Structure-Activity Relationships of Retinoids in Hamster Tracheal Organ Culture

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ABSTRACT

Structure-activity relationships are summarized for 87 retinoids, using reversal of keratinization in the hamster tracheal organ culture system to measure biological activity. Classes of compounds evaluated included all-trans-retinoic acid and its esters, ring-modified analogs of all-trans-retinoic acid and its esters, side-chain-modified analogs of all-trans-retinoic acid and its esters, analogs in which both ring and side chain have been modified, all-trans-retinol and derivatives, all-trans-retiny1 amine derivatives, all-trans-retinal derivatives, all-trans-retinoic acid amides, 13-cis-retinoic acid and derivatives, and 5,6-epoxyretinoids. The activity of many synthetic amide derivatives of all-trans- or 13-cis-retinoic acid approaches that of the parent compounds. No metabolite of all-trans- or 13-cis-retinoic acid has yet been identified which has greater activity than the parent compounds in this assay. New synthetic derivatives with a gem-dimethyl group at position 4 in the cyclohexenyl ring and two aromatic rings in the side chain have activity equal to or greater than that of all-trans- or 13-cis-retinoic acid, with some activity detectable in the $10^{-11}$ M range.

INTRODUCTION

This article summarizes data obtained during the past 7 years in a collaborative testing program which has evaluated the biological activity of new synthetic retinoids, using the hamster tracheal organ culture assay developed in the laboratories of the Lung Cancer Branch and Laboratory of Chemoprevention of the National Cancer Institute. This testing has involved a collaborative effort on the part of the United States government; the pharmaceutical industry in the United States, Switzerland, and Germany; and the academic community and research institutes in America. The eventual goal is to develop a safe and effective pharmacological agent for prevention of common forms of cancer in men and women, one which would be used as a chronic prophylactic measure to inhibit the development of invasive cancer, much in the same manner in which fluoride is used for prevention of dental caries.

Since the tracheal organ culture assay measures the intrinsic ability of retinoids to control epithelial cell differentiation (6, 31), it is believed to have significant predictive value for the potential use of a new retinoid for prevention of epithelial cancer. Obviously, any in vitro test has dangerous liabilities for prediction of in vivo activity; in spite of these limitations, the tracheal organ culture assay is a most valuable procedure for initial evaluation of the biological activity of a new retinoid. It is an extremely sensitive assay and is used routinely to evaluate activity of new retinoids, the concentration of which may be as low as $10^{-10}$ to $10^{-11}$ M during the assay procedure. Thus, it is possible to measure biological activity with less than 1 mg of a new retinoid; with radioactively labeled retinoid metabolites, assays have been performed with only a few gg of material. The length of assay is relatively brief, and test results may be evaluated within 1 month after beginning the assay.

This article provides data on 87 retinoids, from a total set of over 30,000 hamster tracheas which have been used for assays at the National Cancer Institute. Each trachea has come from a separate animal which had been raised on a vitamin A-deficient diet. From each hamster, the trachea was removed under sterile conditions and maintained in organ culture in vitro for 3 to 10 days, as described below. Data are shown for retinoids in which the ring, side-chain, and polar terminal group of the basic molecule have been modified.

MATERIALS AND METHODS

Standard Assay Procedure. The standard assay procedure measures the ability of retinoids to reverse keratinization in a defined in vitro system (6, 19). Tracheas from hamsters that were in very early stages of vitamin A deficiency (5) were placed in organ culture. At the time of culture, the animals were 29 to 31 days old (they had been weaned at 21 days) and were still gaining some weight, with an average weight of 47 to 52 g. Their tracheal epithelium was generally low columnar or cuboidal, with only occasional patches of squamous metaplasia. Each trachea was opened from the larynx to the carina along the membranous dorsal wall and cultured in a serum-free medium (CMRL-1066 supplemented with crystalline bovine insulin, 1.0 $\mu$g/ml; hydrocortisone hemisuccinate, 0.1 $\mu$g/ml; glucose, 2 mm; penicillin, 100 units/ml; and streptomycin, 100 $\mu$g/ml). Cultures were gassed with 50% oxygen, 45% nitrogen, and 5% CO$_2$. The culture dishes were rocked at 35.5 to 36.0 degrees to allow the tracheas contact with both gas and medium. All tracheas were maintained in medium containing no retinoid for the first 3 days. At the end of 3 days, some tracheas were harvested; almost all of these tracheas had significant squamous metaplasia; in a set of several thousand such cultures approximately 60% had keratinized lesions. The remaining tracheas were then divided into different groups which were then treated with either: (a) retinoid dissolved in spectrograde DMSO$^2$ (final concentration of DMSO in culture medium was never greater than 0.1%) or (b) an equivalent amount of DMSO alone. Culture medium was changed 3 times a week, and all of the remaining tracheas were harvested at the end of 10 days in culture. Tracheas were fixed in 10% buffered formalin and embedded in paraffin. Cross-sections of 5 $\mu$m were made through the midportion, stained with hema-

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1 To whom requests for reprints should be addressed.
Received April 24, 1980; accepted July 2, 1980.

2 The abbreviation used is: DMSO, dimethyl sulfoxide.
toxylin and eosin, and then scored with a microscope for the presence of keratin and keratohyaline granules, both of which were found in approximately 90% of control cultures (in a set of several thousand such cultures) that had received no retinoid for the entire 10-day culture period. Analogs were scored as “inactive” if both keratin and keratohyaline granules were seen; they were scored as “active” if neither keratin nor keratohyaline granules were seen.

**Retinoids.** Retinoids were received in sealed ampuls, under argon or nitrogen. These sealed ampuls were stored at −20°C or below. Once opened, ampuls were stored in a vapor-phase nitrogen freezer. Stock solutions of retinoids were made in DMSO, at 0.1 to 1.0 mg/ml, and vials were stored in a vapor-phase nitrogen freezer. After thawing, contents of a given vial were used only once and then discarded. All work with retinoids was performed under subdued light or in rooms with “gold” fluorescent lamps.

**RESULTS**

Charts 1 to 11 show the activity of 87 retinoids in the hamster tracheal organ culture assay. Dose-response curves (19) were made for each retinoid, and the values derived for the dose effective in suppressing keratinization in one-half of the cultures have been tabulated. All-trans-retinoic acid [50% effective dose, 3 × 10−11 M in a total of 1853 cultures (Chart 1)] has been used as the reference substance. Most of the analogs in which the ring (Chart 2) or the side chain (Chart 3) has been modified are significantly less active in the tracheal organ culture assay. Retinol, retinyl acetate, and retinyl ethers (Chart 4) are all less active than retinoic acid, as are retinyl amine derivatives (Chart 5). A large number of retinoid derivatives have been made, some of which have been useful in various biological studies (3, 7, 25), but none are as active as retinoic acid in tracheal organ culture (Chart 6). In view of their relative ease of synthesis from either retinolyl chloride or retinolyl imidazole and the appropriate amine, a particularly large number of amide derivatives have been synthesized and tested (Chart 7). Derivatization of the polar terminus of the retinoid molecule to form amides offers the possibility of synthesizing a set of compounds with an especially wide range of chemical properties, including derivatives which have the interesting property of being soluble in both water and chloroform, as in the case of 2-retinamidoethyl sodium sulfate (Chart 7). Most of these compounds have appreciable activity in the 10−9 to 10−10 M range, with the notable exception of sterically hindered amides such as tert-butyl retinamide or 1-methyl-3-hydroxypropyl retinamide, suggesting that, as a class, the amides must be hydrolyzed to retinoic acid before exerting their biological activity. Several of the retinamides have been reported to be significantly less toxic than is all-trans-retinoic acid and to be useful for inhibition of carcinogenesis in experimental animals (11, 18, 24). Other modifications of the polar terminus (Chart 8), particularly the addition of 1 or 2 carbon atoms, have led to analogs in which activity has been significantly diminished.

13-cis-Retinoic acid and several of its amide derivatives (Chart 9) are highly active in tracheal organ culture. It has recently been suggested (9) that isomerization of all-trans-retinoic acid to the 13-cis-isomer may occur during physiological action of all-trans-retinoic acid. Newly synthesized 13-cis-retinamides (Chart 9) are currently in initial stages of evaluation to determine if they will be more useful than all-trans-retinamides as inhibitors of carcinogenesis. Chart 10 shows 2 new structures, in which both the ring and the side chain of the retinoid molecule have been modified. In these retinoids, a gem-dimethyl group has been inserted at position 4 of the cyclohexenyl ring and 2 aromatic rings have been introduced into the side chain; these new structures are intensely active. In view of the suggestion that 5,6-epoxyretinoic acid might be an activated form of retinoic acid (16, 29), this compound, as well as 3 related analogs (5, 6-epoxyretinoic acid methyl ester, 5,6-epoxyretinyl acetate, and the 5,6-epoxy analog of retinylidene dimedone), were tested (Chart 11). All of these epoxides are significantly less active in the tracheal organ culture assay than were their respective parent compounds.

The tracheal organ culture assay is highly specific for retinoid structures. A wide variety of other terpenoid and related structures have been tested and been found to be inactive (data not shown); these compounds include juvenile hormones I, II, and III; mycophenolic acid, and abscisic acid. Although β-ionone has not been tested, it is apparent that the side chain is of major importance for biological activity, since the C10 and C17 analogs of retinoic acid (Chart 3) are almost totally inactive, and each of the 4 isomeric dihydroretinoic acid analogs (Chart 5) is markedly less active than is all-trans-retinoic acid or its methyl and ethyl esters. Similarly, the C18 ketone (Chart 8) was totally inactive at 1 × 10−8 M.

**DISCUSSION**

The data shown in Charts 1 to 11 indicate that a very wide range of modifications in the ring, side chain, or polar terminus of the retinoid molecule can be made and still allow expression of biological activity. However, it is noteworthy that few analogs have been made that are more active than either all-trans- or 13-cis-retinoic acid. In particular, we find no evidence that a 5,6-epoxide is an activated form (16, 29), although such a molecule might require synthesis in situ in a cell in order to demonstrate enhanced biological activity. Similarly, both 4-hydroxy- and 4-ketoretinoids, which are early products of oxidative metabolism of retinoic acid (8), were found to be markedly less active than all-trans-retinoic acid. The possibility that retinoic acid might also be activated by epoxidation at the 9, 10 or 11,12 double bonds cannot presently be ruled out, since these compounds have not yet been definitively synthesized and tested for biological activity. However, there is little or no evidence at present that any natural, known retinoid metabolite is more active in the tracheal organ culture assay than are either all-trans- or 13-cis-retinoic acid. It is interesting that in many other in vitro test systems retinoic acid has consistently been more active than retinol, retinyl esters, or retinal (4, 14, 23, 27, 30). The correlations of activity of retinoids in this tracheal system with many other in vitro test systems are excellent. Such test systems include reversal of changes induced by carcinogens in mouse prostate organ cultures (4, 12), suppression of keratinization in chick skin organ cultures (30), inhibition of induction of ornithine decarboxylase in mouse skin (27, 28), inhibition of growth of murine melanoma cells (13, 14), enhancement of adhesion of transformed mouse fibroblasts (1), suppression of malignant transformation in a cloned mouse fibroblast cell line (3), and binding to cellular retinoic acid-binding proteins (26). It is of interest that the present tracheal assay system gives a broader range of positive...
results than do some of the other test systems, particularly with amide derivatives, which show rather low activity in several other assays (1, 14, 23, 27, 30). Presumably, the trabecular system has the enzymes necessary to hydrolyze retinamides; the low activity of a sterically hindered amide such as tert-butyl retinamide is again noteworthy in this context. Finally, the intense activity of the retinoids in Chart 10 suggests that chemical synthesis can provide new molecules which may have a stronger interaction with a receptor molecule or site than does all-trans-retinoic acid, in a manner analogous to the enhanced binding of 9-$\alpha$-fluoroglutocorticoids to hydrocortisone receptors.

Although the successful use of retinoids for prevention of lethal cancer in men and women has yet to be demonstrated, recent developments in this field are highly encouraging. It is by now well established that retinoids can prevent cancer of the lung, bladder, breast, and skin in experimental animals (2, 15, 18, 20, 21, 28); more recently, it has been shown in vitro that retinoids can directly suppress malignant transformation of cells caused by chemicals, radiation, or viral transforming factors (3, 10, 17, 25). Very low, nontoxic concentrations have been used in these experiments in cell culture, and these findings raise further hopes that retinoids will be valuable tools to provide needed understanding of the process of carcinogenesis, as well as a practical approach to the prevention of disease. It remains to be determined whether the best approach to cancer prevention with new synthetic retinoids will involve a further increase in their intrinsic biological activity (as can be measured with the present assay) or perhaps will involve a further decrease in their intrinsic toxicity, as we have suggested elsewhere (22).

ACKNOWLEDGMENTS

We would like to express our appreciation to the following: Dr. John J. Burns, Dr. Willy Leimgruber, and Dr. Beverly A. Pawan (Hoffman-La Roche Inc.); Dr. Werner Bollag (Hoffman-La Roche & Co., AG); Dr. Robert J. Gander and Dr. Gavin Hildick-Smith (Johnson & Johnson); and Dr. Wolfgang Blechschmidt, Dr. Fritz Frickel, Dr. Axel Nürenbach, Dr. Joachim Paust, and Dr. Horst Pommer (BASF Aktiengesellschaft) for the immense help which all 3 of these companies have given to this testing program; to Dr. Jerome Berson, Dr. Arnold Brossi, Dr. Guenter Carney, and Dr. John Bieri, Dr. Albert Kapikian, Dr. Gerald Clamon, Dr. John Bieri, Dr. Mary Baker, Dr. William Dauben, Dr. Harian Goering, Dr. Richard K. Hill, and Dr. William C. Hendriksen (BASF Aktiengesellschaft) for their generous advice and counsel on synthesis of retinoids; to Dr. Wolfgang Blechschmidt, Dr. Werner Bollag (F. Hoffmann-La Roche & Co., AG); Dr. Robert J. Gander and Dr. Fritz Frickel, Dr. Axel Nürenbach, Dr. Joachim Paust, and Dr. Horst Pommer for their help with the preparation of this article.

REFERENCES


Key to the Charts

Sources of retinoids were as follows: a, Nancy Acton and Arnold Broissi, National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Md.; b, BASF Aktiengesellschaft, Ludwigshafen am Rhein, Germany; c, Ralph S. Becker, University of Houston, Houston, Texas; d, F. Ivy Carroll, Research Triangle Institute, Research Triangle Park, N. C.; e, Marcia I. Dawson, SRI International, Menlo Park, Calif.; f, Clayton H. Heathcock, University of California, Berkeley, Calif.; g, Hoffmann-La Roche Inc., Nutley, N. J.; and F. Hoffmann-La Roche & Co., AG, Basel, Switzerland; h, Johnson & Johnson, New Brunswick, N. J.; i, Koji Nakanishi, Columbia University, New York, N. Y.; j, Y. Fulmer Shealy, Southern Research Institute, Birmingham, Ala.; k, Steven C. Welch, University of Houston, Houston, Texas. ED_{50}, dose effective in suppressing keratinization in one-half of cultures.

Chart 5. Structure and activity of all-trans-retinyl amine derivatives.
Chart 7. Structure and activity of all-trans-retinoic acid amides.
Chart 8. Structure and activity of additional modifications of the polar terminus of the retinoid molecule.
Chart 11. Structure and activity of 5,6-all-trans-epoxyretinoids.

1. All-trans-Retinoic Acid and Retinoic Acid Esters

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<table>
<thead>
<tr>
<th>Structure, R =</th>
<th>Trivial Name</th>
<th>Source</th>
<th>ED_{50-M}</th>
<th>(Number of Cultures)</th>
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<tbody>
<tr>
<td>H</td>
<td>Retinoic acid, Tretinoin</td>
<td>b, g</td>
<td>3 x 10^{-11}</td>
<td>(1853)</td>
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<td>CH₃</td>
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<td>g</td>
<td>3 x 10^{-10}</td>
<td>(139)</td>
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<tr>
<td>C₂H₅</td>
<td>Retinoic acid ethyl ester</td>
<td>g</td>
<td>5 x 10^{-10}</td>
<td>(124)</td>
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## 2. Ring-modified Analogs of All-\textit{trans}-Retinoic Acid and its Esters

![Structure of retinoic acid analogs](image)

<table>
<thead>
<tr>
<th>Structure, ( R = )</th>
<th>Trivial Name</th>
<th>Source</th>
<th>( \text{ED}_{50}, \text{M} )</th>
<th>(Number of Cultures)</th>
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<td><img src="image" alt="Structure" /></td>
<td>alpha-Retinoic acid</td>
<td>( g )</td>
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<td>5,6-Dihydroretinoic acid</td>
<td>( g )</td>
<td>(&lt; 1 \times 10^{-9})</td>
<td>(45)</td>
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<td><img src="image" alt="Structure" /></td>
<td>5,6-Dihydroxyretinoic acid methyl ester</td>
<td>( g )</td>
<td>(2 \times 10^{-7})</td>
<td>(12)</td>
</tr>
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<td>4-Hydroxyretinoic acid</td>
<td>( g )</td>
<td>(7 \times 10^{-10})</td>
<td>(43)</td>
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<tr>
<td><img src="image" alt="Structure" /></td>
<td>4-Ketoretinoic acid</td>
<td>( g )</td>
<td>(7 \times 10^{-10})</td>
<td>(35)</td>
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<tr>
<td><img src="image" alt="Structure" /></td>
<td>Phenyl analog of retinoic Acid</td>
<td>( g )</td>
<td>(1 \times 10^{-6})</td>
<td>(29)</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>4-Methoxy-2,3,6-trimethylphenyl analog of retinoic acid; TMMP analog of retinoic acid</td>
<td>( g )</td>
<td>(5 \times 10^{-9})</td>
<td>(228)</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>4-Methoxy-2,3,6-trimethylphenyl analog of retinoic acid ethyl ester; TMMP analog of retinoic acid ethyl ester; Etretinate</td>
<td>( g )</td>
<td>(2 \times 10^{-8})</td>
<td>(92)</td>
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<tr>
<td><img src="image" alt="Structure" /></td>
<td>4-Hydroxy-2,3,6-trimethylphenyl analog of retinoic acid ethyl ester</td>
<td>( g )</td>
<td>Inactive, 12/13 cultures, (1 \times 10^{-7})</td>
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<tr>
<td><img src="image" alt="Structure" /></td>
<td>Dimethylacetyl cyclopentenyl analog of retinoic acid</td>
<td>( g )</td>
<td>(5 \times 10^{-10})</td>
<td>(103)</td>
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## 2. Ring-modified Analogs of All-trans-Retinoic Acid and its Esters (Cont’d)

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<th>Structure, R =</th>
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<th>( \text{ED}_{50}, \text{ M} )</th>
<th>(Number of Cultures)</th>
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<tr>
<td><img src="image1" alt="Structure" /></td>
<td>Dimethylmethoxyethyl cyclopentenyl analog of retinoic acid</td>
<td>( g )</td>
<td>( 2 \times 10^{-10} )</td>
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<tr>
<td><img src="image2" alt="Structure" /></td>
<td>2-Furyl analog of retinoic acid</td>
<td>( g )</td>
<td>Inactive, 10/13 cultures, ( 1 \times 10^{-6} )</td>
<td>(27)</td>
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<tr>
<td><img src="image3" alt="Structure" /></td>
<td>3-Thienyl analog of retinoic acid</td>
<td>( b )</td>
<td>( &gt;1 \times 10^{-8} )</td>
<td>(23)</td>
</tr>
<tr>
<td><img src="image4" alt="Structure" /></td>
<td>3-Pyridyl analog of retinoic acid</td>
<td>( g )</td>
<td>( 3 \times 10^{-7} )</td>
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### 3. Side Chain-modified Analogs of All-trans-Retinoic Acid and its Esters

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<tr>
<td></td>
<td>7,8-Dehydroretinoic acid</td>
<td>b</td>
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<tr>
<td></td>
<td>7,8-Dihydroretinoic acid</td>
<td>g</td>
<td>$1 \times 10^{-8}$</td>
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<td></td>
<td>11,12-Dihydroretinoic acid methyl ester</td>
<td>g</td>
<td>$4 \times 10^{-8}$</td>
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<td>13,14-Dihydroretinoic acid ethyl ester</td>
<td>g</td>
<td>$3 \times 10^{-8}$</td>
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<tr>
<td></td>
<td>C$_{15}$ analog of retinoic acid</td>
<td>g</td>
<td>Inactive, 7/7 cultures, $1 \times 10^{-6}$ M</td>
<td>(13)</td>
</tr>
<tr>
<td></td>
<td>C$_{17}$ analog of retinoic acid</td>
<td>f</td>
<td>Inactive, 6/6 cultures, $1 \times 10^{-8}$ M</td>
<td>(12)</td>
</tr>
<tr>
<td></td>
<td>C$_{22}$ analog of retinoic acid</td>
<td>c, g</td>
<td>$3 \times 10^{-9}$</td>
<td>(17)</td>
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<tr>
<td></td>
<td>Aryltiene analog of retinoic acid (trans)</td>
<td>e</td>
<td>$2 \times 10^{-10}$</td>
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<tr>
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<td>Aryltiene analog of retinoic acid (cis)</td>
<td>e</td>
<td>$&gt;1 \times 10^{-9}$</td>
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4. All-trans-Retinol, Retinyl Esters, and Retinyl Ethers

![Structure](image)

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<tr>
<td>H</td>
<td>Retinol</td>
<td>$b,g$</td>
<td>$7 \times 10^{-10}$</td>
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<td>COCH$_3$</td>
<td>Retinyl acetate</td>
<td>$b,g$</td>
<td>$1 \times 10^{-9}$</td>
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<td>CH$_3$</td>
<td>Retinyl methyl ether</td>
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<td>C$_4$H$_9$</td>
<td>Retinyl butyl ether</td>
<td>$b,g$</td>
<td>$3 \times 10^{-8}$</td>
<td>(71)</td>
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<td>C$_6$H$_5$</td>
<td>Retinyl phenyl ether</td>
<td>$g$</td>
<td>$8 \times 10^{-8}$</td>
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5. All-trans-Retinyl Amine Derivatives

![Structure](image)

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<tr>
<th>Structure, R$_1$, R$_2$</th>
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<tr>
<td>H, COCH$_3$</td>
<td>N-Acetyl retinyl amine</td>
<td>$b,g$</td>
<td>$9 \times 10^{-9}$</td>
<td>(113)</td>
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<tr>
<td>H, COC$_6$H$_5$</td>
<td>N-Benzoyl retinyl amine</td>
<td>$b$</td>
<td>$2 \times 10^{-9}$</td>
<td>(61)</td>
</tr>
<tr>
<td>CH$_3$, COCH$_3$</td>
<td>N-Methyl N-acetyl retinyl amine</td>
<td>$d$</td>
<td>$&gt;1 \times 10^{-9}$</td>
<td>(35)</td>
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<tr>
<td>CH$_3$, COC$_6$H$_5$</td>
<td>N-Methyl N-benzoyl retinyl amine</td>
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<td>$1 \times 10^{-9}$</td>
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## 6. All-trans-Retinal and Derivatives

![Structure diagram](image)

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<td>O</td>
<td>Retinal</td>
<td>b, g</td>
<td>3 × 10^{-10}</td>
<td>(98)</td>
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<tr>
<td>NNHCOCH₃</td>
<td>Retinal acetylhydrazone</td>
<td>g</td>
<td>4 × 10^{-10}</td>
<td>(54)</td>
</tr>
<tr>
<td>NOH</td>
<td>Retinal oxime</td>
<td>g</td>
<td>1 × 10^{-8}</td>
<td>(61)</td>
</tr>
<tr>
<td>C(COCH₃)₂</td>
<td>3-Retinylidene-2,4-pentanедione; retinylidene acetylacetone</td>
<td>a</td>
<td>2 × 10^{-9}</td>
<td>(86)</td>
</tr>
<tr>
<td>C(COCH₂CH₃)₂</td>
<td>4-Retinylidene-3,5-heptanедione</td>
<td>a</td>
<td>4 × 10^{-9}</td>
<td>(12)</td>
</tr>
<tr>
<td>C(COCH₂CH₂CH₃)₂</td>
<td>5-Retinylidene-4,6-nonanедione</td>
<td>a</td>
<td>Inactive, 6/7 cultures, 1 × 10^{-8} M</td>
<td>(14)</td>
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<tr>
<td>COCH₂</td>
<td>2-Retinylidene-1,3-cycloпentанедione</td>
<td>a</td>
<td>Inactive, 16/17 cultures, 1 × 10^{-8} M</td>
<td>(29)</td>
</tr>
<tr>
<td>COCH₂</td>
<td>2-Retinylidene-1,3-cyclohexанедione</td>
<td>a</td>
<td>1 × 10^{-10}</td>
<td>(107)</td>
</tr>
<tr>
<td>COCH₂</td>
<td>2-Retinylidene-5, 5-dimethyl 1,3-cyclohexанедione; Retinylidene dimesdon</td>
<td>a</td>
<td>2 × 10^{-10}</td>
<td>(150)</td>
</tr>
<tr>
<td>COCH₂CH₂</td>
<td>2-Retinylidene-1,3-cycloheptанедione</td>
<td>a</td>
<td>4 × 10^{-10}</td>
<td>(51)</td>
</tr>
<tr>
<td>COCH₂CH₂</td>
<td>2-Retinylidene-1,3-cyclooctanедione</td>
<td>a</td>
<td>2 × 10^{-10}</td>
<td>(37)</td>
</tr>
<tr>
<td>COCH₂CH₂CH₂</td>
<td>2-Retinylidene-1,3-cyclonанедione</td>
<td>a</td>
<td>2 × 10^{-10}</td>
<td>(36)</td>
</tr>
</tbody>
</table>
### 7. All-trans-Retinoic Acid Amides

![Chemical Structure of Retinoic Acid Amides](image)

<table>
<thead>
<tr>
<th>Structure, R =</th>
<th>Trivial Name</th>
<th>Source</th>
<th>ED$_{50}$ M</th>
<th>(Number of Cultures)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_2$H$_5$</td>
<td>N-Ethyl retinamide</td>
<td>$g$</td>
<td>$1 \times 10^{-9}$</td>
<td>(86)</td>
</tr>
<tr>
<td>n-C$_3$H$_7$</td>
<td>N-Propyl retinamide</td>
<td>$g,h$</td>
<td>$2 \times 10^{-10}$</td>
<td>(62)</td>
</tr>
<tr>
<td>n-C$_4$H$_9$</td>
<td>N-n-Butyl retinamide</td>
<td>$g,h$</td>
<td>$7 \times 10^{-10}$</td>
<td>(39)</td>
</tr>
<tr>
<td>t-C$_4$H$_9$</td>
<td>N-t-Butyl retinamide</td>
<td>$h$</td>
<td>$&gt; 1 \times 10^{-8}$</td>
<td>(22)</td>
</tr>
<tr>
<td>2-C$_2$H$_4$OH</td>
<td>N-2-Hydroxyethyl retinamide</td>
<td>$g,h$</td>
<td>$1 \times 10^{-10}$</td>
<td>(68)</td>
</tr>
<tr>
<td>2-C$_3$H$_6$OH</td>
<td>N-2-Hydroxypropyl retinamide</td>
<td>$b$</td>
<td>$3 \times 10^{-10}$</td>
<td>(22)</td>
</tr>
<tr>
<td>3-C$_3$H$_6$OH</td>
<td>N-3-Hydroxypropyl retinamide</td>
<td>$b$</td>
<td>$2 \times 10^{-10}$</td>
<td>(21)</td>
</tr>
<tr>
<td>2,3-C$_3$H$_5$(OH)$_2$</td>
<td>N-2,3-Dihydroxypropyl retinamide</td>
<td>$b$</td>
<td>$2 \times 10^{-10}$</td>
<td>(22)</td>
</tr>
<tr>
<td>CH(CH$_3$)CH$_2$CH$_2$OH</td>
<td>N-1-Methyl-3-hydroxypropyl retinamide</td>
<td>$b$</td>
<td>$&gt; 1 \times 10^{-9}$</td>
<td>(40)</td>
</tr>
<tr>
<td>4-n-C$_4$H$_8$OH</td>
<td>N-4-Hydroxybutyl retinamide</td>
<td>$b$</td>
<td>$2 \times 10^{-10}$</td>
<td>(41)</td>
</tr>
<tr>
<td>C$_6$H$_5$</td>
<td>N-Phenyl retinamide</td>
<td>$g,h$</td>
<td>$4 \times 10^{-10}$</td>
<td>(31)</td>
</tr>
<tr>
<td>2-C$_6$H$_4$OH</td>
<td>N-2-Hydroxyphenyl retinamide</td>
<td>$h$</td>
<td>$1 \times 10^{-9}$</td>
<td>(60)</td>
</tr>
<tr>
<td>3-C$_6$H$_4$OH</td>
<td>N-3-Hydroxyphenyl retinamide</td>
<td>$h$</td>
<td>$3 \times 10^{-10}$</td>
<td>(55)</td>
</tr>
<tr>
<td>4-C$_6$H$_4$OH</td>
<td>N-4-Hydroxyphenyl retinamide</td>
<td>$h$</td>
<td>$3 \times 10^{-10}$</td>
<td>(77)</td>
</tr>
<tr>
<td>4-C$_6$H$_4$OCOC$_4$H$_9$</td>
<td>N-4-Pivaloyloxyphenyl retinamide</td>
<td>$h$</td>
<td>$2 \times 10^{-10}$</td>
<td>(41)</td>
</tr>
</tbody>
</table>
Structure-Activity Relationships of Retinoids

7. All-trans-Retinoic Acid Amides (Cont’d)

<table>
<thead>
<tr>
<th>Structure, R =</th>
<th>Trivial Name</th>
<th>Source</th>
<th>ED$_{50}$·M</th>
<th>(Number of Cultures)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-C$_6$H$_4$COOH</td>
<td>N-2 Carboxyphenyl retinamide</td>
<td>b</td>
<td>$&lt; 1 \times 10^{-10}$</td>
<td>(57)</td>
</tr>
<tr>
<td>3-C$_6$H$_4$COOH</td>
<td>N-3 Carboxyphenyl retinamide</td>
<td>b</td>
<td>$1 \times 10^{-10}$</td>
<td>(42)</td>
</tr>
<tr>
<td>4-C$_6$H$_4$COOH</td>
<td>N-4 Carboxyphenyl retinamide</td>
<td>b</td>
<td>$1 \times 10^{-10}$</td>
<td>(70)</td>
</tr>
<tr>
<td>CN$_4$H</td>
<td>N-5-Tetrazolyl retinamide</td>
<td>b</td>
<td>$&lt; 1 \times 10^{-10}$</td>
<td>(65)</td>
</tr>
<tr>
<td>CH$_2$CH$_2$OSO$_3$Na</td>
<td>N-2 Retinamidoethyl sodium sulfate</td>
<td>e</td>
<td>$2 \times 10^{-10}$</td>
<td>(14)</td>
</tr>
</tbody>
</table>

8. Additional Modifications of the Polar Terminus of the Retinoid Molecule

<table>
<thead>
<tr>
<th>Structure, R =</th>
<th>Trivial Name</th>
<th>Source</th>
<th>ED$_{50}$·M</th>
<th>(Number of Cultures)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHCH$_3$</td>
<td>Axerophthene</td>
<td>b</td>
<td>$2 \times 10^{-9}$</td>
<td>(86)</td>
</tr>
<tr>
<td>CHCH$_2$CH$_3$</td>
<td>Retinyl methane; 15-Methyl axerophthene</td>
<td>b</td>
<td>$&gt;1 \times 10^{-8}$</td>
<td>(11)</td>
</tr>
<tr>
<td>CICH$_3$</td>
<td>14-Methyl axerophthene</td>
<td>b</td>
<td>$&gt;1 \times 10^{-8}$</td>
<td>(12)</td>
</tr>
<tr>
<td>CHCOCH$_3$</td>
<td>Methyl retinone</td>
<td>i</td>
<td>$2 \times 10^{-7}$</td>
<td>(37)</td>
</tr>
<tr>
<td>CHC(CH$_3$)$_2$OH</td>
<td>15-Dimethyl retinol</td>
<td>a</td>
<td>$&gt;1 \times 10^{-8}$</td>
<td>(9)</td>
</tr>
<tr>
<td>CHCH$_2$SCOCH$_3$</td>
<td>Retinyl thioacetate</td>
<td>k</td>
<td>$&gt;1 \times 10^{-8}$</td>
<td>(13)</td>
</tr>
<tr>
<td>0</td>
<td>C$_{18}$ Ketone</td>
<td>c</td>
<td>Inactive, 8/8 cultures, $1 \times 10^{-8}$ M</td>
<td>(8)</td>
</tr>
</tbody>
</table>
9. 13-cis-Retinoic Acid and Derivatives

![Chemical structure of 13-cis-Retinoic Acid and Derivatives]

<table>
<thead>
<tr>
<th>Structure, R =</th>
<th>Trivial Name</th>
<th>Source</th>
<th>ED_{50, M}</th>
<th>(Number of Cultures)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH</td>
<td>13-cis-Retinoic acid; Isotretinoin</td>
<td>b, g</td>
<td>$3 \times 10^{-11}$</td>
<td>(69)</td>
</tr>
<tr>
<td>NH(C_{2}H_{5})</td>
<td>N-Ethyl 13-cis-retinamide</td>
<td>j</td>
<td>$3 \times 10^{-10}$</td>
<td>(37)</td>
</tr>
<tr>
<td>NH(2-C_{2}H_{4}OH)</td>
<td>N-2-Hydroxyethyl 13-cis-retinamide</td>
<td>j</td>
<td>$3 \times 10^{-10}$</td>
<td>(40)</td>
</tr>
<tr>
<td>NH(2,3-C_{3}H_{5}(OH)_{2})</td>
<td>N-2,3-Dihydroxypropyl 13-cis-retinamide</td>
<td>b</td>
<td>$1 \times 10^{-10}$</td>
<td>(29)</td>
</tr>
<tr>
<td>NH(4-C_{6}H_{4}OH)</td>
<td>N-4-Hydroxyphenyl 13-cis-retinamide</td>
<td>j</td>
<td>$&gt;1 \times 10^{-9}$</td>
<td>(88)</td>
</tr>
<tr>
<td>NH(CN_{4}H)</td>
<td>N-5-Tetrazolyl 13-cis-retinamide</td>
<td>b</td>
<td>$2 \times 10^{-10}$</td>
<td>(63)</td>
</tr>
</tbody>
</table>

10. Ring- and Side Chain-modified Analogs of All-trans-Retinoic Acid and its Esters

![Chemical structure of Ring- and Side Chain-modified Analogs]

<table>
<thead>
<tr>
<th>Structure, R =</th>
<th>Trivial Name</th>
<th>Source</th>
<th>ED_{50, M}</th>
<th>(Number of Cultures)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>Ro 13-7410</td>
<td>g</td>
<td>$1 \times 10^{-11}$</td>
<td>(50)</td>
</tr>
<tr>
<td>C_{2}H_{5}</td>
<td>Ro 13-6298</td>
<td>g</td>
<td>$1 \times 10^{-11}$</td>
<td>(80)</td>
</tr>
</tbody>
</table>
## 11. 5,6-All-trans-Epoxyretinoids

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Structure, R =</th>
<th>Trivial Name</th>
<th>Source</th>
<th>ED$_{50}$ M</th>
<th>(Number of Cultures)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COOH</td>
<td>5,6-Epoxyretinoic acid</td>
<td>g</td>
<td>$2 \times 10^{-9}$</td>
<td>44</td>
</tr>
<tr>
<td>COOCH$_3$</td>
<td>5,6-Epoxyretinoic acid methyl ester</td>
<td>k</td>
<td>$3 \times 10^{-9}$</td>
<td>45</td>
</tr>
<tr>
<td>CH$_2$COOCH$_3$</td>
<td>5,6-Epoxyretinyl acetate</td>
<td>k</td>
<td>$4 \times 10^{-9}$</td>
<td>35</td>
</tr>
<tr>
<td>CH (C$<em>6$H$</em>{10}$O$_2$)</td>
<td>5,6-Epoxide of retinylidene dimeredone</td>
<td>a</td>
<td>$3 \times 10^{-9}$</td>
<td>46</td>
</tr>
</tbody>
</table>
Structure-Activity Relationships of Retinoids in Hamster Tracheal Organ Culture

Dianne L. Newton, William R. Henderson and Michael B. Sporn


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