Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1583–1586

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

An Unhydrolyzable Analogue of *N*-(4-Hydroxyphenyl)retinamide: Synthesis and Preliminary Biological Studies

Kevin L. Weiss,[a] Galal Alshafie,[b] Jason S. Chapman,[c] Serena M. Mershon,[a] Hussein Abou-Issa,[b] Margaret Clagett-Dame[c] and Robert W. Curley, Jr.[a,*]

[a]*Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA*
[b]*Department of Surgery, College of Medicine, The Ohio State University, Columbus, OH 43210, USA*
[c]*Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706, USA*

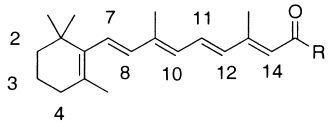
Received 28 December 2000; accepted 3 April 2001

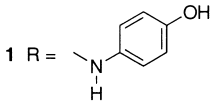
Abstract—The synthesis of a nonhydrolyzable, carbon-linked analogue (4-HBR) of the retinoid *N*-(4-hydroxyphenyl)retinamide (4-HPR) using *Umpolung* methods is described. Preliminary studies of biological activity show 4-HBR is similar to 4-HPR in its actions although a potentially relevant and desirable difference is its reduced suppression of plasma vitamin A levels. These results show that 4-HPR does not have to be hydrolyzed to retinoic acid to produce its chemotherapeutic effects. © 2001 Elsevier Science Ltd. All rights reserved.

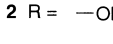
The synthetic retinoid *N*-(4-hydroxyphenyl)retinamide (4-HPR; **1**) was developed a number of years ago and showed promise as, in particular, a breast cancer chemopreventive agent in animals.[1] While this analogue is derived from the natural retinoid, retinoic acid (RA; **2**), it is less toxic and substantially less teratogenic.[2] Continued interest in this retinamide has led to its exploration in a clinical trial as a breast cancer chemopreventive agent[3] and in animal studies as an antitumor agent.[4]

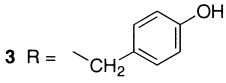
The mechanism through which 4-HPR functions remains unclear. When prepared, it was assumed that 4-HPR was an amide analogue that acted in a RA-like manner. Subsequently, nuclear retinoic acid receptors (RARs), which bind RA, and retinoid X receptors (RXRs), which bind 9-*cis*-retinoic acid, were discovered. These RARs function as ligand dependent transcription factors mediating most or all of the effects of RA.[5] While some researchers have reported that 4-HPR can activate RARs using transactivation assays,[6] we find that 4-HPR has very low affinity for RAR and RXR proteins.[7] 4-HPR has also been shown to induce apoptosis in tumor cells that typically respond to RA by differentiating,[8] and this can even occur in RA-resistant cells.[9] Recently, based on studies in wild-type and receptor knockout F9 murine teratocarcinoma cells, Clifford and co-workers have suggested there are early RAR-independent and late RAR-dependent actions of 4-HPR in these cells.[10]

Certainly it is plausible that 4-HPR might serve as a prodrug which liberates RA in vivo. There is limited evidence that 4-HPR may be metabolized to RA in vivo,[11] but there is also in vivo and in vitro work where hydrolysis could not be detected.[8,12] Thus, some of the RA-like effects of 4-HPR could be due to hydrolysis of 4-HPR. To explore this possibility we have prepared and are studying the nonhydrolyzable, carbon-linked 4-HPR analogue 4-hydroxybenzylretinone (**3**; 4-HBR).







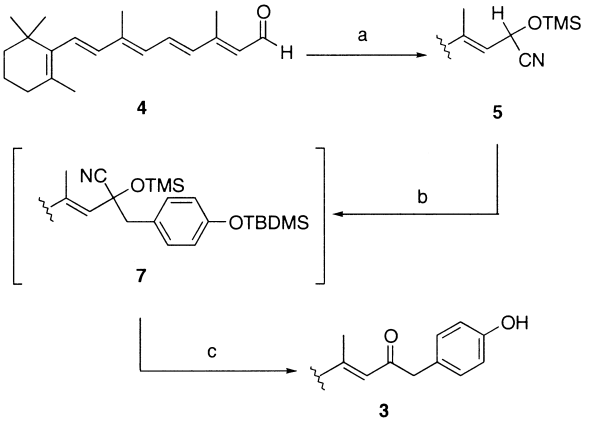


After exploring a number of approaches to 4-HBR, an *Umpolung*, or dipole inversion strategy,[13] was ultimately used in which a retinoid acyl anion equivalent was reacted with a suitable benzyl halide. As shown in

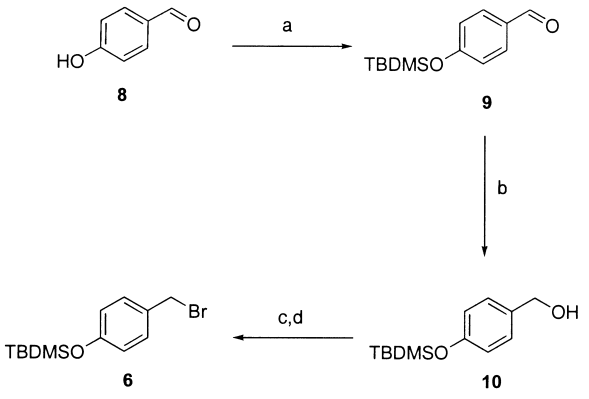
*Corresponding author. Fax: +1-614-292-2435; e-mail: curley.1@osu.edu

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00230-X

Scheme 1, reaction of retinal (**4**) with $TMSCN/Et_3N$[14] afforded the labile silylcyanohydrin **5**, which was used as obtained. Crude **5** was deprotonated (NaHMDS) and the resulting anion was alkylated with benzyl bromide **6**. The resulting compound **7** is deprotected (TBAF) as obtained to provide target **3**. The benzyl bromide **6**, which was employed as the electrophile, was prepared as shown in Scheme 2.[15] Surprisingly, **3** shows no evidence of existing in the enol form as determined by ^1H NMR in $CDCl_3$ or ethanol-d_6. However, it should be noted that once prepared, ketone **3** was found to undergo thermal and acid-catalyzed isomerization to the 13-*cis* isomer much more easily than 4-HPR, producing a 60:40 *trans*/*cis* mixture at equilibrium. With care to avoid exposure to excess acid or heat, or by using preparative reversed-phase HPLC (85% methanol/water), 4-HBR with no more than 5–10% *cis* isomer was obtained.



Scheme 1. (a) TMSCN, TEA (cat), CH_2Cl_2 (quant); (b) NaHMDS, THF then **6**; (c) TBAF, 9:1 THF/H_2O (65% from **5**).



Scheme 2. (a) NaH; TBDMSCl, THF (quant); (b) $NaBH_4$, EtOH (94%); (c) TFAA, THF (95%); (d) LiBr, THF (73%).

To justify detailed biological studies of 4-HBR and its use as a probe for the mechanism of action of 4-HPR, preliminary investigations of its actions have been conducted. Previously, we have found that 4-HPR and its analogues can shrink preformed mammary tumors.[4] Therefore, a pilot study of the relative antitumor activity of 4-HBR was undertaken in female rats treated ca. 50 days earlier with dimethylbenz[*a*]anthracene (DMBA) using previously described methods.[4] In this pilot study, three mammary tumor bearing rats/group were sacrificed after 1, 7, 14, and 21 days of consuming diet mixed with vehicle control or 2 mmol/kg of test retinoid. Blood and liver were collected at each time point and tumor volumes measured. The time course of the tumor volume changes for the three rats surviving the full 21 day experiment are shown in Table 1. While the sample size is small, it appears that 4-HPR and 4-HBR are indistinguishable in their substantial anti-

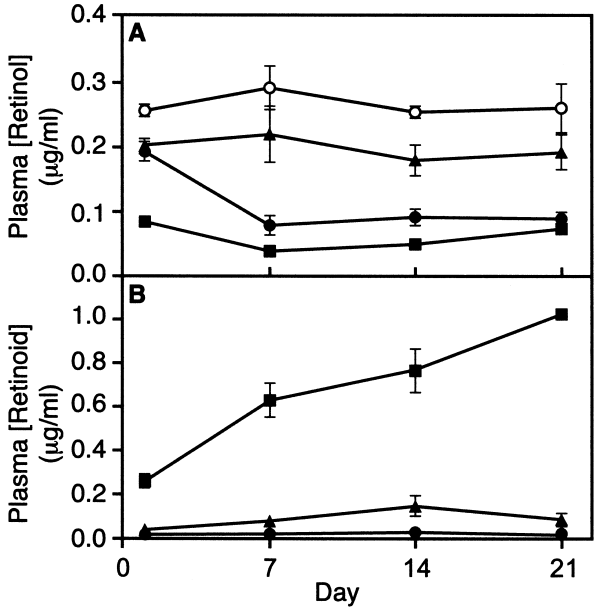


Figure 1. Plasma concentrations of: (a) retinol in control rats (○) and rats treated with 4-HPR (■), 4-HBR (▲) and RA (●) assessed by modifications of the method in ref 18; and (b) treatment retinoid (4-HPR, ■; 4-HBR, ▲; RA, ●). Values = mean ± SE; $N = 3$.

Table 1. Effect of retinoids on total mammary tumor volume[a]

Diet additive (2 mmol/kg)	Mean tumor volume (cm^3)[b]				
	Day 1	Day 7	Day 14	Day 21	% change[c]
Vehicle control	0.06 ± 0.01	0.08 ± 0.03	0.11 ± 0.05	0.15 ± 0.06	+257
4-HBR (**3**)	0.59 ± 0.43	0.47 ± 0.59	0.15 ± 0.17	0.09 ± 0.07	−85
4-HPR (**1**)	0.20 ± 0.09	0.17 ± 0.13	0.10 ± 0.08	0.05 ± 0.03	−77
RA (**2**)	0.69 ± 0.18	0.46 ± 0.06	0.27 ± 0.11	0.18 ± 0.08	−74

[a]At 21 days of feeding diet to three rats.
[b]Values = mean ± SE. Initial mean tumor volumes in control rats are smaller due to requirements of our approved animal use protocol which mandate that tumors not be necrotic nor too large by the experiment's end.
[c]For changes in tumor volume from day 1 to day 21, $p < 0.05$ versus control.

tumor activity in this model. In fact, compared to the control group, each treatment group had a significantly steep decline in tumor volume (all p values <0.05). During the course of this experiment, the diet that had been held for a maximum of 7 days in the animal room was extracted and analyzed by HPLC and showed no evidence of retinoid isomerization or decomposition (93% *trans* at start; 92% *trans* at day 7).

Previously, we have found that the body weight of animals eating retinoid-containing diets reflects retinoid toxicity[4] as does liver weight (unpublished results). While we found no difference in the liver weights among the treatment groups (data not shown), the body weights of the RA and 4-HPR-fed groups showed a substantial (5–6%) decline compared to the virtually unaffected 4-HBR and control-fed groups. This indicates that 4-HBR does not demonstrate any untoward toxicity at this dose and duration of feeding. Of perhaps greater importance, one of the major toxicities associated with 4-HPR therapy in humans is night blindness resulting from displacement of vitamin A (retinol) from its serum retinol binding protein (RBP),[16] thereby minimizing retinol delivery to the eye. As shown in Figure 1, there is much less of a reduction in plasma retinol concentration in animals fed 4-HBR when compared to 4-HPR and RA.[17] Although 4-HBR appears to compete as effectively as 4-HPR for [^{3}H]-retinol binding to RBP (Fig. 2), 4-HBR may cause less effect on plasma retinol levels in vivo because it achieves a lower concentration in the circulation than does 4-HPR (Fig. 1b).

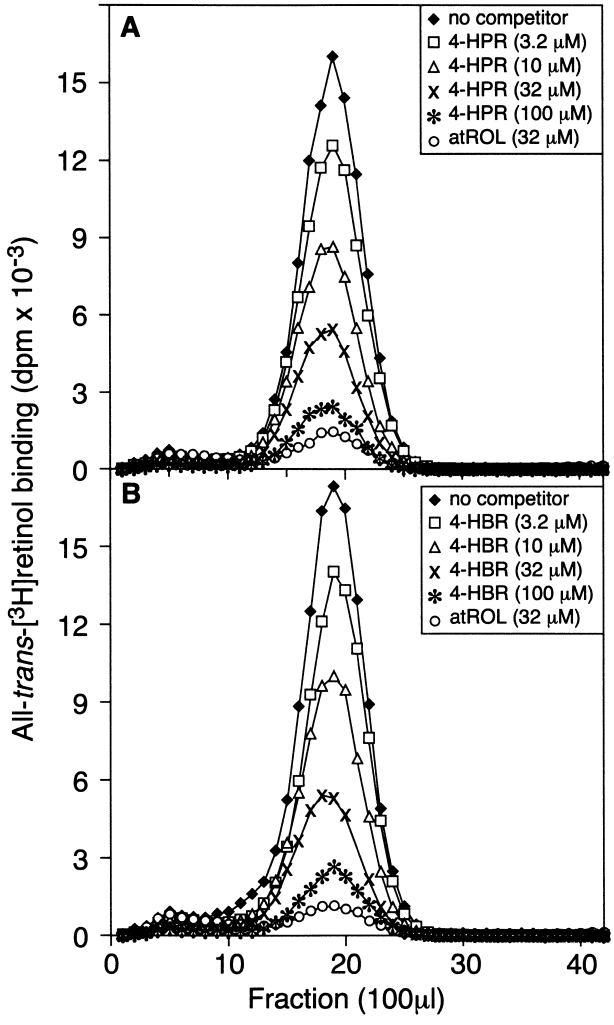


Figure 2. Sedimentation analysis of RBP binding to all-*trans*-[^{3}H]-retinol (250 nM; 4.7 Ci/mmol) in the absence and presence of unlabeled retinoid. RBP-containing samples were incubated with ligands on ice for 3 h, treated with dextran-coated charcoal and analyzed on 4–20% sucrose gradients.

Finally, in preliminary studies, 4-HBR has been found to bind poorly to RARs α, β, and γ, with an affinity similar to that of 4-HPR (for example, K_i's of 4-HBR, 4-HPR, and RA for RARγ are >4000, >4000, and 0.7 nM, respectively). In conclusion, 4-HBR appears to share many of the biological properties of 4-HPR, including its effectiveness as an antitumor agent. However, 4-HBR may have a significant advantage over 4-HPR since the nonhydrolyzable analogue causes a much reduced decline in serum retinol concentration which may lessen the risk of developing night blindness at therapeutic doses. Details of the chemistry, biochemistry, and biological activity of 4-HBR will be reported in due course as further studies are conducted.

Acknowledgements

Financial support in the form of a grant (CA49837) from the National Cancer Institute is gratefully acknowledged.

References and Notes

1. Moon, R. C.; Thompson, H. J.; Becci, P. J.; Grubb, C. J.; Gander, R. J.; Newton, D. L.; Smith, J. M.; Phillips, S. L.; Henderson, W. R.; Mullen, L. T.; Brown, C. C.; Sporn, M. B. *Cancer Res.* **1979**, *39*, 1339.
2. Kenel, M. F.; Krayer, J. H.; Merz, E. A.; Pritchard, J. F. *Teratog. Carcinog. Mutag.* **1988**, *8*, 1.
3. Veronesi, U.; DePalo, G.; Marubini, E.; Costa, A.; Formelli, F.; Mariani, L.; Decensi, A.; Camerini, T.; Rosselli Del Turco, M.; Gaetana Di Mauro, M.; Grazia Muraca, M.; Del Vecchio, M.; Pinto, C.; D'Aiuto, G.; Boni, C.; Campa, T.; Magni, A.; Miceli, R.; Perloff, M.; Malone, W. F.; Sporn, M. B. *J. Natl. Cancer Inst.* **1999**, *91*, 1847.
4. Abou-Issa, H.; Curley, R. W., Jr.; Panigot, M. J.; Tanagho, S. N.; Sidhu, B. S.; Alshafie, G. A. *Anticancer Res.* **1997**, *17*, 3335.
5. Chambon, P. A. *FASEB J.* **1996**, *10*, 940.
6. Fanjul, A. N.; Delia, D.; Pierotti, M. A.; Rideout, D.; Ya, J.-Q.; Pfahl, M. *J. Biol. Chem.* **1996**, *271*, 22441.
7. Abou-Issa, H. M.; Alshafie, G. A.; Curley, R. W., Jr.; Wong, M. F.; Clagett-Dame, M.; Repa, J. J.; Sikri, V. *Anticancer Res.* **1999**, *19*, 999.
8. Sheikh, M. S.; Shao, Z.-M.; Li, X.-S.; Ordonez, T.; Niles, R. M.; Fontana, J. A. *Carcinogenesis* **1995**, *16*, 2477.
9. Delia, D.; Aiello, A.; Lombardi, L.; Pelicci, P. G.; Grignani, F.; Formelli, F.; Menard, S.; Costa, A.; Veronesi, U.; Pierotti, M. A. *Cancer Res.* **1993**, *53*, 6036.
10. Clifford, J. L.; Menter, D. G.; Wang, M.; Lotan, R.; Lippman, S. M. *Cancer Res.* **1999**, *59*, 14.

11. Shih, T.-W.; Shealy, Y. F.; Hill, D. L. *Drug Metab. Disp.* **1988**, *14*, 337.
12. Hultin, T. A.; May, C. M.; Moon, R. C. *Drug Metab. Disp.* **1986**, *14*, 714.
13. Seebach, D. *Angew. Chem.* **1979**, *91*, 259.
14. Kobayashi, S.; Tsuchiya, Y.; Mukaiyama, T. *Chem. Lett.* **1991**, 537.
15. Olszewski, J. D.; Marshalla, M.; Sabat, M.; Sundberg, R. J. *J. Org. Chem.* **1994**, *59*, 4285.
16. Berni, R.; Formelli, F. *FEBS Lett.* **1992**, *308*, 43.
17. Berni, R.; Clerici, M.; Malpeli, G.; Cleris, L.; Formelli, F. *FASEB J.* **1993**, *7*, 1179.
18. Meyer, E.; Lambert, W. E.; DeLeenheer, A. P. *Clin. Chem.* **1994**, *40*, 48.