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Phenylcyclohexene and Phenylcyclohexadiene Substituted Compounds Having Retinoid Antagonist Activity

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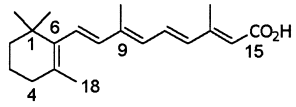
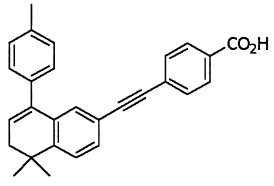
Abstract—Retinoids are natural and synthetic analogues of the hormone retinoic acid. Systemic retinoid agonist therapy is usually associated with toxic side effects, such as mucocutaneous toxicity, which may be alleviated by the use of topical retinoid antagonists. We report the synthesis and biological activity of a new series of potent, RAR-specific antagonists substituted with phenylcyclohexene and phenylcyclohexadiene groups.

Retinoids are natural and synthetic analogues of the hormone retinoic acid.[1] Retinoids are currently being investigated clinically as drugs in several areas, including dermatology and oncology. The therapeutic use of retinoid agonists as drugs is limited because they elicit a number of toxic side effects, such as teratogenicity, headache, and mucocutaneous toxicity.[2] Retinoid antagonists may be useful to counteract some of the adverse effects associated with retinoid therapy. For example, retinoid antagonists may be used as a topical treatment for the mucocutaneous toxicity caused by 13-*cis*-retinoic acid (Accutane™), a systemic retinoid drug used to treat nodulocystic acne.

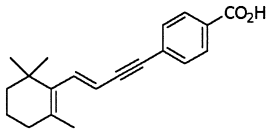
Retinoids belong to the steroid-thyroid-retinoid superfamily of hormones that induce biological responses by binding to and activating nuclear receptors. Retinoids are natural and synthetic ligands that bind to the six known retinoid receptors, the retinoic acid receptors-RARα, β, and γ—and the retinoid X receptors—RXRα, β, and γ. The natural physiological ligand for the RARs is all-*trans*-retinoic acid (ATRA), which binds with high affinity to each of the RAR subtypes but does not bind to the RXRs.[3] The putative hormone for the RXRs is 9-*cis*-retinoic acid, which binds to both RXRs and RARs with high affinity.[4] In biological systems, RARs form functional heterodimers with RXRs. These RAR–RXR heterodimers are effectively activated by RAR-specific agonists, but not by RXR-specific agonists.[5] Depending on the nature of the bound ligand, the receptors undergo distinct conformational changes that allow them to bind to co-activator[6] or co-repressor[7] proteins that either activate or repress gene transcription, respectively.



Retinoic Acid (ATRA)



AGN 193109



AGN 190121

Recently we reported on the identification of AGN 193109, a highly potent and selective RAR antagonist and inverse agonist.[8] We have also reported on several novel structural classes of RAR selective antagonists, which are analogues of AGN 193109.[9] These compounds are similar to one another in that each of them is a highly rigid structure substituted with an aryl group in the 1-position (ATRA numbering). We wished to examine if

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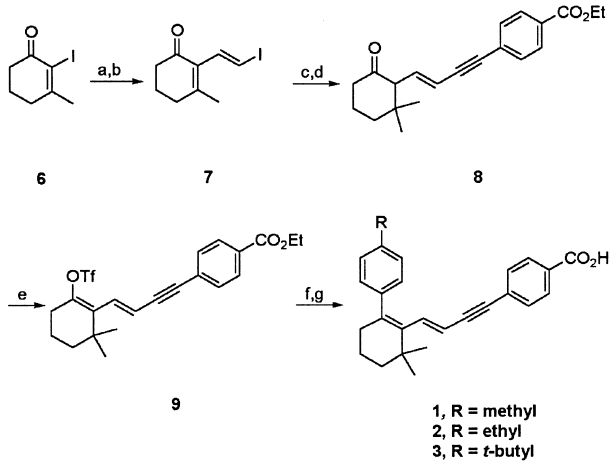
1-phenyl substituents would impart antagonist and/or inverse agonist activity on a series of more flexible retinoid analogues, such as AGN 190121, a potent RAR agonist.[10] Thus, we synthesized two series of new compounds that have structural features common to both AGN 193109 and AGN 190121. The first series includes compounds **1**–**3**, which possess phenyl groups in place of the 18-methyl group of AGN 190121. The second series, which includes compounds **4** and **5**, have a *gem*-dimethyl group at the 4-position and a phenyl group at the 1-position and lack the 18-methyl group. We will show that these compounds act as potent antagonists of the potent RAR agonist, (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-1-yl]benzoic acid (TTNPB)[11] in receptor transactivation assays. Moreover, in a hairless mouse model of retinoid induced topical irritation,[12] compound **1** was able to reverse the topical irritation caused by administration of TTNPB.

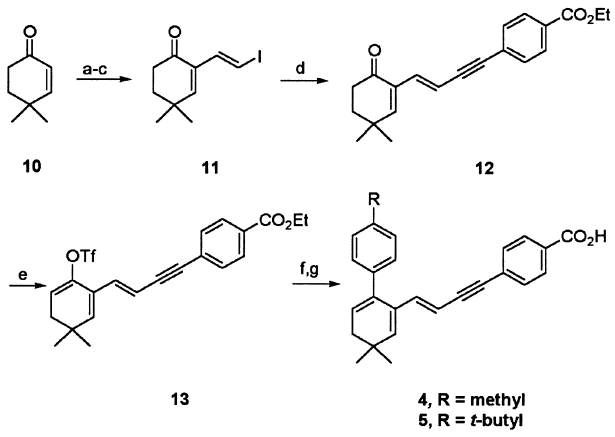
The analogues used in this study were obtained as follows.[13] The cyclohexene derivatives were prepared from 2-iodo-3-methyl-2-cyclohexen-1-one (**6**)[14] as shown in Scheme 1. Palladium catalyzed coupling reaction of **6** with (*E*)-1-(tri-*n*-butylstannyl)-2-(trimethylsilyl)ethylene[15] and subsequent iododesilation reaction gave the (*E*)-vinyl iodide **7** in 30% yield. Compound **7** underwent a Heck reaction with ethyl 4-ethynylbenzoate[15] followed by Michael reaction of the resulting cyclohexenone with methyl cuprate to produce the 3,3-dimethylcyclohexanone derivative, compound **8**, in 42% yield. The ketone was converted into an enol triflate, compound **9**, by treatment with triflic anhydride and a hindered base. This reaction also produced the other enol triflate regioisomer, which was separated by flash column chromatography, thereby lowering the yield of **9** to 42%. The triflate was reacted with three separate arylzinc reagents, 4-tolylzinc bromide, 4-ethylphenylzinc bromide, 4-*tert*-butylphenylzinc bromide, under palladium catalysis to produce the desired 2-phenyl-6,6-dimethyl-1-cyclohexene compounds as benzoate esters, which were hydrolyzed to give the carboxylic acids **1** (R = methyl), **2** (R = ethyl), and **3** (R = *tert*-butyl) in 70%, 76%, and 89% yield, respectively.

A synthesis of the cyclohexadiene analogues used in this study is illustrated in Scheme 2. The starting material, 4,4-dimethyl-2-cyclohexen-1-one (**10**), was iodinated in the 2-position by following the procedure of Sha and Huang.[14] This iodide and (*E*)-1-(tri-*n*-butylstannyl)-2-(trimethylsilyl)ethylene were reacted with a palladium catalyst and the resulting vinylsilane was iodinated to produce vinyl iodide **11** in 24% overall yield from **10**. Compound **11** and ethyl 4-ethynylbenzoate underwent a Heck reaction to produce benzoate **12** in 73% yield. Treatment of cyclohexenone **12** with sodium bis-(trimethylsilyl)amide and *N*-phenyltrifluoromethanesulfonimide gave the cyclohexadienyl triflate **13** in 65% yield. The triflate **13** was reacted separately with 4-tolylzinc bromide and 4-(*tert*-butyl)phenylzinc bromide in the presence of palladium(0) to give the expected C1-phenyl substituted derivatives, which were subsequently hydrolyzed to give the corresponding carboxylic acids, **4** (R = methyl) and **5** (R = *tert*-butyl), in 81% and 53% yield, respectively.

These compounds were tested in RAR binding and transactivation assays as previously described,[16] and the results are summarized in Table 1. None of these compounds exhibited any binding or transactivation activity at the RXRs (data not shown). Although these compounds do not activate any of the RAR subtypes, they bind effectively to all three. With the exception of compound **4**, these compounds bind with highest affinity to the RAR_β subtype, which is consistent with the binding properties of the RAR agonist, AGN 190121. These compounds also have a preference for binding to the RAR_γ subtype relative to RAR_α. The 4-tolyl and 4-ethylphenyl substituted cyclohexene derivatives, compounds **1** and **2**, respectively, have higher binding



Scheme 1. Synthesis of 2-phenyl-6,6-dimethyl-1-cyclohexene retinoid antagonists. (a) $Bu_3SnCHCHSiMe_3$, $Pd(PPh_3)_4$; (b) I_2, THF, 30% (two steps); (c) 4-$EtO_2CPhCCH$, $PdCl_2(PPh_3)_2$, CuI, Et_3N, 50 °C; (d) Me_2CuLi, TMSCl, HMPA; H_3O^+, 42% (two steps); (e) Tf_2O, 2,6-di-*tert*-butyl-4-methylpyridine, 42%; (f) ArZnBr, $Pd(PPh_3)_4$; (g) NaOH, EtOH; H^+, 70–89% (two steps).



Scheme 2. Synthesis of 6-phenyl-3,3-dimethyl-1,5-cyclohexadiene retinoid antagonists. (a) I_2, $TMSN_3$, pyridine, 85%; (b) $Bu_3SnCHCHSiMe_3$, $Pd(PPh_3)_4$, 69%; (c) I_2, THF, 31%; (d) 4-$EtO_2CPhCCH$, $PdCl_2(PPh_3)_2$, CuI, Et_3N, 40 °C, 73%; (e) NaHMDS; Tf_2NPh, 65%; (f) ArZnBr, $Pd(PPh_3)_4$; (g) NaOH, EtOH; H^+, 53–81% (two steps).

affinity to each of the RAR subtypes than the corresponding 4-*tert*-butylphenyl analogue, **3**. Interestingly, the 4-tolyl substituted cyclohexadiene **4** binds equally well to RAR_β and RAR_γ, but the 4-*tert*-butylphenyl substituted cyclohexadiene **5** binds exclusively to RAR_β at the doses tested. Thus, in the cyclohexadiene series, structural changes in this position affect ligand binding to RAR_α and RAR_γ more than to RAR_β. This trend is also observed with dihydronaphthtalene substituted antagonists such as AGN 193109.[17]

We also tested these compounds for their ability to inhibit TTNPB-induced transactivation at each of the RAR subtypes. Each compound was tested at varying concentrations for its ability to inhibit the transcriptional activation caused by TTNPB at a constant concentration of 10 nM as previously described.[9b] The half-maximal inhibition concentration (IC_{50}) values were calculated and are listed in Table 1. Each of these compounds acts as a full antagonist of TTNPB function at all three RARs. Surprisingly, although these compounds were somewhat RAR_β selective in binding, they were most potent at RAR_γ in inhibition of TTNPB transactivation. This discrepancy may arise from the fact that while binding assays are done using monomeric RARs, the transactivation assays are done in the context of RAR–RXR heterodimers.

A possible therapeutic application of retinoid antagonists is to inhibit the skin irritation that is produced in response to retinoid treatment. In particular, it would be desirable to inhibit the mucocutaneous toxicity produced by a systemically applied retinoid agonist. This toxicity is generally limited to small areas around the nose and on the lips. Thus, an antagonist applied topically to this relatively small area would have only minimal absorption and should not interfere with the overall beneficial effects of the retinoid agonist. It is known that RAR_γ is the most abundant receptor in the skin,[18] and convincing evidence exists that the skin irritation induced by retinoids is RAR_γ mediated.[19] Thus, the RAR_γ selectivity displayed by these compounds in the inhibition of TTNPB-induced transactivation suggests that they may be excellent candidates for the topical treatment of the mucocutaneous toxicity that is associated with oral and topical retinoid treatments. In order to determine if the antagonists in this study are able to prevent the cutaneous toxicity produced by a retinoid agonist in an in vivo model of topical irritation, we tested compound **1** for its ability to inhibit the severe cutaneous toxicity produced by the topical administration of TTNPB.[12] As shown in Figure 1, topical application of compound **1** alleviates the severe cutaneous toxicity caused by TTNPB treatment in this clinically relevant model. Thus, the compounds reported here have the potential of being useful in the treatment of the mucocutaneous toxicity that is almost universally associated with both systemic and topical retinoid treatment.

In summary, we have described the synthesis and biological activity of a new series of RAR-specific antagonists, which are structurally less rigid than previously described retinoid antagonists. These bind to the retinoic acid receptors—RARα, β, and γ—with high affinity, and act as antagonists of TTNPB in receptor transactivation assays. Moreover, in a hairless mouse model of retinoid induced topical irritation, a representative compound of this class is able to reverse the topical irritation caused by topical administration of TTNPB. Thus, these compounds may be useful as a topical treatment for the mucocutaneous toxicity caused by systemic retinoid medications such as 13-*cis*-retinoic acid, as well as for treating other retinoid anatagonist responsive diseases.

Table 1. RAR transcriptional activation, competitive binding and antagonist activity for 2-phenyl-6,6-dimethyl-1-cyclohexene (**1**–**3**) and 6-phenyl-3,3-dimethyl-1,5-cyclohexadiene (**4** and **5**) retinoid antagonists[a,b,c]

Compound number	RAR trans. EC_{50} (nM) / RAR bind. K_d (nM)			RAR IC_{50} (nM) / Efficacy (%)		
	α	β	γ	α	β	γ
ATRA	240	38	6	ND[d]	ND	ND
	14	11	16			
TTNPB	30	8	4	ND	ND	ND
	36	5	26			
190121	435	7	10	ND	ND	ND
	135	20	190			
193109	NA[e]	NA	NA	31	20	3
	17	7	6	(91)	(97)	(98)
1	NA	NA	NA	210	22	5
	148	6	21	(93)	(92)	(96)
2	NA	NA	NA	220	40	3
	136	4	36	(97)	(96)	(97)
3	NA	NA	NA	350	27	5
	875	25	331	(94)	(96)	(95)
4	NA	NA	NA	129	35	4
	252	53	31	(96)	(94)	(97)
5	NA	NA	NA	ND	ND	ND
	>1000	87	>1000			

[a]See refs 8 and 16.
[b]Values represent the mean of three determinations. Errors in this assay are approximately 15% of the mean value.
[c]Efficacy (%) is the percent transcriptional activity of 10 nM TTNPB antagonized by each compound at the highest dose tested (10 μM).
[d]ND, not determined.
[e]NA, not active (i.e., 0% efficacy at 10^4 nM).

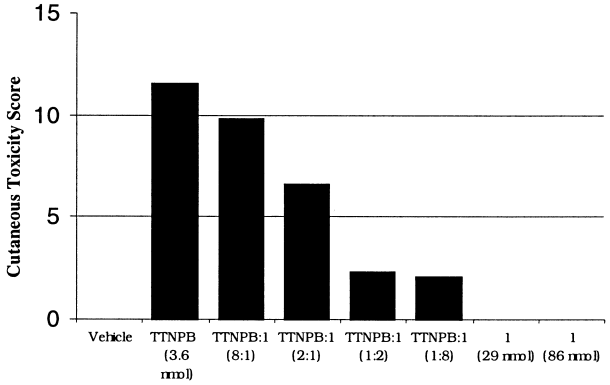


Figure 1. Inhibition of systemic TTNPB-induced topical irritation of hairless mouse skin by compound **1**.[12]

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