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A NEW CLASS OF POTENT RAR ANTAGONISTS: DIHYDROANTHRACENYL, BENZOCHROMENYL AND BENZOTHIOCHROMENYL RETINOIDS

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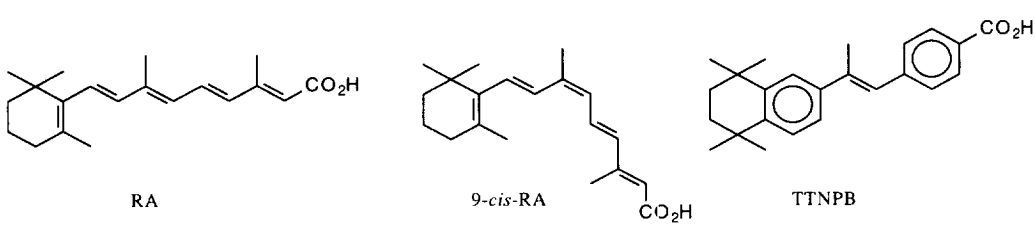
Abstract: The synthesis and biological activity of a novel series of tricyclic retinoic acid receptor antagonists are described. These compounds bind with high affinity to the RARs and are potent antagonists of retinoid function in in vitro and in vivo systems.

Retinoids are small molecule hormones that regulate gene transcription by binding to and activating nuclear receptors.[1] There are two families of retinoid receptors, the retinoic acid receptors (RARs)[2] and retinoid X receptors (RXRs)[3] and each family consists of 3 subtypes (α, β and γ). The natural hormone for the RARs is all *trans* retinoic acid (RA) and 9-*cis* retinoic acid (9-*cis* RA) has been proposed to be the hormone for the RXRs[4] (Figure 1). Under physiological conditions, RARs form heterodimers with RXRs[5] and these RAR-RXR heterodimers can be effectively activated by RA and synthetic RAR agonists. Endogenous RA is believed to have many fundamental biological roles including those in development, maintenance of normal patterns of differentiation and proliferation and immune function.[6] RA and a variety of synthetic RAR agonists have found utility in the treatment of several human diseases such as psoriasis, acne, photoaging and cancer.[7] However, given the broad and fundamental physiological roles ascribed to RAR activation, it is entirely plausible that there are pathological conditions that arise because of inordinate RAR activity or that are dependent for their maintenance on such activity. Such putative diseases should respond to treatment with RAR antagonists. It should be parenthetically noted that other antagonists of the steroid receptor superfamily, such as estrogen receptor antagonists (tamoxifen, raloxifen) and progesterone receptor antagonists (RU 486), have found significant use in clinical medicine.[8]

Figure 1



RA 9-*cis*-RA TTNPB

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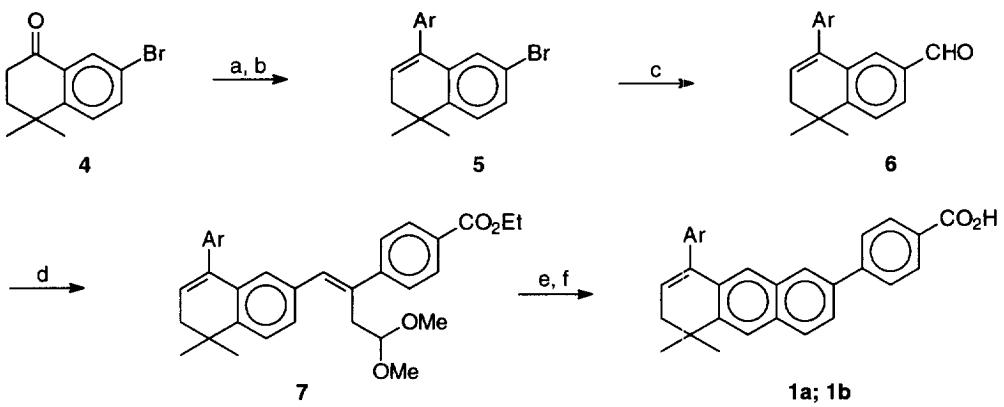
We and others have recently synthesized a variety of RAR antagonists.[9] These compounds will be useful in further elucidating the biology associated with RARs and such studies[10] will be complementary to the molecular biology approaches involving receptor ablation. A deeper understanding of this biology is likely to lead to the identification of newer clinical applications for RAR agonists as well as further the search for therapeutic applications for antagonists. As these therapeutic applications are discovered, it will be useful to have available a variety of structural classes of RAR antagonists so that development candidates with appropriate pharmacokinetic and pharmacodynamic properties can be identified for specific indications. In this regard, we describe a new class of tricyclic RAR antagonists from the dihydroanthracene,[11a] benzochromene and benzothiochromene structural classes. Some of these retinoids, compounds **1a–3a**, incorporate a C-1 tolyl group (retinoid numbering), a structural feature that we had previously used to derive a potent RAR panantagonist[9f] and RARα-specific antagonists.[9g] Here, we have extended the scope of the structural features required for RAR antagonism by introducing a 5-methyl-2-thienyl group at the C-1 position as in compounds **1b–3b**.

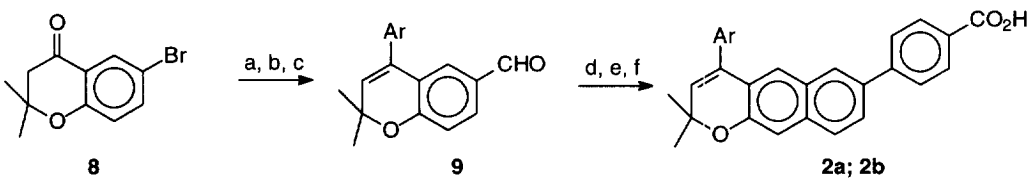
Synthesis

The syntheses of retinoid antagonists **1**, **2**, and **3** are described in Schemes 1-3. Bromoketone **4**[9f] was converted into the dihydronaphthalene derivatives **5** by reaction with aryl magnesium chloride or aryl lithium reagents followed by dehydration of the resultant tertiary alcohols with *p*-TSA. Compounds **5** were treated with *t*-BuLi followed by dimethylformamide (DMF) to obtain the aldehydes **6**. Horner–Emmons reaction of **6** with phosphonate **A**[11b] using *n*-BuLi as base gave a mixture of *E* (compound **7**) and *Z* olefins. The *E* isomers were separated by chromatography and cyclized with $SnCl_4$ and then hydrolyzed to the dihydroanthracene derivatives **1**. The benzochromenes **2** were synthesized starting from bromochromanone **8**[12] using a synthetic route similar to that used for the dihydroanthracenes. The bromochromanone **8** was reacted with the aryl lithium reagents followed by acid treatment to give the aryl chromenes, which were converted to the aldehydes **9** by treatment with *t*-BuLi and DMF. Horner–Emmons reaction of **9** with phosphonate **A** gave *E* and *Z* olefins, and the desired olefins were isolated, cyclized with $SnCl_4$ and then hydrolyzed to compounds **2**. The benzothiochromenes **3** were synthesized starting from 4-bromothiophenol which was condensed with 3,3-dimethyl-acrylic acid in the presence of piperidine. The derived acid was converted to the corresponding acid chloride and cyclized as before to give bromothiochromanone **10**, contaminated with an uncharacterized side product. Compound **10** was converted to the benzothiochromenes **3** using the same sequence of reactions used for the preparation of **1** and **2**.

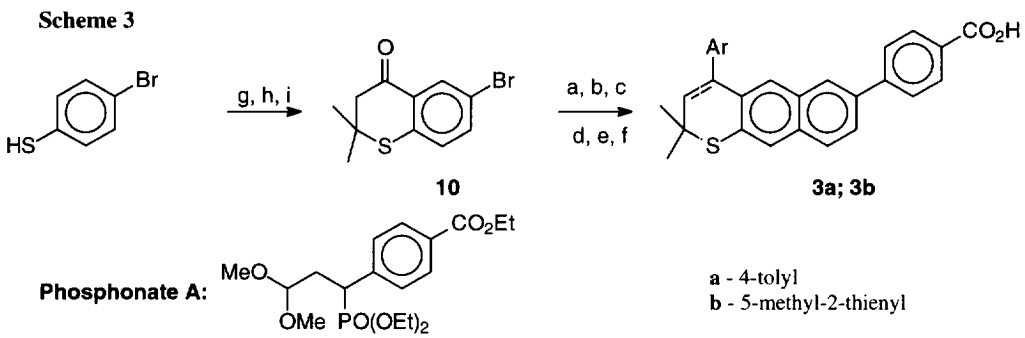
Scheme 1



Scheme 2



Scheme 3



Reagents and conditions: (a) ArMgBr or ArLi, THF, 0 °C; 40–70% (b) *p*-TSA, MeOH; 90–95% (c) *t*-BuLi; DMF, THF, -78 °C; 70–85% (d) Phosphonate **A**, *n*-BuLi, THF, -10 °C; 30–40% (e) $SnCl_4$, CH_2Cl_2, -78 °C 90–95% (f) KOH-H_2O, MeOH; 90–95% (g) Piperidine, 3,3-dimethylacrylic acid; 90% (h) ClCOCOCl, Benzene; 90% (i) $SnCl_4$, CH_2Cl_2, -78 °C; 50%.

Biological activity

The binding affinities of the retinoids **1–3** were measured in assays using baculovirus expressed human, full-length, RARs and RXRs by competition with [^{3}H] RA for RARs and with [^{3}H] 9-*cis* RA for the RXRs.[13] These compounds do not bind to nor transactivate the RXRs (data not shown). However, they bind with high affinity to all three RARs with little selectivity for the different subtypes (Table 1). Compound **3a** is the highest affinity RAR ligand with K_d values comparable to AGN 193109.[9f] Compounds **1–3** were inactive in RAR transactivation assays using transfected CV-1 cells. Functional antagonism was measured by the inhibition with

the test retinoid of transactivation of RARs by TTNPB[14a] (Table 1). Compounds **1–3** are quite potent in suppressing transactivation by TTNPB and they are all full antagonists. Compound **3a** is the most potent antagonist with IC_{50} values of 0.5–2 nM, in keeping with its high binding affinity to the RARs. The compounds show little subtype selectivity although compounds **1** show marginal selectivity for RARγ, a selectivity not reflected in their binding affinities. There is little discernible difference in selectivity or potency between the 5-methyl-2-thienyl antagonists **1b–3b** relative to the tolyl antagonists **1a–3a**.

Table 1 RAR binding affinity (K_d) and antagonism of transactivation (IC_{50}, % Inhibition)[14b] data for the retinoids.

Retinoid		RAR		
		α	β	γ
1a	K_d nM	13	4	7
	IC_{50} nM	20	22	2
	% Inhibition	97	97	98
1b	K_d nM	15	6	11
	IC_{50} nM	20	15	2
	% Inhibition	95	83	96
2a	K_d nM	14	7	14
	IC_{50} nM	5.5	7.5	2
	% Inhibition	97	98	98
2b	K_d nM	17	12	33
	IC_{50} nM	6.5	6.5	4
	% Inhibition	97	98	97
3a	K_d nM	5.5	6	5
	IC_{50} nM	1.5	2	0.5
	% Inhibition	97	98	98
3b	K_d nM	13	13	18
	IC_{50} nM	NT	NT	NT

NT = Not tested.

Some of the RAR antagonists described here were also tested in an in vivo retinoid antagonism model.[15] The cutaneous toxicity score (Table 2) is an aggregate of the flaking and abrasion caused by test compounds. Hairless mice were treated topically with TTNPB or with TTNPB and 2 doses of the antagonist. The antagonists were effective in reducing the cutaneous toxicity caused by TTNPB in a dose-dependent manner (Table 2).

Compound **3a** is the most potent of the compounds tested, once again reflecting the K_d and IC_{50} values. The thienyl analog **2b** is the least potent, again reflecting the in vitro values. The data shown here demonstrate that these antagonists would be effective agents in preventing the mucocutaneous toxicity produced by therapeutic RAR agonists.

Table 2 Inhibition of in vivo toxicity by retinoid antagonists.

Retinoid(s)	Dose nmol/25gm	Bodyweight %gain(loss)	Flaking score	Abrasion Score	Cutaneous Tox. Score
Vehicle		0	0	0	0
TTNPB	3.6	(6 ± 1)	3	2	11 ± 2
TTNPB + **1a** (1:2)	3.6 + 7.2	(2 ± 3)	1	1	2.0 ± 0.6
TTNPB + **1a** (1:8)	3.6 + 28.8	5 ± 3	1	0	1.1 ± 0
TTNPB + **2a** (1:2)	3.6 + 7.2	3 ± 3	1	1	1.9 ± 0.7
TTNPB + **2a** (1:8)	3.6 + 28.8	2 ± 2	1	0	1.0 ± 0.5
TTNPB + **2b** (1:2)	3.6 + 7.2	(6 ± 3)	2	3	4.6 ± 2.6
TTNPB + **2b** (1:8)	3.6 + 28.8	(1 ± 2)	2	1	3.0 ± 1.5
TTNPB + **3a** (1:2)	3.6 + 7.2	(5 ± 4)	1	0	1.4 ± 0
TTNPB + **3a** (1:8)	3.6 + 28.8	(0 ± 2)	1	0	0.7 ± 0.6

Vehicle = Acetone : DMSO (92.5 : 7.5)

In conclusion, we have described here a new series of RAR antagonists, which bind with high affinity to RARs and do not activate transcription. We also demonstrate that these compounds are effective functional antagonists of RAR agonists in in vitro transactivation and in vivo toxicity assays. The benzothiochromene **3a** is a particularly potent and effective antagonist suggesting that it could be a lead compound for development as an antidote to retinoid agonist induced mucocutaneous toxicity and for other future clinical applications for RAR antagonists.

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