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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 1,2,3,4-TETRAHYDROQUINOLINE AND 3,4-(1H)-DIHYDROQUINOLIN-2-ONE ANALOGS OF RETINOIC ACID

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Abstract. Retinoids are natural and synthetic analogs of the hormone retinoic acid. Retinoids are currently being investigated clinically as drugs in several areas, including dermatology and oncology. We report the synthesis and biological activity of a new series of potent, RAR-specific retinoids substituted with a 1,2,3,4-tetrahydroquinoline or a 3,4-(1H)-dihydroquinolin-2-one group. © 1997 Elsevier Science Ltd.

Vitamin A and its natural and synthetic analogs are known as retinoids. Besides their important role in vision, retinoids act as hormones and control a number of biological responses, including cell differentiation and proliferation. Retinoids induce gene transcription in cells by binding to and activating the six known retinoid receptors: the retinoic acid receptors (RAR α , β , and γ) and the retinoid X receptors (RXR α , β , and γ).¹ All-trans-retinoic acid (ATRA) is the physiological hormone for the RARs, and is only able to bind to the RARs.² The putative hormone for the RXRs, 9-cis-retinoic acid (9-cis-RA), binds to and transactivates both RXRs and RARs.³ In addition, the retinoid receptors are able to modulate the activity of other transcription factors such as AP-1, a dimeric protein composed of the oncogenes Jun and Fos.⁴ Thus, retinoids control a wide variety of biological functions and may be useful as therapeutic agents in a number of different areas, including oncology,⁵ immunology,⁶ and ophthalmology.⁷

Currently, retinoids are being used clinically to treat skin diseases⁸ and some cancers⁵ but their therapeutic efficacy is limited because they cause a number of detrimental side effects such as bone and lipid toxicity⁹ and teratogenicity.¹⁰ In order to decrease the number of side effects associated with retinoid treatment, we, and others, have recently developed ligands which are specific for a particular receptor subtype.¹¹ Another approach to discovering drugs with reduced side effects is to design molecules that distinguish between various tissues. Synthetic retinoids with unique pharmacokinetic properties may target specific cell types and have greater efficacy and reduced side effects than currently available retinoids.

To date, most synthetic retinoids, such as (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethy]-2naphthalenyl)propen-1-yl]benzoic acid (TTNPB), were modeled after ATRA, and thus have a large lipophilic 'left-hand' group connected by a lipophilic tether to the polar carboxylic acid terminus. We have designed potent, RAR selective retinoids with polar left-hand groups tethered to the carboxylic acid moiety. In particular, we felt that left-hand groups possessing a trivalent nitrogen would be ideally suited for this purpose since the polarity and size requirements around nitrogen are easily modified by the introduction of various substituents. To this end, we report here the synthesis and biological activity of a new series of retinoids substituted with 1,2,3,4-tetrahydroquinoline (THQ) or 3,4-(1H)-dihydroquinolin-2-one (DHQ) groups.



Retinoic Acid (ATRA)

9-cis-Retinoic Acid (9-cis-RA)

TTNPB

The analogs used in this study were obtained as follows.¹² RA was purchased from Sigma Chemical Co. TTNPB was prepared as described in the literature.¹³ The 6-acetylenic analogs were prepared from 6-bromo-4,4-dimethyl-3,4-dihydro-2(1H)-quinolinone (20)¹⁴ as shown in Scheme 1. The THQ derivatives were prepared by reducing DHQ 20 with lithium aluminum hydride to give THQ 19. Heck coupling reactions of 19 and 20 with (trimethylsilyl)acetylene and subsequent removal of the trimethylsilyl groups gave the terminal acetylenes, 21 and 22, respectively. The acetylenes, 21 and 22, underwent a second Heck reaction using ethyl iodobenzoate and the resulting esters (10 and 13) saponified to afford the carboxylic acids, 1 and 4, respectively. The ester 10 was N-alkylated with either n-heptyl bromide to give ester 11 or with isopropyl iodide to produce ester 12. The esters 11 and 12 were hydrolyzed to give the carboxylic acid derivatives, 2 and 3, respectively. Similarly, DHQ 13 was N-alkylated with isopropyl iodide and the resulting ester, 14, hydrolyzed to give the carboxylic acid, 5.

Scheme 1. Synthesis of 6-Substituted 1,2,3,4-Tetrahydroquinolines and 3,4-(1H)-Dihydroquinolin-2-ones.^a



 $a^{(a)}$ LiAlH₄, THF. (b) TMSCCH, PdCl₂(PPh₃)₂, CuI, Et₃N, 50 °C. (c) MeOH, K₂CO₃. (d) ethyl 4-iodobenzoate, PdCl₂(PPh₃)₂, CuI, Et₃N, 50 °C. (e) 10, RX, DMF, K₂CO₃ or 13, NaH, RX, DMF. (f) NaOH, EtOH, THF; H^{*}.

A synthesis of the 7-acetylenic substituted analogs used in this study is illustrated in Scheme 2. Starting with aryl bromides 23 and 24^{14} we used our standard Heck coupling protocol to produce the respective benzoates, 15 and 17, which were saponified to the carboxylic acid analogs, 6 and 8, respectively. Alternatively, 23 and 24 were first N-alkylated with isopropyl iodide and then converted to the ester derivatives, 16 and 18, respectively. Esters 16 and 18 were then hydrolyzed to give the corresponding carboxylic acids, 7 and 9.

Scheme 2. Synthesis of 7-Substituted 1,2,3,4-Tetrahydroquinolines and 3,4-(1H)-Dihydroquinolin-2-ones.^a



a(a) LiAlH₄, THF. (b) TMSCCH, PdCl₂(PPh₃)₂, CuI, Et₃N, 50 °C. (c) MeOH, K₂CO₃. (d) ethyl 4-iodobenzoate, PdCl₂(PPh₃)₂, CuI, Et₃N, 50 °C. (e) 23, *i*-propyl iodide, DMA or 24, NaH, *I*-propyl iodide, DMF. (f) LiOH, THF; H⁴.

The RAR transactivation potencies and competitive binding affinities for these analogs were determined as previously described¹⁵ and the results are summarized in Table 1. These compounds do not bind to or activate any of the RXRs. As reported for other acetylene-linked retinoids, these compounds for the most part selectively transactivate through RAR β and RAR γ relative to RAR α .¹⁶ In considering the 6-substituted series (compounds 1-5), the N-isopropyl derivatives appear to be the most potent in terms of receptor transactivation activity. The THQ 1 is approximately 20-fold less potent at RAR β and RAR γ than the N-isopropyl derivative 3, indicating the need for lipophilicity around nitrogen. Receptor activity is decreased in compounds with very large groups as evidenced by compound 2, the N-heptyl derivative, which is 28- and 40-fold less potent at RAR β and RAR γ , respectively, than 3. The highly polar DHQ, 4, has minimal receptor activity while the more lipophilic N-isopropyl derivative, 5, is a relatively potent transactivator with EC₅₀ values of 4 nM at both RAR β and RAR γ . Structure-activity relationships for the 7-substituted compounds, 6-9, are similar to the 6-substituted compounds. Thus, the N-isopropyl analog, 7, is approximately 10- and 20-fold more potent at RAR $_{\beta}$ and RAR $_{\gamma}$. Also, respectively, than the hydrogen substituted compound, 6. Likewise, the unsubstituted DHQ, 8, is a poor transactivator while the N-isopropyl derivative, 9, has EC₅₀ values of 4 nM at RAR β and 20 nM at RAR γ . Also, compound 7 is an exception within this series since it potently activates all three RARs.

 Table 1. RAR Transcriptional Activation and Competitive Binding Data for 1,2,3,4-Tetrahydroquinoline and 3,4-(1*H*)-Dihydroquinolin-2-one Retinoid Analogs.¹⁷



| compound | substitution | | | F | EC50 (nM) | | K _d (nM) | | |
|----------|--------------|----------------|------------------|------|-----------|------|---------------------|------------------|------------------|
| number | position | Х | <u>R</u> | RARa | RARβ | RARγ | RARα | RARβ | RARγ |
| ATRA | | _ | | 350 | 80 | 10 | 15 | 13 | 18 |
| TTNPB | | | | 30 | 3 | 2 | 36 | 5 | 26 |
| 1 | 6 | H ₂ | н | 2300 | 170 | 130 | 5202 | 714 | 2421 |
| 2 | 6 | H_2 | n-heptyl | NA | 280 | 260 | >103 | >103 | >10 ³ |
| 3 | 6 | H_2 | <i>i</i> -propyl | NA | 10 | 6 | 627 | 60 | 72 |
| 4 | 6 | 0 | Н | NA | NA | NA | >10 ³ | >10 ³ | >10 ³ |
| 5 | 6 | 0 | <i>i</i> -propyl | NA | 4 | 4 | 136 | 17 | 17 |
| 6 | 7 | H_2 | Н | NA | 36 | 54 | NA | 211 | 233 |
| 7 | 7 | H_2 | <i>i</i> -propyl | 17 | 3 | 3 | 52 | 18 | 52 |
| 8 | 7 | 0 | н | NA | 1000 | 200 | >103 | >10 ³ | >10 ³ |
| 9 | 7 | 0 | <i>i</i> -propyl | NA | 4 | 20 | 58 | 18 | 23 |

NA indicates <u>Not Active</u> (i.e., $> 10^4$ nM)

We also wanted to test these compounds in an in vivo assay of retinoid activity. The inhibition of tumor promoter induced ornithine decarboxylase (ODC) activity in hairless mouse skin is a classic in vivo model of the anti-proliferative activity of retinoids.¹⁸ We have tested hundreds of compounds in this assay and it is our experience that retinoid benzoic acids and their corresponding ethyl ester derivatives have very similar activities.¹⁹ In addition, we have found that the ester analogs tend to be less topically irritating and have a better topical therapeutic index than the parent carboxylic acids. Thus, the ethyl ester derivatives of these retinoids were tested for their ability to inhibit ODC activity and the data are presented in Table 2. In the 6-substituted series, the unsubstituted THQ, **10**, is a moderately potent inhibitor of ODC activity (IC₆₀ = 5 nM) whereas the N-n-heptyl derivative, **11**, is completely inactive. Compound **12**, the N-isopropyl derivative of **10**, has an IC₆₀ value of 0.4 nM, and is comparable in activity to the highly potent arotinoid, TTNPB. For the DHQs, the N-isopropyl derivative, **14**, is active, having an IC₆₀ value of 6 nM, while the unsubstituted compounds in the 6-substituted series. Thus, the unsubstituted THQ, **15**, is only a moderately potent inhibitor of ODC, with an IC₆₀ value of 7 nM, while the N-isopropyl analog, **16**, is one of the most potent ODC inhibitors

known, having an IC₆₀ value of less than 0.1 nM. Likewise, the unsubstituted DHQ, 17, is inactive while 18, the N-isopropyl substituted DHQ, inhibits ODC activity with a potency of 2.9 nM.

Table 2. Inhibition of Ornithine Decarboxylase Activity in Hairless Mouse Skin by 1,2,3,4-Tetrahydroquinoline and 3,4-(1*H*)-Dihydroquinolin-2-one Esters.

| Compound | ODC | Compound | ODC | |
|----------|-----------|----------|-----------|--|
| number | IC60 (nM) | number | IC60 (nM) | |
| ATRA | 1.4 | | | |
| TTNPB* | 0.33 | 14 | 6 | |
| 10 | 5 | 15 | 7 | |
| 11 | >300 | 16 | <0.1 | |
| 12 | 0.4 | 17 | >300 | |
| 13 | >300 | 18 | 2.9 | |

* ethyl ester

Because RAR_{γ} is the most abundant retinoid receptor in the skin²⁰ we would expect that RAR_{γ} transactivation potency is an important factor in determining a compound's activity in the ODC inhibition assay. The data in Tables 1 and 2 seem to support this notion, although it is also clear that other factors influence ODC inhibition. Thus, compounds 2, 4, and 8 with RAR_{γ} EC₅₀ values over 200 nM, correspond to ester derivatives 11, 13, and 17, respectively, which are inactive in the ODC assay. Conversely, with the exception of 5, carboxylic acid analogs with single digit RAR_{γ} EC₅₀ values (i.e., 3 and 7) have esters (12 and 16, respectively) that inhibit ODC activity at sub-nanomolar concentrations.

It is interesting to compare the ODC data of the N-isopropyl THQs, 12 and 16, to the N-isopropyl DHQs, 14 and 18. The carboxylic acid analogs of THQs 12 and 16, 3 and 7, respectively, bind to RAR γ with lower affinity than the carboxylic acid analogs of DHQs 14 and 18, 5 and 9, respectively, but the THQ esters, 12 and 16, are about 10-fold more potent inhibitors of ODC activity than are the corresponding DHQ esters, 14 and 18. The increased potency in the ODC assay displayed by the THQ derivatives may be linked to the increased lipophilicity of these retinoids relative to that of the DHQ derivatives.

In summary, we have described the synthesis and biological activity of a new series of RAR β , γ selective retinoids substituted with THQ or DHQ groups. The ODC inhibition potency of these analogs is most closely related to their ability to bind to and transactivate through RAR γ , the most abundant retinoid receptor in the skin.. We have identified two THQ analogs, **12** and **16**, that are highly potent inhibitors of tumor promoter induced ODC activity in hairless mouse skin. Furthermore, we have demonstrated that THQ and DHQ analogs, which have similar receptor binding and transactivation profiles, have different potencies in the inhibition of ODC activity in the skin. Thus, these retinoids, which may possess unique pharmacokinetic properties, may have advantages over the currently available retinoids in terms of increased efficacy and reduced side effects.

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