Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 18, pp. 2373-2378, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00435-6

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

Richard L. Beard,*[,a] Min Teng,[a] Diana F. Colon,[a] Tien T. Duong,[a] Scott M. Thacher,[b] Taghreed Arefieg,[b] and Roshantha A.S. Chandraratna*[,a,b]

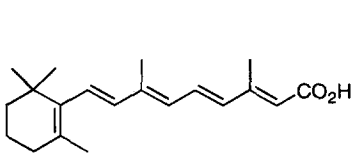
Retinoid Research, Departments of [a]Chemistry and [b]Biology, Allergan, Incorporated, Irvine, California 92623-9534.

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-(1*H*)-dihydroquinolin-2-one group. © 1997 Elsevier Science Ltd.

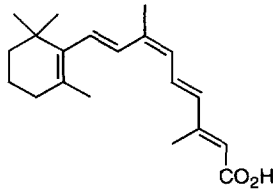
Vitamin A and its natural and synthetic analogs are known as retinoids. Besides their important role in vision, retinoids act as hormones and control a number of biological responses, including cell differentiation and proliferation. Retinoids induce gene transcription in cells by binding to and activating the six known retinoid receptors: the retinoic acid receptors (RARα, β, and γ) and the retinoid X receptors (RXRα, 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 diseases[8] and some cancers[5] but their therapeutic efficacy is limited because they cause a number of detrimental side effects such as bone and lipid toxicity[9] 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.

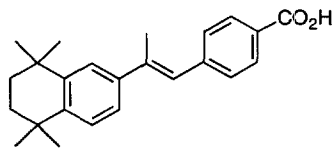
To date, most synthetic retinoids, such as (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-1-yl]benzoic acid (TTNPB), were modeled after ATRA, and thus have a large lipophilic 'left-hand' group connected by a lipophilic tether to the polar carboxylic acid terminus. We have designed 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-(1*H*)-dihydroquinolin-2-one (DHQ) groups.



Retinoic Acid (ATRA)



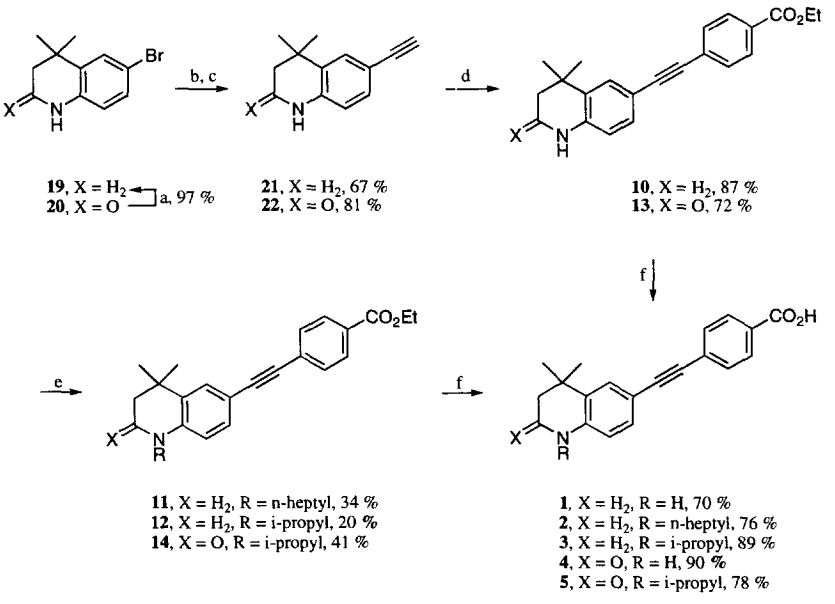
9-*cis*-Retinoic Acid (9-cis-RA)



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(1*H*)-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**.

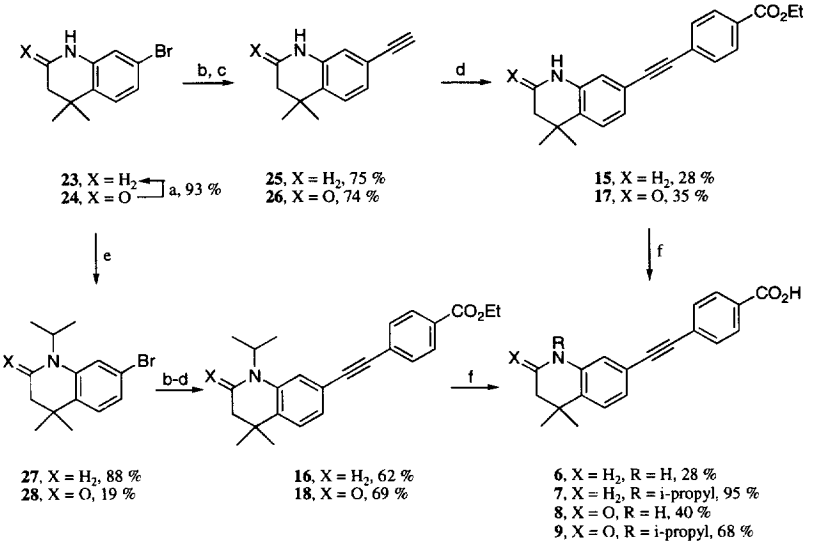
Scheme 1. Synthesis of 6-Substituted 1,2,3,4-Tetrahydroquinolines and 3,4-(1*H*)-Dihydroquinolin-2-ones.[a]



[a](a) $LiAlH_4$, THF. (b) TMSCCH, $PdCl_2(PPh_3)_2$, CuI, Et_3N, 50 °C. (c) MeOH, K_2CO_3. (d) ethyl 4-iodobenzoate, $PdCl_2(PPh_3)_2$, CuI, Et_3N, 50 °C. (e) **10**, RX, DMF, K_2CO_3 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**.

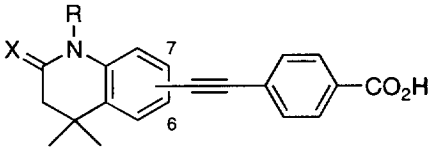
Scheme 2. Synthesis of 7-Substituted 1,2,3,4-Tetrahydroquinolines and 3,4-(1*H*)-Dihydroquinolin-2-ones.[a]



[a](a) $LiAlH_4$, THF. (b) TMSCCH, $PdCl_2(PPh_3)_2$, CuI, Et_3N, 50 °C. (c) MeOH, K_2CO_3. (d) ethyl 4-iodobenzoate, $PdCl_2(PPh_3)_2$, CuI, Et_3N, 50 °C. (e) **23**, *i*-propyl iodide, DMA or **24**, NaH, *I*-propyl iodide, DMF. (f) LiOH, THF; H^+.

The RAR transactivation potencies and competitive binding affinities for these analogs were determined as previously described[15] 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 RARβ and RARγ relative to RARα.[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 RARβ and RARγ 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 RARβ and RARγ, 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 EC_{50} values of 4 nM at both RARβ and RARγ. 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_β and RARγ, respectively, than the hydrogen substituted compound, **6**. Likewise, the unsubstituted DHQ, **8**, is a poor transactivator while the N-isopropyl derivative, **9**, has EC_{50} values of 4 nM at RARβ and 20 nM at RARγ. 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			EC50 (nM)			Kd (nM)		
number	position	X	R	RARα	RARβ	RARγ	RARα	RARβ	RARγ
ATRA				350	80	10	15	13	18
TTNPB				30	3	2	36	5	26
1	6	H_2	H	2300	170	130	5202	714	2421
2	6	H_2	*n*-heptyl	NA	280	260	$>10^3$	$>10^3$	$>10^3$
3	6	H_2	*i*-propyl	NA	10	6	627	60	72
4	6	O	H	NA	NA	NA	$>10^3$	$>10^3$	$>10^3$
5	6	O	*i*-propyl	NA	4	4	136	17	17
6	7	H_2	H	NA	36	54	NA	211	233
7	7	H_2	*i*-propyl	17	3	3	52	18	52
8	7	O	H	NA	1000	200	$>10^3$	$>10^3$	$>10^3$
9	7	O	*i*-propyl	NA	4	20	58	18	23

NA indicates Not Active (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 (IC_{60} = 5 nM) whereas the N-n-heptyl derivative, **11**, is completely inactive. Compound **12**, the N-isopropyl derivative of **10**, has an IC_{60} 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 IC_{60} value of 6 nM, while the unsubstituted compound, **13**, is inactive. The ODC activities of compounds in the 7-substituted series are similar to compounds in the 6-substituted series. Thus, the unsubstituted THQ, **15**, is only a moderately potent inhibitor of ODC, with an IC_{60} value of 7 nM, while the N-isopropyl analog, **16**, is one of the most potent ODC inhibitors

known, having an IC_{60} 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 number	ODC IC_{60} (nM)	Compound number	ODC IC_{60} (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 RARγ is the most abundant retinoid receptor in the skin[20] we would expect that RARγ 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 RARγ EC_{50} 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 RARγ EC_{50} 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 RARγ 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 RARβ,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 RARγ, 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.

Acknowledgment. We are grateful to G. Croston for providing the receptor transactivation assay data, and to D. Mais, E. Berger and K. Flatten for providing the ligand binding data. We also thank Mr. Hai Nguyen for mass spectral analyses.

References and Notes

1. Review: *The Retinoids: Biology, Chemistry, and Medicine*; 2nd ed.; Sporn, M. B.; Roberts, A. B.; Goodman, D. S., Eds.; Raven: New York, 1994.
2. (a) Petkiovich, M.; Brand, N. J.; Krust, A.; Chambon, P. *Nature* **1987**, *330*, 444. (b) Giguere, V.; Ong, E. S.; Segui, P.; Evans, R. M. *Nature* **1987**, *330*, 624. (c) Brand, N.; Petkovich, M. Krust, A.; Chambon, M.; de The, H.; Marchio, A.; Dejean, A. *Nature* **1988**, *332*, 850. (d) Krust, A. Kastner, P.; Petkovich, M.; Zelent, A.; Chambon, P. *Proc, Natl. Acad. U.S.A.* **1989**, *86*, 5310.
3. (a) Heyman, R. A.; Mangelsdorf, D. J.; Dyck, J. A., Stein, R.B.; Eichele, G.; Evans, R. M.; Thaller, C. *Cell* **1992**, *68*, 397. (b) Levin, A. A.; Sturzenbecker, L. J.; Kazmer, S.; Bosakowski, T; Huselton, C.; Allenby, G.; Speck, J.; Kratzeisen, Cl.; Rosenberger, M.; Lovey, A.; Grippo, J. F. *Nature* **1992**, *355*, 359.
4. Yang-Yen, H.-F.; Zhang, X.-K.; Graupner, G.; Tzukerman, , M.; Sakamoto, B.; Karin, M.; Phahl, M. *New Biologist* **1991**, *3*, 1206.
5. (a) Ref 1, pp 573-630; (b) Tallman, M. S.; Wiernik, P. H. *J. Clin. Pharmacol.* **1992**, *32*, 868.
6. (a) Ref 1, pp. 373-390. (b) Hope, W. C.; Patel, B. J.; Fiedler-Nagy, C.; Wittreich, B. H. *Inflamation* **1990**, *14*, 543. (c) Hanglow, A. C.; Bachmann, H.; Rosenberger, M.; Coffey, J. F. *Int. J. Immunopharmac.* **1990**, *12*, 703. (d) Sidell, N.; Chang, B.; Bhatti, L. *Cellular Immunology* **1993**, *146*, 28.
7. Verstraeten, T.; Hartzer, M.; Wilcox, D. K.; Cheng, M. *Invest. Ophthalmol. Vis. Sci.* **1992**, *33*, 2830.
8. (a) Ref 1, pp 631-638. (b) *Retinoids: Present and Future. Proceedings of a Sympisium Held at the 18th World Congress of Dermatology.* Shalita, A. R.; Fritsch, P. O., Eds.; Supplement to *J. Am. Acad. Dermatol.* **1992**, *27* (6), part 2.
9. Review: Vahlquist, A. In *Retinoids: Present and Future. Proceedings of a Sympisium Held at the 18th World Congress of Dermatology.* Shalita, A. R.; Fritsch, P. O., Eds.; Supplement to *J. Am. Acad. Dermatol.* **1992**, *27* (6), pp S29 - 233.
10. Agnish, N. D.; Kochhar, D. M. In *Retinoids and Clinical Practice*; Korean, G., Ed.; Mercel Dekker: New York 1992; pp 47-76.
11. (a) Teng, M.; Duong, T. T., Klein, E. S.; Pino, M. E.; Chandraratna, R. A. S. *J. Med. Chem.* **1996**, *39*, 3035. (b) Johnson, A. T.; Klein, E. S.; Wang, L. Pino, M. E.; Chandraratna, R. A. S. *J.Med. Chem.* **1996**, *39*, 5027, and references therein.
12. All new compounds gave ^{1}H NMR, ^{13}C NMR, IR, and HRMS data consistent with their structures.
13. Frickel, F.-F.; Nurrenbach, A. *U.S Patent* 4,578,498, Mar. 25, 1986.
14. The starting aryl bromides **20** and **24** were prepared according to the literature procedure: Teng, M.; Beard, R. L.; Duong, T. T.; Colon, D. F.; Chandraratna, R. A. *U.S. Patent* 5,616,712, April 1, 1997.
15. (a) Giguere, V.; Ong, E. S.; Segui, P.; Evans, R. M. *Nature* **1987**, *330*, 624. (b) Allegretto, E. A.; McClurg, M. R.; Lazarchik, S. B.; Clemm, D. L.; Kerner, S. A.; Elgrot. M. G.; Boehm, M. F.; White, S. K.; Pike, J. W.; Heyman, R. A. *J. Biol. Chem.* **1993**, *268*, 26625.
16. Chandraratna, R. A. S.; Henry, E.; Attard, J.; Gillett, S. J.; Song, T.; Garst, M. E.; Nagpal, S.; Athanikar, Arefieg, T.; Gil, D. W.; Wheeler, L. A.; Lew-Kaya, D.; Sefton, J. In *Proceedings of the XIIIth International Symposium on Medicinal Chemistry.* Muller, J.-C., Ed., Supplement to *Eur. J. Med. Chem.* **1995**, *30*, 505s.
17. EC_{50} values were determined from full dose-response curves ranging from 10^{-12} to 10^{-5} M. Retinoid activity was normalized to that of all-*trans* RA and is expressed as EC_{50} values, which is the concentration of retinoid required to produce 50% of the maximal observed response. Values represent the EC_{50} determination of a single experiment with triplicate determinations. Standard errors for this assay system are, on average, approximately 15% of the mean values.
18. Verma, A. K.; Rice, H. M.; Shapas, B. G.; Boutwell, R. K. Analogs.*Cancer Research* **1978**, *38*, 793.
19. For example, the ODC IC_{60} values for the benzoic acid derivatives TTNPB, **10**, and **16** are 0.75 nM, 9.1 nM, and < 0.06 nM, respectively, which correspond to ethyl ester derivatives with IC_{60} values of 0.33 nM, 5.0 nM, and < 0.1 nM, respectively.
20. Elder, J. T.; Fisher, G. J.; Zhang, Q.-Y.; Eisen, D.; Krust, A.; Kastner, P.; Chambon, P.; Voorhees, J. J. *J. Invest. Dermatol.* **1991**, *96*, 425.

(*Received in USA* 19 *June* 1997; *accepted* 18 *August* 1997)