OTHER PUBLICATIONS


* cited by examiner
TTNPB

4-HPTTNPB

4-HBTTNPB

3-Propyla Analog of 4-HPTTNPB

Retinoic Acid (RA)

4-Hydroxyphenylretinamide (4-HPR)

4-HBR

Sugar Analogs of 4-HBTTNPB

FIG. 1A

C-Linked

O-Linked
Aldose Sugar Analogs of 4-HBTTNPB

R = \text{Oxygen-linked)

R = \text{Methylene-linked)

FIG. 1B
FIG. 2
FIG. 3
FIG. 4

Chemical structures and reactions:

13 \[ \text{HO-CO} \rightarrow \text{HO-COOCH}_3 \]

14

15 \[ \text{RO-COOCH}_3 \]

16 \[ \text{R} = \text{CH}_2\text{C}_6\text{H}_5 \]

17 \[ \text{R} = \text{CH}(\text{CH}_3)_2 \]

18 \[ \text{R} = \text{CH}_2\text{C}_6\text{H}_5 \]

19 \[ \text{R} = \text{CH}_3 \]

20 \[ \text{R} = \text{CH}_3 \]

21 \[ \text{R} = \text{CH}(\text{CH}_3)_2 \]

22 \[ \text{R} = \text{CH}_2\text{C}_6\text{H}_5 \]

23

Chemical equations and reactions:

- Reaction 13 to 14
- Reaction 15 to 16
- Reaction 17 to 18
- Reaction 19 to 20
- Reaction 21 to 22
- Reaction 23
FIG. 5
FIG. 8A
Fig. 8B
RAR\(\alpha\) Competition Binding

% Specific [3H]atRA Binding

Log Concentration (M)

- atRA
- 4HPR
- TTNPB
- 4HTTNPB
- 4HBTTPNB

FIG. 9
RARβ Competition Binding

% Specific [3H]aTRA Binding

Log Concentration (M)

- atRA
- 4HPR
- TTNPB
- 4HTTNPB
- 4HBTTNPB

FIG. 10
RARγ Competition Binding

% Specific [3H]atRA Binding

Log [Retinoid] (M)

- atRA
- 4HPR
- TTNPB
- 4HPTTNPB
- 4HBTTNPB

FIG. 11
In the above Figure, 4-HTTNPB = 4-HPTTNPB

FIG. 12
In the above Figure, 4HTTNPB = 4-HPTTNPB
FIG. 14
FIG. 15

Log Drug [M]

% Labeled Cells

TUNEL
PI Only

Vehicle
4HPR (-5.0)
4HPR (-4.5)
TTNPB (4.5)
4HPTTNPB (-5.0)
4HPTTNPB (4.9)
4HTTNPB (-5.0)
4HTTNPB (4.5)
1

4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-napthalenyl)-1-propenyl]benzoic acid analogs and method of manufacture and use thereof.

PRIORITY APPLICATIONS


STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under CA049837 awarded by the National Institutes of Health. The government has certain rights in the invention.

RELATED PATENTS AND RELATED COPENDING APPLICATIONS

The present invention is related to U.S. Pat. Nos. 6,117,254; 6,129,339; 6,117,253; 6,117,252; 5,855,026; 5,835,507; 5,759,727; 5,740,639; 5,735,885; 5,710,147; 5,695,925; 5,663,377; 5,599,953; 5,574,177; and 5,516,792, which are incorporated herein by reference. The present invention is also related to co-pending U.S. patent application Ser. No. 11/416,907, filed on May 3, 2006, which is also incorporated herein by reference.

FIELD OF THE INVENTION

The inventive field includes analogs of 4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-napthalenyl)-1-propenyl]benzoic acid and methods of manufacture and use thereof, such as for use in cancer prevention and treatment.

BACKGROUND OF THE INVENTION

Breast cancer is the cause of thousands of deaths among females every year. (See A Snapshot of Breast Cancer, National Cancer Institute (August 2005)). Surgical intervention has saved the lives of many women. However, radical and partial mastectomies can be physically and emotionally debilitating. Surgery in combination with chemotherapy may still expose patients to the possibility of recurrence.

Massive research efforts have been directed to researching and developing drugs and other therapies (such as radiation, immunotherapy and vaccine) to treat and/or prevent breast cancer. Retinoic acid is a known metabolite of vitamin A. Retinoic acid and certain analogs thereof appear to be necessary for maintenance of normal epithelial tissue differentiation. Such analogs of retinoic acid may also be able to reverse the metaplastic condition of hamster trachea in vitamin A deficient epithelial tissue. (See Newton et al., Cancer Res 40:3413-3425 (1980)). Retinoic acid and certain amide analogs thereof have also been investigated for use as chemotherapeutic agents. (See Moon et al., Cancer Res 39:1339-1346 (1979)). Other retinoic acid analogs such as retinyl acetate, 13-cis-retinoic acid and glucuronide analogs have also been shown to display some cancer preventive activity including breast cancer preventive activity. (See Hill D L et al., Ann Rev Nutrition, 12:161-181 (1992))(See also Mehta R G et al., Oncology, 48:1505-1509 (1991)). Stilbene derivatives of retinoic acid have been suggested as possible agents for use in oncology. (See Simoni D et al., Retinoic Acid and Teratogenicity of N-(4-hydroxyphenyl)-all-trans Retinamide in Rats and Rabbits, Teratogenesis, Carcinogenesis and Mutagenesis, 8:1-11 (1988)). It has also been reported that N-(4-hydroxyphenyl) retinamide ("4-HPR") displays chemopreventive activity toward breast cancer. (See Moon et al., Cancer Res, 39:1339-1346 (1979)). It has also been reported that the combination of 4-HPR and calcium glucarate has increased and synergistic breast cancer chemopreventive activity in carcinogen-induced rat mammary tumors. (See, Abou-Issa H M et al., Proc Nail Acad Sci USA, 85:4181-4184 (1988). However, the 4-HPR still displayed teratogenic potential in rat, mice and rabbit studies. (See Kenel M F et al., Teratogenicity of N-(4-hydroxyphenyl)-all-trans Retinamid in Rats and Rabbits, Teratogenesis, Carcinogenesis and Mutagenesis, 8:1-11 (1988)).It has also been reported that 4-HPR impairs night vision in human patients. (See Kaiser-Kupter M I et al., Abnormal Retinal Function Associated with Fenretinide, A Synthetic Retinoid Renretinide (HPR), Eur J Cancer Clin Oncol, 25:805-808 (1989)).

Toxicity has, however, been a significant obstacle to developing retinoic acid and the analogs thereof. (See Biesalski H K, Toxicology, 57:117-161 (1989)). The observed side effects include: teratogenicity, hepatotoxicity, blood lipid abnormalities, scaly skin, hair loss and headaches. Other research has sought to discover new retinoic acid analogs having increased potency and/or reduced toxicity for use as cancer preventative agents. For example, it has been reported that 4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-napthalenyl)-1-propenyl]benzoic acid ("TTNPB") was also disclosed in Simoni et al.

The structure of retinoic acid ("RA") is shown below.

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\includegraphics[width=0.5\textwidth]{retinoic_acid.png}
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Analogs as Potent Inducers of Differentiation and Apoptosis:

New Promising Chemopreventive and Chemotherapeutic Agents in Oncology, Pure Appl Chem, 73:1437-1444 (2001)). Aromatic analogs of retinoic acid, such as 4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-napthalenyl)-1-propenyl]benzoic acid ("TTNPB"), were also disclosed in Simoni et al.

The structure of retinoic acid ("RA") is shown below.
The structure of 4-HPR is shown below.

The ketone analog of 4-HPR is 4-HBR. The structure of 4-HBR is shown below. The properties (and synthesis thereof) of 4-HBR is disclosed in U.S. Pat. No. 6,117,845 to Clagett-Dame et al., which also discloses synthesis of various sugar-analogs of 4-HBR.

4-HBR and analogs thereof possess anti-neoplastic activity and are useful in preventing (i.e., prophylactic treatment) and/or treating neoplastic growth in mammals.

Despite recent advances in synthesis of stable and active analogs of retinoid acid, there remains a need for more potent and less toxic analogs and derivatives of retinoic acid and TTNPB, particularly such drugs useful for prophylactic treatment and treatment of many cancers including breast cancer.

SUMMARY OF THE INVENTION

One aspect of the invention is a compound according to the formula

\[ \text{(4-HBTTNPB)} \]

wherein \( L \) is a member selected from the group consisting of a single bond, \( \text{CH}_2 \), and \( \text{O} \), and wherein \( R^1 \) is a member selected from the group consisting of an aldose moiety.
and, C₁₋₆ straight or branched chain alkyl group, and salts, esters and solvates thereof.

Preferably, the aldose residue moiety is:

The compound may also be pharmaceutically suitable salts, esters or solvates thereof, whereby such compounds or such salts, esters or solvates thereof may be administered to a human for the prophylactic treatment of breast cancer or for treating breast cancer. Where L is O, the C₁₋₆ straight or branched chain alkyl group may be:

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A shows the structure of various compounds referred to in the specification.

FIG. 1B shows the structure of oxygen and methylene linked sugars of 4-HBTTNPB.

FIG. 2 shows a reaction scheme for the synthesis of TTNPB and 4-HPTTNPB.

FIG. 3 shows a reaction scheme for the synthesis of propyl analogs of TTNPB and 4-HPTTNPB.

FIG. 4 shows a reaction scheme for the synthesis of 4-HBTTNPB.

FIG. 5 shows a reaction scheme for the synthesis of an oxygen-linked sugar analog of 4-HBTTNPB.

FIG. 6 shows a reaction scheme for the synthesis of CH₂-linked (methylene-linked) sugar analogs of 4-HBTTNPB.

FIG. 7 shows a reaction scheme for another synthesis of CH₂-linked sugar analogs of 4-HBTTNPB.

FIG. 8B shows a reaction scheme for the synthesis of another oxygen-linked sugar analog of 4-HBTTNPB.

FIG. 9 is a graph comparing the ability of various compounds to interact with the RAR-α receptor as determined by competition binding assay.

FIG. 10 is a graph comparing the ability of various compounds to interact with the RAR-β receptor as determined by a competition binding assay.

FIG. 11 is a graph comparing the ability of various compounds to interact with the RAR-γ receptor as determined by a competition binding assay.

FIG. 12 is a graph comparing the ability of compounds to interact with the RAR-γ receptor as determined using a competition binding assay.

FIG. 13 is a graph of the results obtained in an F9 gene reporter assay plotting the relative light units as a function of the log of the retinoid concentration.

FIG. 14 is a graph of the results obtained from a cell growth inhibition assay, whereby MCF-7 cells were treated once with varying concentrations of retinoids to determine the relative potency of each compound to inhibit cell growth 72 hours later.

FIG. 15 is a graph showing data obtained using a TUNEL assay to investigate cell apoptosis in MCF-7 cells after exposure to a single dose of various retinoid compounds for 72 hours.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The structure of 4-[(E)-2-(5,6,7,8-tetramethyl-2-naphthyl)-1-propenyl]benzoic acid (TTNPB) is set forth in FIG. 1A. TTNPB and other analogs of retinoic acid show resistance to metabolism and possess significant chemotherapeutic activity modulating cell growth and apoptosis. TTNPB is a potent but toxic aromatic analog of retinoic acid. TTNPB selectively binds to the retinoic acid receptor ("RAR"). However, TTNPB does not bind to the retinoid X receptor ("RAR"). As such, TTNPB is advantageous over retinoic acid in that it selectively binds to the RAR. Retinoic acid does, however, isomerize and bind to the RXR.

The carboxylic acid group in the retinoic acid molecule is covalently linked (via an amide linkage) to a para-hydroxyphenyl amine thus yielding the 4-hydroxyphenylretinamide ("4-HPR"). 4-HPR has chemopreventative-chemotherapeutic activity against breast cancer. 4-HPR is also significantly less toxic than retinoic acid. In culture, 4-HPR inhibits growth of cancer cells by apoptosis. In contrast, retinoic acid and TTNPB cause growth arrest by causing differentiation. 4-HPR may also show RAR-independent activity. (See U.S. Pat. No. 6,117,845).

As shown in FIG. 2, the carboxylic acid group of TTNPB is covalently linked (via an amide linkage) to a para-hydroxyphenyl amine thus yielding the 4-HPTTNPB. As shown in FIG. 3, 3-propyl analogs of TTNPB and 4-HPTTNPB were synthesized (see, compounds 11 and 12 herein below). Without being bound to any theory, it appears that the 3-propyl analog of 4-HPTTNPB (compound 12) will not bind to the RAR even if it is hydrolyzed in vivo to compound 11. It is theorized that the 3-propyl analog of 4-HPTTNPB is sufficiently non-toxic for pharmaceutical use in humans (i.e., pharmaceutically suitable) because it will not bind to the RAR or RXR proteins.

Alternatively, it is theorized that the ketone group in compounds 20, 21, 22, 23 (4-HBTTNPB), 25 (oxygen-linked
glucuronide of 4-HBTTNPB), 29 (methylene-linked glucuronide of 4-HBTTNPB), and 33 (methylene-linked glucuronide of 4-HBTTNPB) functions similar to the amide group in 4-HPTTNPB. It is understood that the glucose moiety may be any of the various moieties derived from an aldose such as a monosaccharide, a monobasic acid, a dicarboxylic acid, an aldehyde alcohol or an aldehyde acid. (See Morrison and Boyd, Organic Chemistry, 3rd:1075 (1973)). For example, within the family of aldose derivatives, glucuronic acid is an aldehyde acid that forms a glucuronide derivative moiety. The ketone group will also not bind to the RAR or RXR proteins. Compound 20 is E-4-[2-methyl-2-(1,1,4,4-tetramethyl-1,2,3,4-tetrahydronapth-7-yl)-vinyl]-phenyl-2'-(4'-methoxyphenyl)-1'-ethanone. Compound 21 is E-4-[2-methyl-2-(1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalen-7-yl)-vinyl]-phenyl-2'-(4'-isopropoxyphenyl)-1'-ethanone. These ketone-containing compounds were synthesized as set forth herein below and exhibit potent activity inhibiting tumor cell proliferation such that they are suitable for the prophylactic treatment of breast cancer and for treating breast cancer. Unexpectedly, sugar-containing analog compounds 25, 29, 30 and 33 also have enhanced desirable properties such as solubility, bioavailability and low toxicity.

EXAMPLES

Synthesis and characterization of various retinoic acid derivatives is described in U.S. Pat. Nos. 6,117,845; 4,578,498; and 4,326,055, whereby the entire disclosure of each hereby incorporated herein by reference. In addition, instant FICOS. 2-8 set forth additional synthetic methods for making the various novel compounds disclosed herein.

2.5-Dimethylhexane-2,5-diol and propyl benzene are commercially available from Aldrich Chemical Company, Milwaukee, Wis. Other reagents may be commercially available from, among others, Sigma-Aldrich, St. Louis, Mo. and Aldrich Chemical Company. Such reagents were prepared using known procedures or as set forth herein.

2,5-Dichloro-2,5-dimethylhexane (compound 5) was synthesized using a known procedure set forth in Bruson H et al., J Am Chem Soc, 62:36 (1940), whereby 50 g of 2,5-dimethylhexane-2,5-diol (compound 1) and 1 L of concentrated hydrochloric acid was used. Yield was measured quantitatively. Properties of compound 2 matched that reported in Bruson et al. 1,1,4,4-Tetramethyl-1,2,3,4-tetrahydronapthalene (compound 4) was synthesized using a known procedure set forth in Bruson et al. J Am Chem Soc, 76:16 (1973), whereby 30 g of 2,5-dichloro-2,5-dimethylhexane (compound 2), 38 g of benzene, and 16 g of aluminum chloride was combined resulting in a 60% yield. Properties of compound 3 matched that reported in Bruson et al.

7-Acetyl-1,1,4,4-tetramethyltetralin (compound 4) was synthesized using a known procedure set forth in Wood T F et al., J Org Chem, 28(9):2248 (1963), whereby 16 g of 1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene, 7.4 g acetyl chloride and 13.6 g of aluminum chloride was used resulting in a 92% yield. 1H NMR was used to confirm formation of compound 3.

Ethyl E-4-[2-methyl-2-(1,1,4,4-tetramethyl-1,2,3,4-tetrahydronapth-7-yl)-vinyl]-benzoate (compound 5) was synthesized using a known procedure set forth in Dawson M et al., J Org Chem, 49:5265 (1984), whereby 30 g of 1.0 M NaCH$_2$SOH (or, 27 mmol) was added to 10 g diethyl p-carboxybenzylphosphonate with stirring. After 0.5 hours, the red-brown solution of phosphonate anion was added to 3.45 g of 7-acety1-1,1,4,4-tetramethyltetralin (compound 4) in 45 mL of Me$_2$SO. The reaction mixture was stirred for 4 hours. Then, 9 mL of 2.0 M NaOEt (or, 18 mmol) in EtOH was added, and the red-brown reaction mixture was stirred overnight. The reaction mixture was worked up by pouring into 10% aqueous NaHCO$_3$ (900 mL), extracting with Et$_2$O, washing with brine; and, drying over MgSO$_4$.

After filtration and removal of Et$_2$O, the residue was suspended in 6 mL hexane and allowed to stand for 4-6 hours producing gummy crystals. Recrystallization from 20% hexane/CH$_2$OH yielded 4.68 g (83% yield) of ethyl E-4-[2-methyl-(1,1,4,4-tetramethyl-1,2,3,4-tetrahydronapth-7-yl)-vinyl]-benzoate (compound 5) which was a colorless solid. Properties of compound 5 matched that reported in Dawson et al. HPLC (octadecylsiline 4.6x250 mm column, CH$_3$OH/H$_2$O 85:15, UV 310 nm, flow rate 1.0 mL/min, tR=8.5 min.). E-4-[2-methyl-2-(1,1,4,4-tetramethyl-1,2,3,4-tetrahydronapth-7-yl)-vinyl]-benzoate (compound 5) in 6M aqueous KOH. Yield was 90%. m.p. was 247-248° C. (from acetic acid). HPLC (octadecylsiline 4.6x250 mm column, CH$_3$OH/H$_2$O 85:15, UV 310 nm, flow rate 1.0 mL/min, tR=8.5 min.).: 1H-NMR (300 MHz, (CD$_3$)$_2$CO), H 1.27 (s, 3H), 1.31 (s, 3H), 1.70 (s, 4H), 2.29 (s, 3H), 6.83 (s, 1H), 7.28 (s, 2H), 7.44 (s, 1H), 7.70 (AB, 4H, JAB=8.06 Hz), acid OH exchanges with H$_2$O from the solvent.

E-4-[2-methyl-2-(1,1,4,4-tetramethyl-1,2,3,4-tetrahydronapth-7-yl)-vinyl]-benzoic acid 4-hydroxyanilide (4-HPTTNPB; compound 7) was synthesized by hydrolyzing ethyl E-4-[2-methyl-2-(1,1,4,4-tetramethyl-1,2,3,4-tetrahydronapth-7-yl)-vinyl]-benzoate (compound 5) in 6M aqueous KOH. Yield was 90%. m.p. was 252-253° C. HPLC (octadecylsiline 4.6x250 mm column, CH$_3$OH/H$_2$O 85:15, UV 310 nm, flow rate 1.0 mL/min, tR=8.5 min.).: 1H-NMR (300 MHz, (CD$_3$)$_2$CO), H 1.21 (s, 6H), 1.25 (s, 6H), 1.62 (s, 4H), 2.21 (s, 3H), 6.83 (s, 1H), 7.28 (s, 2H), 7.44 (s, 1H), 7.70 (AB, 4H, JAB=8.06 Hz), acid OH exchanges with H$_2$O from the solvent.
Ethyl E.Z-4-[2-methyl-1,1,4,4-tetramethyl-2,3,4-tetrahydronaphthal-7-yl]-vinyl]-benzoic acid (compound 11) was synthesized according to the procedure set forth herein respecting compound 5. The resultant mixture was worked up and chromatographed using silica gel and 10% EtOAc/90% hexane to produce a mixture containing compound 10 as a colorless oil at a 16% yield. 1H-NMR was used to confirm the identity of the product. 

E-4-[2-methyl-2-(6-propyl-1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthal-7-yl)-vinyl]-benzoic acid 4-hydroxyanilides (compound 17) were synthesized by hydrolyzing ethyl compound 10 in 0.5M aqueous KOH producing an 80% yield. 1H-NMR was used to confirm the identity of the product (see Frickel F. et al., U.S. Pat. No. 4,578,498) whereby the HPLC included the following materials and settings: octadecylsilane 4.6x250 mm column, CH3OH/H2O 85:15, UV 310 nm, flow rate 1.0 mL/min, and t retention = 24 min.

E-4-
[2-methyl-2-(1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthal-7-yl)-vinyl]-phenyl-2'(4-methoxyphenyl)-1'-ethanone (compound 20), E-4-
[2-methyl-2-(1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthal-7-yl)-vinyl]-phenyl-2'(4-isopropoxyphenyl)-1'-ethanone (compound 21), and, E-4-
[2-methyl-2-(1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthal-7-yl)-vinyl]-phenyl-2'(4-benzyloxyphenyl)-1'-ethanone (compound 22). A solution of 1.25 equivalents of 4-substituted phenylacetic acid in dry tetrahydrofuran (“THF”) was slowly added with stirring to 2.45 equivalents of 2M lithium diisopropylamide in THF at room temperature. The reaction mixture was stirred at room temperature for 1.5 hours and then slowly added with stirring to a solution of 1.0 equivalent of compound 5 in THF at room temperature. The reaction was stirred at room temperature overnight and then slowly added with stirring to 6M hydrochloric acid at room temperature. The organic phase was separated and washed with using 1.2 M aqueous sodium carbonate and water and then concentrated to 1/5 volume. The hot solution was diluted with 5 volumes of isopropanol. The mixture was cooled to 0°C, and the product was collected by filtration and washed using cold isopropanol to produce compound 20 in the form of colorless needles (from acetic acid) at a 30% yield. The structure was confirmed by COSY and HMQC. HPLC using octadecylsilane 4.6x250 mm column, CH3OH/water 85:15, UV 310 nm, flow rate 1.0 mL/min., and t retention = 8.69 Hz. HPLC using octadecylsilane 4.6x250 mm column, CH3OH/water 85:15, UV 310 nm, flow rate 1.0 mL/min, and t retention = 8.69 Hz. The characterizations data included: 1H-NMR (300 MHz, (CD3)2SO), δ 17.9 (C31), 32.1 (C27, C28, C29, C30, C33, C34), 34.2 (C2, C3), 34.8 (C20), 55.7 (C32), 124.5 (C12), 158 (C19), 114.2, 123.8, 124.2, 127, 127.5, 128.7, 129.4, 130.9, 134.4, 139.8, 140.3, 143.4, 143.2, 144.6 (C2w).

Compound 21 was prepared as colorless needles from acetic acid at a 43% yield. HPLC confirmed the identity (octadecylsilane 4.6x250 mm column, CH3OH/water 85:15, UV 310 nm and flow rate 1.0 mL/min) at t = 49 min.: 1H-NMR (400 MHz, (CD3)2SO), δ 1.31 (s, 6H), 2.09 (s, 6H), 2.86 (s, 3H), 4.23 (s, 2H), 4.51 (sept. 1H, J=6 Hz), 6.84 (s, 1H), 7.29 (s, 2H), 7.49 (s, 1H), 7.75 (AB, 4H, J=8.42 Hz); 13C-NMR (75 MHz, (CD3)2SO), δ 17.9 (C27), 31.9 (C34), 34.2 (C2, C3), 34.2 (C20), 34.9 (C1, C4), 44.1 (C2w), 70.2 (C32), 127 (C12), 157.8 (C19), 116.5, 124.2, 124.8, 126.6, 127.3, 129.3, 130.1, 131.4, 135.5, 140.7, 141.6, 144.1, 145.1, 145.3 (C2w). Compound 22 was a white powder from acetic acid produced at a 60% yield. HPLC (octadecylsilane 4.6x250 mm column, CH3OH/water 85:15, UV 310 nm and flow rate 1.0 mL/min) at t = 66 min.: 1H-NMR (400 MHz, (CD3)2SO), δ 1.30 (d, 6H, J=6 Hz), 7.41 (s, 1H), 7.75 (AB, 4H, J=8.42 Hz), 7.25-7.42 (m, total of 8H), 7.72 (AB, 4H, J=8.42 Hz); 13C-NMR (75 MHz, (CD3)2SO), δ 17.9 (C27), 31.9 (C34), 34.2 (C2, C3), 34.2 (C20), 34.9 (C1, C4), 44.1 (C2w), 70.2 (C32), 127 (C12), 157.8 (C19), 116.5, 124.2, 124.8, 126.6, 127.3, 129.3, 130.1, 131.4, 135.5, 140.7, 141.6, 144.1, 145.1, 145.3 (C2w).
Compound 26.1 is available from Sigma-Aldrich, St. Louis, Mo. Upon cooling the suspension using an ice bath, diisopropylethylamine (57.6 mL, 331 mmol) was added drop wise. Then, chloromethyl methyl ether (50 g, 621 mmol) was carefully added via an addition funnel. A significant amount of white smoke formed in the reaction vessel. Solid tetrabutylammonium iodide (50 g, 134 mmol) was added, and the solution was allowed to warm to room temperature. The reaction was stirred in darkness for 48 hours, and the solution gradually turned red in color. After cooling the vessel to 0° C., saturated aqueous NH₄Cl (75 mL) was added. Then, the mixture was diluted with brine, and the combined aqueous layers were extracted with CH₂Cl₂ (3x). The combined organic layers were dried over MgSO₄, filtered, and concentrated. Then, the solids were triturated using ether (4x), and the ether was concentrated. The resultant oil was chromatographed on silica gel (1:1 hexanes/ethyl acetate) to afford 12.4 g of orange solid. Dry toluene (100 mL) was added, and the reagent was stored at 4° C. and used without characterization.

Preparation of 2,6-anhydro-1-deoxy-3,4,5,7-tetra-O-(methoxymethyl)-D-gluco-hept-1-enitol (compound 26.3). To a flame dried flask under argon atmosphere was added dimethyl titanocene Cp₂Ti(CH₃)₂ (Petasis’ reagent). To a flame dried flask under argon atmosphere was added to diisopropylethylamine (57.6 mL, 331 mmol) and absolute ether (300 mL), which was cooled to 10° C. Dimethyl lithium (100 mL, 140 mmol, 1.4 M) was carefully added drop wise via an addition funnel under darkness. The cold bath was removed, and the red solution was allowed to stir for 10 min. The solution was cooled to 0° C., and ice water (25 mL) was carefully added to quench the unreacted methyl lithium. The layers were separated, and the aqueous layer was extracted using ether (2x). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated in darkness at 20°C to produce 12.4 g of orange solid. Dry toluene (100 mL) was added, and the reagent was stored at 4° C. and used without characterization.

Preparation of 2,6-anhydro-1-deoxy-3,4,5,7-tetra-O-(methoxymethyl)-D-gluco-hept-1-enitol (compound 26.3). To a flame dried flask under argon atmosphere was added 12.4 g of 2,6-anhydro-1-deoxy-3,4,5,7-tetra-O-(methoxymethyl)-D-gluco-hept-1-enitol. To a flame dried flask under argon atmosphere was added diisopropylethylamine (57.6 mL, 331 mmol) and absolute ether (300 mL), which was cooled to 10°C. Dimethyl lithium (100 mL, 140 mmol, 1.4 M) was carefully added drop wise via an addition funnel under darkness. The cold bath was removed, and the red solution was allowed to stir for 10 min. The solution was cooled to 0°C, and ice water (25 mL) was carefully added to quench the unreacted methyl lithium. The layers were separated, and the aqueous layer was extracted using ether (2x). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated in darkness at 20°C to produce 12.4 g of orange solid. Dry toluene (100 mL) was added, and the reagent was stored at 4°C and used without characterization.

Preparation of dimethyl titanocene Cp₂Ti(CH₃)₂ (Petasis’ reagent). To a flame dried flask under argon atmosphere was added diisopropylethylamine (14.63 g, 58.8 mmol) and absolute ether (300 mL), which was cooled to 10°C. Methyl lithium (100 mL, 140 mmol, 1.4M) was carefully added drop wise via an addition funnel under darkness. The cold bath was removed, and the red solution was allowed to stir for 10 min. The solution was cooled to 0°C, and ice water (25 mL) was carefully added to quench the unreacted methyl lithium. The layers were separated, and the aqueous layer was extracted using ether (2x). The combined organic layers were dried over Na₂SO₄ under argon for 1 hour and concentrated in darkness at 20°C to produce 12.4 g of orange solid. Dry toluene (100 mL) was added, and the reagent was stored at 4°C and used without characterization.

Preparation of dimethyl titanocene Cp₂Ti(CH₃)₂ (Petasis’ reagent). To a flame dried flask under argon atmosphere was added diisopropylethylamine (14.63 g, 58.8 mmol) and absolute ether (300 mL), which was cooled to 10°C. Methyl lithium (100 mL, 140 mmol, 1.4 M) was carefully added drop wise via an addition funnel under darkness. The cold bath was removed, and the red solution was allowed to stir for 10 min. The solution was cooled to 0°C, and ice water (25 mL) was carefully added to quench the unreacted methyl lithium. The layers were separated, and the aqueous layer was extracted using ether (2x). The combined organic layers were dried over Na₂SO₄ under argon for 1 hour and concentrated in darkness at 20°C to produce 12.4 g of orange solid. Dry toluene (100 mL) was added, and the reagent was stored at 4°C and used without characterization.

Preparation of dimethyl titanocene Cp₂Ti(CH₃)₂ (Petasis’ reagent). To a flame dried flask under argon atmosphere was added diisopropylethylamine (14.63 g, 58.8 mmol) and absolute ether (300 mL), which was cooled to 10°C. Methyl lithium (100 mL, 140 mmol, 1.4 M) was carefully added drop wise via an addition funnel under darkness. The cold bath was removed, and the red solution was allowed to stir for 10 min. The solution was cooled to 0°C, and ice water (25 mL) was carefully added to quench the unreacted methyl lithium. The layers were separated, and the aqueous layer was extracted using ether (2x). The combined organic layers were dried over Na₂SO₄ under argon for 1 hour and concentrated in darkness at 20°C to produce 12.4 g of orange solid. Dry toluene (100 mL) was added, and the reagent was stored at 4°C and used without characterization.
heated to 70 °C and stirred in darkness for 18 hours. The resultant black solution was cooled and poured into hexanes (−500 mL). The formed precipitate was filtered through Celite. The supernatant was concentrated yielding a red oil that was chromatographed on silica gel (4:1 when 2 hexanes/ethyl acetate) producing 8.66 g (87% yield) of yellowish oil. [α]D 46.8 (c 2.33, Cl2CHCl); IR (cm−1) 2940 (m), 2895 (m), 1750 (w), 1440 (w), 1154 (s), 1032 (s), 918 (m), 1H NMR (DMK-d6) δ 3.31–3.37 (m, 12H), 3.64–3.71 (m, 2H), 3.78–3.83 (m, 2H), 3.88–3.89 (m, 1H) 4.12 (d, 1H, J=5.4 Hz), 4.35 (s, 1H), 4.51 (s, 1H), 4.62 (s, 2H), 6.64–4.84 (m, 6H), 13C NMR (DMK-d6) δ 55.15, 55.87, 56.04, 56.19, 67.50, 75.42, 76.68, 77.36, 81.08, 93.43, 95.35, 97.23, 96.74, 97.81, 135.69; HRMS (ESI) calculated for C13H12O6 (M+Na)+. 375.1631, found 375.1628.

Preparation of 2,6-anhydro-1-deoxy-1-[4-(hydroxymethyl)phenyl]-3,4,5,7-tetra-O-(methoxymethyl)-D-glycero-D-gulo-heptitol (compound 26.4). To a flame dried flask under argon atmosphere was added compound 26.4 (2.44 g, 5.12 mmol) and Pdel (2.37 g, 94% yield) of clear oil. [α]D −27.0 (c 4.70, CH2Cl2). 1H NMR (DMK-d6) δ 3.19–3.42 (m, 5H), 3.24 (s, 3H), 3.30 (s, 3H), 3.35 (s, 3H), 3.39 (s, 3H), 3.44 (s, 3H), 3.54–3.64 (m, 2H), 3.73 (dd, 1H, J=2.6, 13.5 Hz), 4.38 (s, 2H), 4.50 (d, 1H, J=6.4 Hz), 4.54 (d, 1H, J=6.4 Hz), 4.70 (d, 1H, J=6.5 Hz), 4.77–4.85 (m, 4H, J=8.0 Hz), 7.26 (d, 2H, J=8.0 Hz), 7.29 (d, 2H, J=8.0 Hz); 13C NMR (DMK-d6) δ 33.39, 55.05, 56.47, 56.49, 56.57, 57.97, 67.46, 74.84, 78.00, 79.10, 80.23, 81.66, 84.86, 97.20, 99.11, 99.32, 128.15, 130.19, 137.23, 139.45; HRMS (ESI) calculated for C22H36O10 (M+Na)+ 497.2563, found 497.2384.

Preparation of 2,6-anhydro-7-deoxy-7-[4-(methoxymethyl)phenyl]-3,4,5-tri-O-acyetyl-L-glycero-L-gulo-heptonoic acid methyl ester (compound 26.6). The MOM-protected glucoside compound 26.5 (2.43 g, 5.12 mmol) was dissolved in methanol (500 mL) and placed in a flask at room temperature. Aqueous NaOH (6 N, 26 mL) was added, and the solution was stirred for 18 hours. Then, the mixture was concentrated to dryness. In a separate flask, KBr (2.42 g, 156 mmol) was added via addition funnel. The flask was equipped with a reflux condenser, heated to 70° C and stirred in darkness for 18 hours. The reaction was diluted with water and brine. The combined aqueous layers were washed with ether in a separatory funnel. The aqueous layer was concentrated to dryness, and the resulting solid was exhaustively triturated with methanol. The methanol was concentrated and dried. The dried residue was suspended in DMF (180 mL). Iodomethane (6.4 g) dissolved in DMF (10 mL) was added, and the reaction mixture was stirred for 20 hours under argon at room temperature. The reaction mixture was supplemented with acetic anhydride (40 mL), pyridine (20 mL), and DMAP (15 mg) and stirred for 18 hours. The reaction mixture was diluted with water and extracted using ethyl acetate (3×). The organic layers were washed using water and brine, and the combined aqueous layers were extracted with ether (3×). The organic layers were combined, dried over MgSO4, concentrated, and chromatographed (1:1 then 1:2 hexanes/ethyl acetate) to produce 3.29 g (67% yield) of orange oil. [α]D 26.6 (c 1.15, DMK); IR (cm−1) 3470 (w), 2923 (m), 2887 (m), 1692 (m), 1444 (m), 1414 (w), 1150 (s), 1101 (s), 1024 (s), 918 (m), 1H NMR (DMK-d6) δ 2.60 (dd, 1H, J=9.4, 14.4 Hz), 3.18–3.42 (m, 5H), 3.25 (s, 3H), 3.35 (s, 3H) 3.40 (s, 3H), 3.44 (s, 3H), 3.54–3.61 (m, 2H), 3.73 (dd, 1H, J=1.8, 11.3 Hz), 4.51–4.58 (m, 4H), 4.70 (d, 1H, J=6.5 Hz), 4.77–4.85 (m, 4H), 4.93 (d, 1H, J=6.5 Hz), 7.25 (s, 4H); 13C NMR (DMK-d6) δ 38.35, 55.04, 56.45, 56.55, 64.44, 64.57, 67.42, 77.97, 79.07, 80.32, 81.63, 84.83, 97.20, 99.01, 99.19, 99.32, 127.15, 130.11, 138.75, 141.03; HRMS (ESI) calculated for C9H13O9 (M+Na)+ 483.2188, found 483.2186.

Preparation of 2,6-anhydro-1-deoxy-1-[4-(hydroxymethyl)ethyl phenyl]-3,4,5,7-tetra-O-(methoxymethyl)-D-glycero-D-gulo-heptitol (compound 26.5). To a flame dried flask equipped with a flame aron atmosphere was added compound 26.4 (2.44 g, 5.12 mmol) and Pdel (2.37 g, 94% yield) of clear oil. [α]D −26.2 (c 1.15, DMK); IR (cm−1) 2956 (w), 2818 (w), 1698 (s), 1567 (s), 1444 (m), 1370 (s), 1211 (s), 1105 (m), 1028 (m); 1H NMR (DMK-d6) δ 1.94 (s, 3H), 1.95 (s, 3H), 2.74–2.81 (m, 1H), 2.90 (dd, 1H, J=3.4, 7.3 Hz), 3.30 (s, 3H), 3.39–3.49 (m, 4H), 4.18 (d, 1H, J=9.8 Hz), 4.38 (s, 2H), 4.90 (t, 1H, J=9.8 Hz), 5.05 (t, 1H, J=9.8 Hz), 5.29 (t, 1H, J=9.8 Hz), 7.22 (s, 4H); 13C NMR (DMK-d6) δ 20.39, 20.52, 20.60, 38.12, 52.67, 58.03, 70.62, 72.53, 74.09, 74.73, 76.41, 78.62, 128.25, 130.16, 137.43, 137.76, 168.40, 169.89, 170.07, 170.30; HRMS (ESI) calculated for C16H23O7 (M+Na)+ 475.1580, found 475.1577.
2.76-2.83 (m, 1H), 2.92 (dd, 1H, J = 3.5, 7.3 Hz), 3.64 (s, 3H), 3.96-3.99 (m, 1H), 4.20 (d, 1H, J = 9.7 Hz), 4.62 (s, 2H), 4.90 (t, 1H, J = 9.7 Hz), 5.05 (t, 1H, J = 9.7 Hz), 5.29 (t, 1H, J = 9.7 Hz), 7.25 (d, 2H, J = 8.2 Hz), 7.36 (d, 2H, J = 8.2 Hz); 13C NMR (DMK-d6) δ 20.40, 20.52, 20.63, 34.37, 38.12, 52.69, 70.58, 72.52, 74.04, 76.35, 78.43, 129.88, 130.68, 137.26, 138.67, 168.39, 169.91, 170.09, 170.29; HRMS (ES) calculated for C13H12BrO4 (M+Na+) 494.0257, found 494.0278.

Preparation of 2,6-anhydro-1-deoxy-1-[4-(methoxymethyl)phenyl]-3,4,5,7-tetra-O-acetyl-d-glycero-D-gulo-heptitol (compound 26.8). The MOM-protected glucoside compound 26.5 (0.643 g, 1.35 mmol) was dissolved in methanol (34 mL) and placed in a flask at room temperature. Aqueous HCl (6 N, 6.7 mL) was added, and the mixture was stirred for 18 hours. The mixture was concentrated to dryness. Acetic anhydride (4 mL) and pyridine (3 mL) were added to the pasted along with a catalytic amount of DMAP, and the mixture was stirred for 18 hours at room temperature. The reaction mixture was diluted with water and extracted with ethyl acetate (3x). The organic layers were combined, washed with water and dried over MgSO4, filtered, concentrated, and chromatographed (1:1 hexanes/ethyl acetate) to produce 570 mg (90% yield) of compound 26.8 in the form of a white solid, m.p. = 120-122°C. [α]D = -4.0 (c 0.78, DMK); IR (cm⁻¹) 2940 (w), 2936 (w), 2862 (w), 1750 (s), 1630 (w), 1580 (w), 1436 (w), 1370 (m), 1224 (s), 1105 (w), 1032 (m); 1H NMR (CDCl3) 1H 1.96-2.02 (m, 12H), 2.72 (dd, 1H, J = 5.3, 12.2 Hz), 4.40 (s, 4H), 4.92 (t, 1H, J = 9.6 Hz), 5.03 (t, 1H, J = 9.6 Hz), 5.15 (t, 1H, J = 9.6 Hz), 7.16 (d, 2H, J = 8.0 Hz), 7.23 (d, 2H, J = 8.0 Hz); 35 [1H NMR (CDCl3)] 1H 1.92-1.98 (m, 12H), 2.72 (dd, 1H, J = 7.3, 6.8 Hz), 2.88 (dd, 1H, J = 3.2, 7.3 Hz), 3.77-3.85 (m, 2H), 5.00 (dd, 1H, J = 2.4, 6.1 Hz), 4.21 (dd, 1H, J = 5.9, 6.1 Hz), 4.63 (s, 2H), 4.86 (t, 1H, J = 9.6 Hz), 4.97 (t, 1H, J = 9.6 Hz), 5.22 (d, 1H, J = 6.8 Hz), 7.25 (d, 2H, J = 8.2 Hz), 7.37 (d, 2H, J = 8.2 Hz); 13C NMR (DMK-d6) δ 20.49, 20.59, 34.32, 80.31, 87.54, 73.72, 73.82, 74.58, 76.07, 78.40, 130.68, 137.12, 138.92, 169.97, 170.10, 170.30, 170.52; HRMS (ES) calculated for C13H12BrO4 (M+Na+) 523.0580, found 523.0602.

Preparation of tert-butyl-dimethylsilylcyanohydrin (compound 28) from aldehyde compound 27. A mixture compound 27 (3.62 mmol) in dry CH2Cl2 (50 mL) was added to a flame-dried flask under argon atmosphere. A catalytic amount of Et3N (0.1 mL) was added. Tert-butyl(dimethylsilyl) cyanide (1.0 g, 7.08 mmol) dissolved in CH2Cl2 (10 mL) was added by cannula. The reaction was stirred for 20 hours. Then, the solution was concentrated, chromatographed (95:5 hexanes/ethyl acetate), dried over Na2SO4 under argon, and subjected to vacuum overnight producing compound 28.

Preparation of compound 29. To a flame-dried flask under argon atmosphere was added THF (40 mL) along with LiHMDS (1.0 M in hexanes, 3.8 mL, 3.8 mmol). The mixture was cooled to -78°C. Compound 28 (2.54 mmol) in THF (15 mL) was added by cannula into the flask. The solution was stirred for 30 minutes at -78°C. Crystaline bromoglucoronide (compound 26.7) (2.78 g, 5.56 mmol) in THF (15 mL) was added to the flask. The mixture was stirred for 3 hours at -78°C. The mixture was added to the cold bath and quenched with 1 M NH4Cl (10 mL). The mixture was extracted with ethyl acetate (3x), the organic layers were combined, washed with brine, dried over Na2SO4, filtered, concentrated, and chromatographed (1:1 hexanes/ethyl acetate) to produce the alkylation product and some recovered compound 26.7. The alkylation product was taken up in 1% aqueous THF (200 mL) and chilled to 0°C. Tetra-n-butylammonium fluoride (309 mg, 1.18 mmol) was added which darkened the solution. The solution was stirred for 1 hour. The reaction mixture was diluted with water and extracted with ethyl acetate (3x). The organic layers were combined, washed with brine, dried over Na2SO4, filtered, concentrated, and chromatographed (2:1 hexanes/ethyl acetate) producing the protected methyl ester of compound 29.

The protected methyl ester of compound 29 (1.64 mmol) was dissolved in methanol (500 mL) and added to a flask. The mixture was cooled to 4°C. Potassium carbonate (136 mg, 0.98 mmol) was added to the flask and stirred for 2 hours. The reaction mixture was concentrated to about 200 mL at 25-30°C. Adjustment to the original volume using methanol was followed by adding 1 N KOH (14 mL, 14 mmol). After stirring for 20 hours at 4°C, the reaction mixture was warmed and stirred for 5 hours at room temperature. The reaction mixture was cooled to 0°C and adjusted to 7 pH using 4 N HCl. The reaction mixture was concentrated to about 100 mL at 25-30°C, cooled to 0°C, and adjusted to 3 pH using 1 N HCl. The suspension was extracted using ethyl acetate. The organic layers were combined, dried over Na2SO4 under argon for 2 hours, and concentrated. The residue was chromatographed on reverse phase silica gel (gradient 70:30 to 85:15 methanol/water) producing compound 29.

Preparation of compound 30. THF (10 mL) and LiHMDS (1.0 M in hexanes, 0.78 mL, 0.78 mmol) were added to a flame dried flask under argon atmosphere. The mixture was cooled to -78°C. Compound 28 (0.51 mmol) in THF (5 mL) was added by cannula into the flask. The solution was stirred for 30 minutes at -78°C. Compound 29 (8.0 mg, 0.53 mmol) in THF (5 mL) was added to the flask. The mixture was stirred for 2 hours at -78°C. The flask was removed from the cold bath and quenched with 1 M NH4Cl (1 mL). The reaction mixture was extracted using ethyl acetate (3x), the organic layers were combined, washed with brine, dried over Na2SO4, filtered, concentrated, and chromatographed (2:1 hexanes/ethyl acetate) producing the alky-
lated product and some recovered compound 26.9. The alkylated product was taken up in 1% aqueous THF (20 mL) and chilled to 0°C. TBAF (134 mg, 0.51 mmol) was added and the solution darkened. The darkened solution was stirred overnight while warming to room temperature. The reaction mixture was diluted with water and extracted with ethyl acetate (3x). The organic layers were combined, washed with brine, dried over Na₂SO₄, filtered, and concentrated. The resultant oil was chromatographed (2:1 hexanes/ethyl acetate) producing the acetate-protected analog of compound 30.

The acetate protected analog of compound 30 (0.18 mmol) was dissolved in methanol (75 mL) and added to a flask. The mixture was cooled to 4°C. Potassium carbonate (25 mg, 0.18 mmol) was added to the flask, and the reaction was stirred for 20 hours. The reaction mixture was cooled to 0°C. The pH was adjusted to 5 using 1 N HCl. The mixture was extracted using ethyl acetate, and the organic layers were combined, dried over Na₂SO₄, filtered, and concentrated. The residue was chromato graphed on reverse phase silica gel (gradient 70:30 to 85:15 methanol/water) to provide compound 30.

With reference to the synthetic route shown in FIG. 8B, glycosyl trichloroacetimidate (compound 31) was synthesized according to the procedure set forth in Cheng H et al., J Med Chem, 48(2):645 (2005) with a quantitative yield. NMR 1H.

Preparation of E-4-[2-methyl-2-(1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphth-7-yl)-vinyl]-phenyl-2’-(4’-tetra-O-acetyl-beta-D-glucopyranosyloxyphenyl)-1’-ethanone (compound 32). To a solution of 0.14 g (0.319 mmol) of compound 31 and 4 Ångstrom molecular sieves. The reaction mixture was stirred for 30 min. at room temperature. After cooling to -20°C., 7.85 µL (0.0638 mmol) of BF₃·Et₂O was added and continuously stirred for 2 hours. Molecular sieves were filtered off, and the filtrate was washed with saturated NaHCO₃ and brine; and then dried over Na₂SO₄ under argon for 2 hours and concentrated. The residue was chromato graphed on reverse phase silica gel (gradient 70:30 to 85:15 methanol/water) to provide compound 30.

Preparation of E-4-[2-methyl-2-(1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphth-7-yl)-vinyl]-phenyl-2’-(4’-tetra-O-acetyl-beta-D-glucopyranosyloxyphenyl)-1’-ethanone (compound 33). To a solution of 0.070 g (0.091 mmol) of compound 32 in 5 mL of dry CH₂Cl₂ were added 0.236 g (0.479 mmol) of NaH. The reaction mixture was stirred for 18 hours. The reaction mixture was concentrated, and the contents were dissolved in ethyl acetate, washed with water and brine, dried over MgSO₄, and concentrated to produce 0.018 g (33% yield) of white precipitate. NMR 1H.

Biological Activity

Methods

Nuclear Retinoid Receptor (RAR/RXR) Binding. Competition of retinoids with [3H]-all-trans-RA for binding to RARα, RARβ, RARγ, and RARγ was determined using an in vitro ligand binding assay. (See Clagett-Dame et al., Methods for generating and characterizing retinoid receptors from E. coli and insect cell expression systems, Meth. Enzymol., 282:13-14 (1997)). Recombinant human RARα was expressed as a fusion protein in E. coli (see Repa J J et al., All-trans retinol is a ligand for the retinoic acid receptors, Proc Natl Acad Sci USA, 90:7293-7297 (1993)). The human RARγ was determined using an in vitro ligand binding assay. (See Robarge M J et al., N-linked analogs of retinoid O-glycosides: Potential cancer chemopreventive-chemotherapeutic agents, Bioorg Med Chem Lett, 4(17):2117-2122 (1994)), murine RARγ, (see Repa J J et al., All-trans 3,4-didehydroretinoic acid equals all-trans retinoic acid in support of chick neuronal development, FASEB J, 10:1078-1084 (1996)) and murine RXRγ was determined using an in vitro ligand binding assay. (see Munder M et al., Identification of nicotine intestinal accessory factor that enables DNA sequence recognition by vitamin D receptor, Proc Natl Acad Sci USA, 92:2795-2799 (1995)).

Cell growth inhibition and TUNEL assays. MCF-7 breast cancer cells were maintained as described in Chapman A S et al., Hydrolysis of 4-IPR to atRA occurs in vivo but is not required for retinamide-induced apoptosis, Arch Biochem Biophys, 419:234-243 (2003). The cells were plated at 30,000 cells/well in 24-well plates. The wells were dosed with varying amounts of retinoid compounds. At cell harvest, propidium iodide was added. Half of the cells were used to determine the number of live and dead cells after adding fluorescein diacetate, which yields a fluorescent product upon cleavage by metabolically active cells. The other half were fixed in 4% paraformaldehyde, dried on aminosilane coated slides and used to determine TUNEL and propidium iodide staining.

Reporter gene assay. The F9-RARE-lacZ reporter cell line (see Wagner M et al., Development, 116:55-66 (1992)) was cultured in serum-free L15 medium (15,000 cells/well); dosed with retinoid compounds or vehicle; and assayed for chemiluminescence as described in Chapman J S et al., Hydrolysis of 4-IPR to atRA occurs in vivo but is not required for retinamide-induced apoptosis, Arch Biochem Biophys, 419:234-243 (2003). Data were normalized for total protein using the method set forth in Bradford M M, Anal Biochem, 72:248-254 (1976).

Assessment of Compound Teratogenicity. Female rats (Harlan Sprague-Dawley, Madison, Wis. approximately 200 g in weight) were maintained on normal laboratory rat chow and mated with normal rats of the same strain. Pregnant rats were given a single oral bolus dose of compound dissolved in Wesson® corn oil. Each dose was 60-100 µL. The doses were given at day 9.25. Each dose was maintained under standard laboratory conditions until euthanasia at embryonic day 21.5. The fetuses were removed. Fetuses with a heartbeat were determined to be alive. The number of live and dead fetuses were recorded. The number of resorption sites was also recorded. The live fetuses were examined for gross external malformations. All animals were maintained in accordance with protocol approved by the University of Wisconsin-Madison animal care committee.
The teratogenicity data is set forth in Table 1.

### TABLE 1

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<th>Live*</th>
<th>Dead &amp; Resorbed</th>
<th>Neural Tube</th>
<th>Cleft Palate</th>
<th>Memadism</th>
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<td>Vehicle</td>
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<td>0/13 (0%)</td>
<td>0/13 (0%)</td>
<td>0/13 (0%)</td>
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<tr>
<td>4HPTTPNB (0.66 micromoles/kg at E9.25)</td>
<td>3 (20%)</td>
<td>12 (80%)</td>
<td>3/3 (100%)</td>
<td>3/3 (100%)</td>
<td>3/3 (100%)</td>
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<tr>
<td>4HBTTNPB (0.66 micromoles/kg at E9.25)</td>
<td>1 (7%)</td>
<td>14 (93%)</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
<td>na**</td>
</tr>
<tr>
<td>5</td>
<td>0 (0%)</td>
<td>14 (100%)</td>
<td>na**</td>
<td>na</td>
<td>na</td>
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<tr>
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<td>7</td>
<td>12 (92%)</td>
<td>1 (8%)</td>
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<tr>
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<td>0/16 (0%)</td>
<td>0/16 (0%)</td>
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</table>

*Only fetuses that were alive at the time of dissection were scored for gross malformations
**na = not applicable; no fetuses were live at dissection

### Results

**Nuclear RAR Binding.** The ability of compounds to interact with the retinoic acid receptor types alpha, beta and gamma was tested using a competition binding assay. atRA (all-trans retinoic acid) is commercially available and was obtained from Eastman Kodak (Rochester, N.Y.). TTNPB was very effective at competing with [3H]all-trans-RA for binding to all of the RARs whereas 4-HP showed only weak competition (i.e., 3-4 orders of magnitude less potent). Similarly, both 4-HPTTPNB and 4-HBTTNPB competed poorly for [3H]atRA binding to the human RARs. (See FIGS. 9, 10 and 11.)

**Nuclear RXR Binding.** The ability of compounds to interact with the retinoid X receptor type gamma was tested using a competition binding assay. The control synthetic RXR ligand, LGD1069 (see Boehm M F et al., Synthesis and structure-activity relationships of novel retinoid X receptor-selective retinoids, *J Med Chem*, 37:2930-2941 (1994)) was very effective at competing with [3H]-9-cis-RA for binding to RXRgamma (Kp, 36 nM). TTNPB showed very weak competition at concentrations above 10-5 M (44% at 10-6 M) whereas 4-HPTTPNB and 4-HBTTNPB showed almost no competition with [3H]-9-cis-RA for binding to the human RXRgamma at the highest concentration. (See FIG. 12.)

**Reporter gene assay.** The F9 cell reporter assay yielded beta-galactosidase activity in response to exposure of cells to atRA or any compound with similar transactivation potential. That results from ligands binding to the RAR. It also results from activation of R-galactosidase via the retinoid receptor complex binding to the RARE enhancer (hormone response element) situated upstream of the promoter/enhancer cassette. Both atRA and TTNPB were highly active producing activity in that assay; both compounds interact well with the RARs (Ki values in the 0.2 to 5 nM range). 4-HP was approximately 1000-fold less potent than atRA and TTNPB in activating the reporter system. 4-HPTTPNB was around 100-fold less potent than atRA and TTNPB in the reporter assay. Because 4-HPTTPNB did not show any significant binding to the RAR proteins, the activity in the reporter gene assay may be due to partial hydrolysis of 4-HPTTPNB to TTNPB, which is active in this system. (See FIG. 13.)

**Cell growth inhibition assay.** MCF-7 cells were treated with varying concentrations of retinoids at the start of the assay and maintained in culture for 72 hours after which the number of live (viable) and total (viable+dead) cells were determined. The response of cells to micromolar doses of 4-HP was cell death whereas TTNPB was nearly ineffective in causing the death of cells. Exposure of cells to increasing concentrations of 4-HPTTPNB or 4-HBTTNPB resulted in a dose-dependent decrease in the total number of live cells after 72 hours. (See FIG. 14.)

**DNA fragmentation.** The TUNEL assay (which measures DNA fragmentation) was used to determine whether any of the tested compounds induced cellular apoptosis. Propidium iodide staining (in the absence of TUNEL labeling) was used to measure the number of cells exhibiting necrosis or late-stage apoptosis. As previously described, 4-HP induced a high degree of apoptosis in MCF-7 cells at the concentrations tested. (See Chapman J S et al., Hydrolysis of 4-HP to atRA in vivo but is not required for retinamide-induced apoptosis, *Arch Biochem Biophys*, 419, pp. 234-243 (2003)). In contrast, TTNPB was ineffective at inducing DNA fragmentation after 72 hours, whereas both 4-HPTTPNB and 4-HBTTNPB at the highest doses were very active at inducing apoptosis after 72 hours. (See FIG. 15.) Thus, modification of the TTNPB molecule in that fashion produced a dramatic and unexpectedly superior improvement in biological characteristics and activity.

**Teratogenicity testing.** The ability of compounds to induce fetal malformations was assessed by giving pregnant rats a single oral dose of compound at the stage of embryogenesis when development is highly perturbed by administration of retinoid compounds. A 0.66 micromoles/kg dose of 4-HPTTPNB was administered as a single oral bolus dose to three pregnant rats at embryonic day 9.25. Such dosing produced profound effects on the outcome of pregnancy in all animals. A very high percentage (>80%) of the fetuses at embryonic
day 21.5 were either dead or resorbed. (See Table 1). The few that were alive were all profoundly malformed (e.g., open neural tube, cleft palate and mermaidism). In contrast, the non-hydrolyzable analog, 4-HBTTNPB, administered at the same molar concentration and at the exact same time during pregnancy did not produce any similar adverse effects, and it led to the production of fetuses at E21.5 that were indistinguishable from the vehicle-treated control fetuses. The number of dead and resorbed fetuses also did not differ between the vehicle and 4-HBTTNPB groups. Thus, 4-HBTTNPB at such dose was devoid of any observable teratogenic activity.

Collectively, these data suggest that 4-HPTTNPB shares some characteristics inherent in TTNPB and 4-HPR whereas 4-HBTTNPB is most like the non-hydrolyzable 4-HPR analog, 4-HBR. Without being bound to any theory, TTNPB-like activity in cell culture may result from hydrolysis of 4-HPTTNPB liberating small amounts of TTNPB. In addition, 4-HPTTNPB may induce cell apoptosis reflecting activity of intact 4-HPTTNPB or an unknown metabolite thereof mirroring activity similar to 4-HPR. This is supported by the activity of the non-hydrolyzable analog, 4-HBTTNPB, which induces apoptosis at similar concentrations, but cannot liberate TTNPB. Finally, the potent effect of 4-HPTTNPB on producing teratogenic effects and the complete absence of such effects due to 4-HBTTNPB supports the hypothesis that TTNPB-like activity in vivo results from the hydrolysis of 4-HPTTNPB to liberate small amounts of TTNPB.

Cell culture. The human mammary carcinoma cell line, MCF-7, may be obtained from the American Type Culture Collection (Manassus, Va.). MCF-7 cells may be maintained in DMEM (Sigma-Aldrich) medium supplemented with 4 g/L glucose, 3.7 g/L sodium bicarbonate and 10% fetal calf serum.

Assay for cell growth inhibition. Cells are plated in flasks/wells of a tissue culture dish. After 24 hours, cells are dosed with a compound of the present invention at varying concentrations, and after 72 hours, the cells are removed from flasks/wells and counted. Fluorescence (due to staining of cells with fluorescein diacetate) was used to count the total number of cells. Phase-contrast microscopy is used to count the total number of cells.

Anti-proliferative activity of compounds 12, 23, 25, 29, 30 and 33 (against MCF-7 human mammary tumor cell culture models) may be determined using the protocol set forth herein. The data would show that each compound inhibits growth of MCF-7 cells in a dose-dependent manner. The data would also demonstrate the utility of each compound to inhibit cancer cell growth.

Competition binding studies may also be performed as set forth herein using compounds 23 (4-HBTTNPB), 12 (propyl analog of 4-HPTTNPB), 25 (O-linked glucuronide of 4-HBTTNPB), 29 (CH₂-linked glucuronide of 4-HBTTNPB), 30 (CH₂-linked glucose of 4-HBTTNPB) and 33 (O-linked glucose of 4-HBTTNPB) and comparing these results to those obtained for 4-HPTTNPB.

Competition binding to RARs. Compounds 12 and 23 bind only very weakly to RARs, and are thus comparable to 4-HPTTNPB. Compounds 25, 29, 30 and 33 are only slightly better at binding to RARs than 4-HPTTNPB.

Competition binding to RXRs. Compounds 12 and 23 would be comparable to 4-HPTTNPB and show only slight binding competition. Compounds 25, 29, 30 and 33 are no better at binding to RXRs than 4-HPTTNPB.

Reporter gene assay studies may be performed as set forth herein using compounds 23 (4-HBTTNPB), 12 (propyl analog of 4-HPTTNPB), 25 (O-linked glucuronide of 4-HBTTNPB), 29 (CH₂-linked glucuronide of 4-HBTTNPB), 30 (CH₂-linked glucose of 4-HBTTNPB) and 33 (O-linked glucose of 4-HBTTNPB). The results may be compared to data obtained concerning 4-HPTTNPB which is set forth below.

Reporter Gene Assay: Compound 23 may be less active than 4-HPTTNPB (unable to undergo hydrolysis). Compound 12 may also be less active than 4-HPTTNPB, and it may liberate a metabolite that is inactive at RAR binding. Compounds 25, 29, 30 and 33 may be less active than 4-HPTTNPB.

Cell growth inhibition assays may be performed as set forth herein using compounds 23 (4-HBTTNPB), 12 (propyl analog of 4-HPTTNPB), 25 (O-linked glucuronide of 4-HBTTNPB), 29 (CH₂-linked glucuronide of 4-HBTTNPB), 30 (CH₂-linked glucose of 4-HBTTNPB) and 33 (O-linked glucose of 4-HBTTNPB). The data may be compared to data concerning 4-HPTTNPB which is set forth below.

Cell growth inhibition assay. Compound 23 is similar in activity to 4-HPTTNPB (unable to undergo hydrolysis). Compound 12 may also be less active than 4-HPTTNPB, and it may liberate a metabolite that was RAR inactive at binding. Compounds 25 and 29 are less active than 4-HPTTNPB, and compounds 30 and 33 are similar in activity to 4-HPTTNPB.

DNA fragmentation studies may be performed as set forth herein using compound 23 (4-HBTTNPB). Compound 23 may possess activity similar to 4-HPTTNPB in the DNA fragmentation study. Compounds 25 and 29 are less active than 4-HPTTNPB, and compounds 30 and 33 are similar in activity to 4-HPTTNPB.

Studies investigating the inhibition of DMBA-induced mammary tumors may be performed using compounds 23 (4-HBTTNPB), 25 (O-linked glucuronide of 4-HBTTNPB), 29 (CH₂-linked glucuronide of 4-HBTTNPB), 30 (CH₂-linked glucose of 4-HBTTNPB), and 33 (O-linked glucose of 4-HBTTNPB). Compounds 23, 25, 29, 30 and 33 may be active and demonstrate little, if any, toxicity.

Studies investigating the teratogenic potential are performed using compounds 23 (4-HBTTNPB), 25 (O-linked glucuronide of 4-HBTTNPB), 29 (CH₂-linked glucuronide of 4-HBTTNPB), 30 (CH₂-linked glucose of 4-HBTTNPB) and 33 (O-linked glucose of 4-HBTTNPB). Surprisingly and unexpectedly, the data will demonstrate that compounds 23, 25, 29, 30 and 33 possess little, if any, observable embrotoxicity.

The compounds of the present invention are useful in treating cellular proliferative disorders such as breast cancer. All references cited herein are specifically incorporated by reference in their entireties and for all purposes as if fully set forth herein.

We claim:

1. A compound according to the formula

![Chemical Structure](image-url)
2. A method for treating breast cancer in a human comprising administering to the human a therapeutically effective amount of a compound selected from the group consisting of

and salts and solvates thereof.