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Synthesis and Pharmacological Activity of Conformationally Restricted, Acetylenic Retinoid Analogs.

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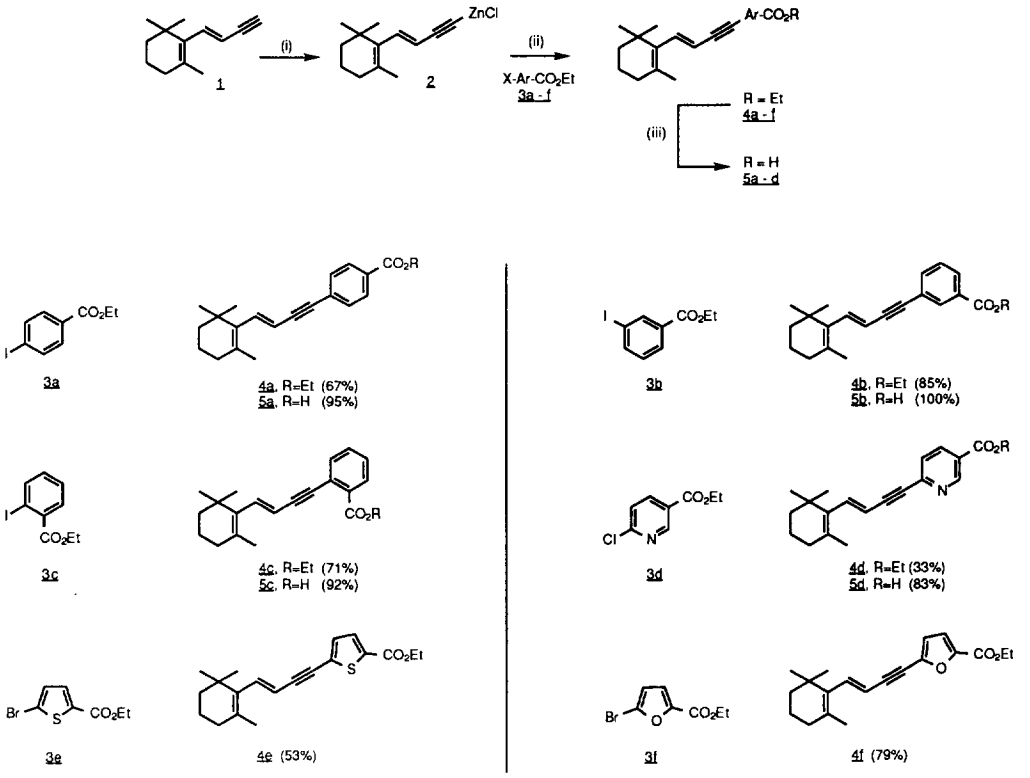
Abstract: The biological activity of retinoids are determined by their ability to activate one or more of six nuclear retinoid receptors (RARs and RXRs). We describe rigid, acetylenic retinoids that selectively transactivate through the RARβ and RARγ subtypes and demonstrate their potent anti-proliferative activity in the skin.

Retinoids have found considerable applications in clinical medicine, particularly in the treatment of dermatological disorders such as psoriasis and acne.[1] They are also of potential use in the treatment and chemoprevention of cancer,[2] repair of photodamage to skin,[3] acceleration of wound healing,[4] and treatment of corneal disease.[5] The wider clinical use of retinoids awaits the development of analogs of improved therapeutic efficacy. Although several alternate mechanisms have been proposed,[6] it now appears most likely that retinoids elicit many of their biological responses by regulating gene transcription through nuclear receptors.[7] Two families of retinoid receptors, the retinoic acid receptors (RARs)[8] and the retinoid X receptors (RXRs),[9] each with three subtypes (α,β,γ), have been described. Retinoic acid (RA), the hormone for RAR, is a potent activator of all three RARs. Very interestingly, 9-*cis*-retinoic acid (9-*cis*-RA), the putative hormone for RXR,[10] activates both RXRs and RARs. These pan-agonist activities for the naturally occurring retinoids are not surprising since these ligands are conformationally very flexible and could interact with different receptors in many different topologies. Thus, conformationally restricted analogs could be valuable tools in probing the binding requirements of the retinoid receptors. Identification of the *active topologies* for retinoids at each receptor would greatly facilitate future design of more selective and effective analogs. In this paper, we report the syntheses and biological activities of several acetylenic retinoids of limited conformational mobility. The potencies of these analogs at the RARs and RXRα were determined in transactivation assays.[11] The clinically relevant anti-proliferative potencies of these analogs were determined by their ability to inhibit induction of ornithine decarboxylase (ODC) by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) in hairless mouse epidermis.[12] The syntheses of several retinoid analogs based on the known dienyne[13] are shown in Scheme 1. After conversion to its zinc salt 2, the alkyne was coupled, according to the procedure of Negishi, to various haloaryl esters 3a-f in the presence of catalytic amounts of Pd (PPh3)4 to give the retinoid esters 4a-f.[14] Base hydrolysis of the esters 4a-d gave the acids 5a-d.

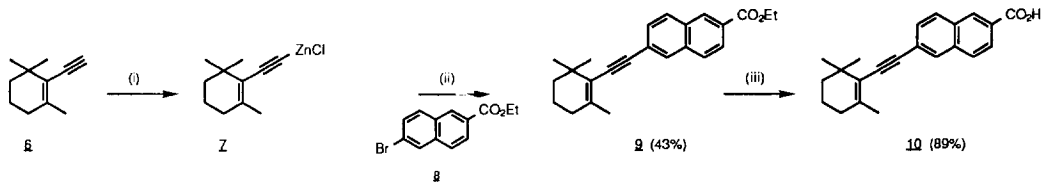
Scheme 1



(i) n-BuLi, THF, $ZnCl_2$, THF. (ii) $Pd(PPh_3)_4$, THF. (iii) KOH, EtOH

The analog 10, which has the triple bond adjacent to the trimethylcyclohexenyl ring, was synthesized as shown in Scheme 2. The known enyne 6[15] was converted to its zinc salt 7 and then coupled with ethyl 6-bromo-2-naphthalenecarboxylate 8[16] to give 9. Base hydrolysis of 9 gave the acid 10.

Scheme 2



(i) n-BuLi, THF; $ZnCl_2$, THF. (ii) $Pd(PPh_3)_4$, THF. (iii) KOH, EtOH.

Table 1. Transcriptional activation assay data for retinoid analogs.

	EC_{50} (nM)			
Agent	RARα	RARβ	RARγ	RXRα
RA	5.0	1.5	0.5	NA
9-*cis*-RA	102	3.3	6.0	13.0
TTNPB	21	4	2.4	NA
5a	598	2	10	NA
5b	NA	NA	NA	NA
5c	NA	NA	NA	NA
5d	NA	5	15	NT
10	NA	355	930	NA

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

The ability of several free acid analogs to activate transcription from individual receptors was determined in assays utilizing chimaeric receptors consisting of the DNA-binding region of the estrogen receptor fused to the ligand-binding region of retinoid receptors (ER-RAR or ER-RXR) and a reporter gene with an estrogen response element (ERE-CAT). Since the endogenous retinoid receptors cannot interact with the ERE-CAT, the assay provides a readout of activity only at the transfected receptor.[17] The data shown in Table 1 indicate that like RA, the synthetic acetylenic retinoids are completely specific for the RAR family of receptors and show no activity at RXRα. Interestingly, the para-substituted phenyl analog 5a is quite selective for subtypes being approximately 300x and 60x more potent at RARβ and RARγ, respectively, than at RARα. RA, on the other hand, is only marginally more potent at RARβ and RARγ than at RARα. It should be noted that the rigid analog 5a can mimic only some of the several topologies available to the flexible RA as regards the relative orientation of the fatty ring and the polar carboxyl group. The corresponding meta and ortho isomers 5b and 5c, respectively, are completely inactive at all the RARs. It should be emphasized that these three analogs essentially differ only in the relative orientation of the fatty and polar groups and should be very similar in pharmacokinetic and metabolic behavior. In contrast, the pyridyl analog 5d which has a conformation identical to 5a but quite distinct physicochemical properties, has transactivation properties similar to 5a. Introduction of the pyridyl moiety results in even greater RARβ/γ selectivity since activity at RARα is essentially abrogated for 5d. The naphthyl analog 10, which deviates from the topology of 5a, is significantly reduced in potency. These data strongly suggest critical topological requirements vis a vis the fatty and polar groups for activity at the RARs. Furthermore, the stratagem of introducing conformational rigidity in order to achieve receptor selectivity has been successful since the acetylenic analogs 5a and 5d exhibit significant RARβ/γ selectivity.

Table 2. Potency of Retinoid Analogs in Inhibition of TPA-Induced ODC Activity

Agent	IC_{80} (a)	Agent	IC_{80}
4a	1.3	5a	0.46
4b	>1000	5b	890
4c	>1000	5c	>1000
4d	5.3	5d	14.8
4e	30	9	131
4f	>300	10	215

(a) IC_{80} is the amount of retinoid (nmoles) which inhibits 80% of TPA-induced ODC activity.

We also evaluated these analogs in an in-vivo model of retinoid anti-proliferative activity. The retinoids were tested for their ability to inhibit TPA-induced ODC activity in hairless mouse skin (Table 2). ODC is a critical enzyme in the polyamine biosynthesis pathway and is elevated in cells prior to a hyperproliferative response.[18] Thus, inhibition of ODC induction in skin is a good measure of the potential clinical utility of retinoid analogs in hyperproliferative skin diseases such as psoriasis. The phenyl and pyridyl analogs, 5a and 5d, are quite potent in the ODC inhibition assay. The isomeric phenyl analogs, 5b and 5c, are essentially inactive and the naphthyl analog 10 is quite reduced in potency. Thus, activity of these analogs in the ODC assay essentially parallels that obtained in the RAR transactivation assay. This is not surprising since RARγ is the predominant RAR subtype in hairless mouse skin[19] and the ODC assay probably reflected the RARγ activity of these analogs. It is interesting to note that the ethyl ester derivatives of the active free acid analogs are quite potent in the ODC assay. Since the free carboxylic acid group is required for efficient binding to the retinoid receptor,[20] the ester derivatives must be delivered to the target cells and act as effective prodrugs in the ODC assay. The thiophene ester 4e is a log order less potent than 4a while the furyl derivative 4f is essentially inactive. These decreased potencies, which can be explained on the basis of deviations from the optimal topology or on the basis of increased susceptibility to metabolic deactivation, made the thiophene and furyl analogs less interesting and were not investigated further.

In summary, the data on these rigid acetylenic analogs reinforce the notion that retinoid-like activity is dependent on a critical spatial relationship between the fatty ring and the polar carboxylic acid group. The conformation defined by 5a is an active topology for eliciting biological activity from the RARβ and RARγ receptors and would serve as an useful template in designing new analogs with even greater selectivity for each of these receptor subtypes.

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