thiazole analogs **6** and **7** were prepared according to the procedure depicted in Scheme 1. Thus, in an one-pot reaction, acetylenes **10a** and **10b**[17] were carboaluminated with trimethylaluminum and coupled to 2-bromothiazole to give isomerically pure stilbenes **11a** and **11b**, respectively. The thiazoles **11a** and **11b** were carboethoxylated at the 5' position (n-BuLi, THF, -78 °C; $ClCO_2Et$) and the corresponding esters saponified to afford carboxylic acids **6** and **7**, respectively. Imidazole analogs **8** and **9** were prepared by a related procedure as shown in Scheme 2. The 2-trimethylstanyl substituted imidazole, **13**, was prepared *in-situ* from imidazole **12**[27] and coupled to vinyl iodide **14**, obtained in 49% yield by carboalumination (Me_3Al, $ZrCl_2Cp_2$) and iodination (I_2) of **10a**, to give stilbene **15**. Esterification of **15** gave stilbene **16**, which was saponified to produce the N-sulfamoyl carboxylic acid, **8**. Alternatively, the N-sulfamoyl group of **16** was removed under acidic conditions and the resulting stilbene ester, **17**, hydrolyzed to give carboxylic acid **9**. The olefin geometry of these analogs was assigned on the basis of NMR experiments as described previously.[17]

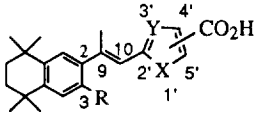
Scheme 2. Synthesis of imidazole substituted stilbenes.



The transactivation data are summarized in Table 1 and illustrate several interesting trends in receptor selectivity. First, consistent with what was described for TTNPB (**1**) and 3-methyl-TTNPB (**2**),[15, 16] removal of the 3-methyl group in the thiophene and thiazole analogs results in dramatically reduced potency at the RXRs and moderately increased potency at the RARs. Thus, compounds **5** and **7** are 40- to 100-fold less potent at the RXRs than their corresponding 3-methyl-substituted analogs, **4** and **6**, respectively. This confirms our previous suggestion that the torsional angle about the C2-C9 bond is an important determinant of RXR selectivity.[15b] Thus, in the 3-methyl-substituted analogs an unfavorable steric interaction between the C3-methyl and the C10-hydrogen causes a pronounced twist about the C2-C9 bond (torsional angle of ~ 70°) which results in the observed selectivity for the RXRs. We had previously reported that a CH to N substitution in the right-hand, six-membered aromatic ring was without effect on receptor selectivity, as was illustrated by the essentially identical receptor activity profiles of 3-methyl-TTNPB (**2**) and its nicotinic acid analog.[17] In contrast, a similar N substitution in the five-membered aromatic ring analogs results in a nearly 10-fold increase in potency at the RXRs and concurrent small decrease in potency at the RARs. Thus, the thiazole analog **6** is a significantly more potent and selective RXR agonist than the thiophene analog **4**. A similar trend is observed in the

desmethyl series as well in that the thiazole analog **7** is an effective transactivator at all of the receptors, while the thiophene analog **5** is a more selective RAR agonist. These changes in receptor selectivity could be ascribed to changes in the dihedral angle about the C10-C2' bond upon nitrogen substitution (which lead to different orientations of the carboxyl group).[17] The presence of a small sp^2 hybridized N in the 5-membered ring minimizes the non-bonded steric interactions between the C9 methyl and effectively locks the ring in the same plane as the double bond and this apparently leads to increased RXR activity. Introduction of a second N into the 5-membered ring, as in the imidazoles **8** and **9**, result in analogs that are specific for RXRs (albeit of reduced potency) with no activity at the RARs. Interestingly, the imidazole **8** which is substituted with the bulky sulfamoyl group is as active as the unsubstituted imidazole **9**. Given that the conformation adopted by **9** would be very similar to that of the thiazole **6**, it appears that the additional polarity of the imidazole is detrimental towards both RAR and RXR activity suggesting that electronic factors can also influence receptor activity.

Table 1. Transcriptional activation assay data for stilbene retinoid analogs.[18]



substituted stilbenes

		stilbene substitution				EC50 (nM)					
entry	number	R	X	Y	CO_2H	RAR_α	RAR_β	RAR_γ	RXR_α	RXR_β	RXR_γ
i	RA					350	80	10	900	1400	1100
ii	9-*cis*-RA					191	50	45	100	200	140
iii	TTNPB					30	3	2	NA	NA	NA
iv	3-Me-TTNPB					340	230	180	1200	1175	1500
v	**3**	Me	S	CH	4'	NA	1990	979	215	180	105
vi	**4**	Me	S	CH	5'	NA	56	131	35	23	33
vii	**5**	H	S	CH	5'	690	29	15	2400	2300	2300
viii	**6**	Me	S	N	5'	NA	220	200	4	3	4
ix	**7**	H	S	N	5'	210	46	34	210	210	160
x	**8**	Me	NR*	N	5'	NA	NA	NA	59	180	86
xi	**9**	Me	NH	N	5'	NA	NA	NA	89	130	170

NA indicates Not Active (i.e., $EC_{50} > 10^4$ nmol)
* R = SO_2NMe_2

In summary, we demonstrate that different structural features of the stilbene retinoids, namely the substituent at the 3-position of the tetrahydronaphthalene ring and the nature of the five-membered aromatic ring can be effectively manipulated to obtain analogs with different levels of RXR and RAR activity. The thiazole analogs described here are of particular interest. The analog **6** is a highly potent RXR agonist which is 25-65 fold more active at the RXRs than the physiological hormone, 9-*cis*-RA. The desmethyl analog **7** is an effective pan-agonist with a receptor activation profile very similar to that of 9-*cis*-RA. The analogs **7** and **5** are also of interest since they are the first examples of stilbene retinoids without a bulky substituent at the 3-position that have significant RXR activity, demonstrating that the right-hand ring alone can be manipulated to achieve RXR activity. These types of structural modifications can potentially be extended to other series, such as the benzophenone derivatives,[28] to obtain new classes of RXR selective analogs as well as pan-agonists with different levels of RAR and RXR activity. These results demonstrate that it should be possible to design analogs that have the optimal mix of receptor activities required for a particular disease application.

Acknowledgment. We are grateful to Dr. Glenn Croston at Ligand Pharmaceuticals, La Jolla, CA for providing the receptor transactivation assay data reported in Table 1.

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