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(54) Title: NOVEL C-4 SUBSTITUTED RETINOIDS

(57) Abstract: Novel C-4 substituted all-trans retinoic acid analogs are synthesized and used to treat various cancers and dermatological diseases and conditions. These novel C-4 substituted all-trans retinoic acid analogs inhibit all-trans retinoic acid (ATRA) 4-hydroxylase activity, thereby inhibiting the catabolism of ATRA. These novel C-4 substituted ATRA analogs also have ATRA-mimetic activity. The substitutions at C-4 comprise azole, sulfur, oxygen, nitrogen, pyridyl, acetylinic, cyclopropyl-amine, ester, oxime, cyano, oxirone and aziridine groups.

NOVEL C-4 SUBSTITUTED RETINOIDS

This application claims priority from Provisional U.S. Application No. 60/217,465, filed July 11, 2000.

Background of Invention

Field of Invention

This invention relates to novel retinoic acid analogs that have substitutions at C-4. More specifically, this invention relates to the method of synthesis of novel C-4 substituted all-*trans* retinoic acid analogs and the method of using these novel compounds as therapeutic agents for cancers and dermatological diseases and conditions. This invention also relates to the pharmaceutical compositions containing these novel C-4 substituted all-*trans* retinoic acid analogs.

Description of the Related Art

All-*trans* retinoic acid (ATRA), the biologically most active metabolite of vitamin A, plays a major role in cellular differentiation and proliferation of epithelial tissues. Differentiating agents, such as ATRA, redirect cells towards their normal phenotype and therefore may reverse or suppress evolving malignant lesions or prevent cancer invasion (Hill DL and Grubbs CJ, Retinoids and cancer prevention, *Annu Rev Nutr* **12:** 161-181, 1992; Hong WK and Itri L, Retinoids and human cancer, In *The Retinoids: Biology, Chemistry and Medicine*, Sporn MB, Roberts AB and Goodman DS (eds), pp 597-630, Raven Press: New York, 1994). However, the therapeutic effects of retinoic acid are undermined by its rapid *in vivo* catabolism by cytochrome P450-dependent enzymes (Muindi J, Frankel SR, Miller WH Jr, Jakubowski A, Scheinberg DA, Young CW, Dmitrovsky E and Warrell RP Jr, Continuous treatment with all-trans retinoic acid causes a progressive reduction in plasma drug concentrations: implications for relapse and

retenoid "resistance" in patients with acute promylocytic leukemia, *Blood* **79:** 299-303, 1992; Smith MA, Parkinson DR, Cheson BD and Friedman MA, Retinoids in cancer chemotherapy, *J Clin Oncol* **10:** 839-864, 1992; Warrell RP Jr., Differentiating agents, In Cancer, principles and practice of oncology; DeVita Jr, Hellman S and Rosenberg SA (eds), Vol. I, pp 483-490, Lippincott: Philadelphia, 1997; Kizaki et al., 1996).

In addition, ATRA is known to have therapeutic effects for many dermatological diseases. Again, the fast catabolism of ATRA has limited the usefulness of the compound for treatment. (Cunliffe, 1986; Griffiths CEM, Fischer GJ, Finkel LJ, Voorhees JJ, Mechanism of action of retinoic acid in skin repair, *BR Journal of Dermatology*, 127 (Suppll):21-24, 1992).

ATRA can be metabolized through several routes. The physiologically most prominent pathway starts with hydroxylation at the 4-position of the cyclohexenyl ring, leading to the formation of 4-hydroxy-ATRA that is converted to more polar metabolites *via* 4-oxo-ATRA (Frolik CA, Roberts AB, Tavela TE, Roller PP, Newton DL and Sporn MB, Isolation and identification of 4-hydroxy- and 4-oxo-retinoic acid, *In vitro* metabolites of all-*trans*-retinoic acid in hamster trachea and liver, *Biochemistry* **18:** 2092-2097, 1979; Frolik CA, Roller PP, Roberts AB and Sporn MB, *In vitro* and *in vivo* metabolism of all-*trans*- and 13- *cis*-retinoic acid in hamsters, *J Biol chem* **255:** 8057-8062, 1980; Roberts AB, Nichols MD, Newton DL and Sporn MB, In vitro metabolism of retinoic acid in hamster intestine and liver, *J Biol Chem* **254:** 6296-6302, 1979; Roberts AB, Lamb LC and Sporn MB, Metabolism of all-*trans*-retinoic acid in Hamster liver microsomes: oxidation of 4-hydroxy- to 4-keto-retinoic acid, *Arch Biochem Biophys* **199:** 374-383, 1980; Van Wauwe J, Coene M-C, Cools W, Goosens J, Lauwers W, Le Jeune L, van Hove C and van Nyen G, Liarozole-fumarate inhibits the metabolism of 4-keto-*all*-trans-retinoic acid, *Biochem Pharmacol* **47:** 737-741j, 1994; Napoli JL, Retinoic acid

biosynthesis and metabolism, *FASEB J* **10:** 993-1001, 1996). The first and third catabolic steps are catalyzed by a cytochrome P450-dependent enzyme complex (Frolik CA, Roller PP, Roberts AB and Sporn MB, *In vitro* and *in vivo* metabolism of all-*trans*- and 13- *cis*-retinoic acid in hamsters, *J Biol chem* **255:** 8057-8062, 1980; Leo MA, Lida S and Lieber CS, Retinoic acid metabolism by a system reconstituted with cytochrome P450, *Arch Biochem Biophys* **243:** 305-312, 1984; Van Heusden J, Wouters W, Ramackers FCS, Krekels MDWG, Dillen L, Borgers M and Smets G, All-*trans*-retinoic acid metabolites significantly inhibit the proliferation of MCF-7 human breast cancer cells *in vitro*, *Br J Cancer* **77:** 26-32, 1998a; Van Heusden J, Wouters W, Ramackers FCS, Krekels MDWG, Dillen L, Borgers M and Smets G, All-*trans*-retinoic acid metabolites significantly inhibit the proliferation of MCF-7 human breast cancer cells *in vitro*, *Br J Cancer* **77:** 1229-1235, 1998b). Although the exact nature of this enzyme remains to be elucidated, a cytochrome P450 enzyme (designated CYP26) with specific ATRA 4-hydroxylase activity, which is also rapidly induced by ATRA has recently been cloned from zebra fish, mouse and man (for reviews, see Haque M, Andreola F, DeLuca LM, The cloning and characterization of a novel cytochrome P450 family, CYP26, with specificity towards retinoic acid, *Nutri Rev* **56**:84-85, 1999; Sonneveld E and Vander Sagg PT, Metabolism of retinoic acid: implications for development and cancer, *Inter. J Vit Nutr Res* **68**: 404-410, 1998).

Initially, the 4-hydroxylase activity was thought to mainly reside in the liver (Roberts AB, Lamb LC and Sporn MB, Metabolism of all-*trans*-retinoic acid in Hamster liver microsomes: oxidation of 4-hydroxy- to 4-keto-retinoic acid, *Arch Biochem Biophys* **199:** 374-383, 1980), but its presence has now been demonstrated in skin and tumor cells and tissues (Vanden Bossche H, Willemsens G, Retinoic acid and cytochrome P450, In *Retinoids: 10 Years On.* Saurat JH (ed). pp 79-88, Karger: Basel, 1990; Varani J, Gendimenico GA, Hhah B, Gibbs

D, Capetola RJ, Mezick JA and Voorhess JJ, A direct comparison of pharmacologic effects of retinoids on skin cells *in vitro* and *in vivo*, *Skin Pharmacol* **4:** 254-261, 1991; Wouters W, Van Dun J, Dillen A, Coene M.-C, Cools W and De Coster R, Effects of liarozole, a new antitumoral compound an retinoic acid-induced inhibition of cell growth and on retinoic acid metabolism in MCF-7 breast cancer cells, *Cancer Res* **52:** 2841-2846, 1992; Krekels MDWG, Zimmerman J, Janssen B, Van Ginckel R, Van Hove C, Coene M.-C and Wouter W, Analysis of the oxidative catabolism of retinoic acid in rat Dunning R 3327G prostate tumors, *Prostate* **29:** 36- 41, 1996).

In principle, inhibitors of 4-hydroxylase should increase endogenous levels of ATRA (acting as 'ATRA-mimetics') and overcome some ATRA-resistance. A number of azole compounds which inhibit several cytochrome P450 enzymes have also been shown to be inhibitors of ATRA 4-hydroxylase (Williams JB and Napoli JL, Metabolism of retinoic acid and retinol during differentiation of F9 embryonal cells, *Proc Natl Acad Sci USA* **82:** 4658-4662, 1985; Williams JB and Napoli JL, Inhibition of retinoic acid metabolism by imidazole antimycotics in F9 embroynal carcinoma cells, *Biochem Pharmacol* **36:** 1386-1388, 1987; Napoli JL, Retinoic acid biosynthesis and metabolism, *FASEB J* **10:** 993-1001, 1996; Roberts AB, Nichols MD, Newton DL and Sporn MB, In vitro metabolism of retinoic acid in hamster intestine and liver, *J Biol Chem* **254:** 6296-6302, 1979; Vanden Bossche H, Willemsens G and Janssen PAJ, Cytochrome-P-450-dependent metabolism of retinoic acid in rat skin microsomes: Inhibition by ketoconazole, *Skin Pharmacology* **1:** 176-185, 1988; Van Wauwe JP, Coene MC, Goossens J, Van Nijen G, Cools W, Lauwers W, Ketoconazole inhibits the in vitro and in vivo metabolism of all-trans-retinoic acid, *J Pharmacol Exp Ther*, 245:718-722, 1988; Freyne E, Raeymaekers A, Venet M, Sanz G, Wouters W, De Coster R and Van Wauwe J, Synthesis of Liazal™, a retinoic acid metabolism blocking agent (RAMBA) with potential clinical

applications in oncology and dermatology, *Bioorg Med Chem Lett* **8:** 267-272, 1998). The discovery of retinoic acid metabolism blocking agents (RAMBAs) have led to interest of using RAMBAs in the treatments of cancers. (Miller, Jr., W.H., The Emerging Role of Retinoids and Retinoic Acid Metabolism Blocking Agents in the Treatment of Cancer, *Cancer*, 83, 1471-1482, 1998). Inhibitors of retinoic acid metabolism are known as retinoic acid metabolism blocking agents or "RAMBAs".

Liarozole fumarate (LIAZAL™), a (1H- imidazol-1-ylmethyl)-1H-benzimidazole derivative, is one of the first new generation RAMBAs in clinical practice. Liarozole fumarate may soon be approved for the treatment of prostate cancer. (see, Waxman J. Roylance R., Editorial: New Drugs for Prostate Cancer? *Eur. J. Cancer*, 34, 437, 1998; and Debruyne, F.J.M. et al., Liarozole-A Novel Treatment Approach for Advanced Prostate Cancer: Results of a Large Randomized Trial versus Cyproterone, *Urology*, 52, 72-81, 1998)

Studies of liarozole's pharmacodynamics revealed that it inhibits ATRA 4-hydroxylase. (De Coster R, Wouters W, Van Ginckel R, End D, Krekels M, Coene M.-C and Bowden C, Experimental studies with liarozole (R75251): an antitumoral agent which inhibits retinoic acid breakdown, *J Steroid Biochem Molec Biol* **43:** 197-201, 1992) However, the FDA's review of phase III trial data for liarozole in prostate cancer was negative. Although clinical efficacy was seen, the activity/toxicity ratio was considered insufficient. Hence Janssen Pharmaceutica NV, liarozole's manufacturer, has discontinued clinical development of liarozole (Wouters W (2000) *Personal communication*; Njar VCO and Brodie AMH, Inhibitors of cytochrome P450 enzymes: Their role in prostae cancer therapy, *I Drugs* **1**: 495-506, 1999c). It appears that the reason for the high toxicity was that liarozole inhibits ATRA 4-hydroxylase only at micromolar concentrations, and at those levels it also exhibits harmful inhibitory activity with other

cytochrome P450 enzymes (Bruynseels et al., 1990). The adverse side-effects of liarozole in the treatment of prostate cancer may be caused by a lack of selectivity for and/or potent inhibition of ATRA 4-hydroxylase enzyme.

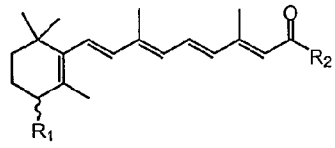
Because of therapeutic benefits of liarozole for prostate cancer are limited by its side-effects, it would be useful to have compounds that inhibit ATRA 4-hydroxylase in nanomolar concentrations and have greater specificity for ATRA 4-hydroxylase than liarozole. Such compounds may avoid the harmful side-effects of liarozole and be tolerated better. Such compounds may also be useful in the treatment of other types of cancers and various dermatological conditions.

Thus, this invention helps overcome the problems of treating cancers and dermatological diseases and dermatological conditions with novel compounds that block catabolism of all-*trans* retinoic acid. These novel compounds have higher specificity to enzymes involved in retinoic acid catabolism and lower toxicity for the patient. Selective and potent inhibitory compounds of ATRA catabolism, using nanomolar concentration of the compounds, result in effective modulation to desirable levels of ATRA, either endogenous ATRA or of ATRA mimetic compounds. With higher levels of ATRA, the patient will have improved prognosis and outcomes.

The novel compounds in this invention are ATRA analogs that have substitutions at the C-4.

Brief Summary of the Invention

This invention is a novel chemical compound having the formula (I)



(I)

In formula (I), R_1 is azole, sulfur, oxygen, nitrogen, pyridyl, acetylinic, cyclopropyl-amine, esters, oxime, cyano, oxirane or aziridine; and R_2 is hydroxyl, aminophenols, esters, or azoles.

R_1 may be a sulfur containing group. Examples of such sulfur containing groups include thiirane, thiol and alkylthiol derivatives. Examples of such alkylthiol derivatives include C_1 to C_{10} alkyl thiols.

R_1 may be an oxygen containing group. Examples of oxygen containing groups include $-OR_4$, where R_4 is hydrogen or an alkyl group (preferably a 1-10 carbon alkyl, more preferably methyl or ethyl), cyclopropylether or an oxygen containing group that forms, together with the 4-position carbon, an oxirane group.

R_1 may be a nitrogen containing group. Examples of such nitrogen containing groups include the formula $-NR_5R_6$, where R_5 and R_6 are independently selected from the group consisting of hydrogen and alkyl groups (preferably a 1-10 carbon alkyl, more preferably methyl or ethyl), or R_5 and R_6 may together form a ring. Preferably the ring formed by R_5 and R_6 is a imidazolyl ring or a triazole ring.

Preferable azole substituent groups include imidazoles and triazoles. More preferably, the azole substituent groups include 1*H*-imidazole-1-yl, 1*H*-1,2,4-triazol-1-yl and 4*H*-1,2,4-triazol-1-yl.

R_1 may be a cyano, amino, azido, cyclopropylamino, or R_1 is a nitrogen containing group that forms, together with the 4-position carbon, an aziridine group or an oxime group.

R_1 may also be a pyridyl group or a allylic azole group, preferably methyleneazolyl.

The definitions for R_1 of an ester includes substituent groups that contain an ester moiety, including substituent groups attached via an ester moiety.

R_2 may be preferably selected from the group consisting of hydroxyl, aminophenol, $-OR_3$ and azole groups, wherein R_3 is selected from the group consisting of alkyl, aryl and heterocyclic groups, more preferably, hydroxyl or $-OCH_3$ (methoxy).

Said alkyl substituents for the above identified substituent groups include substituted and unsubstituted alkyl groups, branched and straight chain and cyclo alkyl groups, such as cyclopropyl.

The term "aryl" includes a phenyl or naphthyl ring.

The term "heterocyclic group" includes an unsubstituted or substituted stable 3- to 7-membered monocyclic or 7- to 10-membered bicyclic heterocyclic ring and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of nitrogen, oxygen or sulfur, and wherein the nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized and including a bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached at any heteroatom or carbon atom which affords a stable structure. The hetercyclic group may be saturated or unsaturated.

Examples of heterocyclic groups include piperidinyl, piperazinyl, azepinyl, pyrrolyl, 4-piperidonyl, pyrrolidinyl, pyrazolyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl,

morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, quinuclidinyl, isothiazolidinyl, indolyl, quinolinyl, isoquinolinyl, benzimidazolyl, thiadiazolyl, benzopyranyl, benzothiazolyl, benzoazolyl, furyl, tetrahydrofuryl, tetrahydropyranyl, thienyl, benzothienyl, thiamorpholinyl, thiamorpholinylsulfoxide, thiamorpholinylsulfone, oxadiazolyl, triazolyl, tetrahydroquinolinyl, and tetrahydroisoquinolinyl.

It is an object of this invention to synthesize novel C-4 substituted retinoic acid analogs.

It is a further object of this invention that the synthesized C-4 retinoic acid analogs inhibit ATRA 4-hydroxylase. The terms "inhibit" and "inhibition" include total inhibition and less than total inhibition of ATRA 4-hydroxylase.

It is another object of this invention to use the novel C-4 substituted ATRA analogs to inhibit ATRA 4-hydroxylase.

It is another object of this invention to use the novel C-4 substituted ATRA analogs alone or in combination with other compounds, including retinoic acid, to treat cancer. It is another object of this invention to use the novel C-4 substituted ATRA analogs alone or in combination with other compounds to treat melanoma, leukemia, lymphoma, breast, prostate, ovarian, lung, or other types of cancers.

It is another object of this invention to use the novel C-4 substituted ATRA analogs alone or in combination with other compounds, including retinoic acid, to treat dermatologic diseases or dermatologic conditions. It is another object of this invention to use the novel C-4 substituted ATRA analogs alone or in combination with other compounds to treat acne, psoriasis, wrinkling, photoaged skin, and other dermatologic conditions or diseases.

It is an object of this invention to synthesis an all-*trans* retinoic acid analog with azole substituted at C-4. It is a further object of this invention to use the C-4 substituted azole all-*trans*

retinoic acid analog to treat cancer. It is a further object of this invention to use the C-4 substituted azole all-*trans* retinoic acid analog to treat melanoma, leukemia, lymphoma, breast, prostate, ovarian, lung or other types of cancers. It is a further object of this invention to use the C-4 substituted azole all-*trans* retinoic acid analog to treat dermatological diseases and dermatological conditions. It is a further object of this invention to use the C-4 substituted azole all-*trans* retinoic acid analog to treat psoriasis, and dermatological conditions ranging from acne to photoaged skin to wrinkling.

It is an object of this invention to synthesis an all-*trans* retinoic acid analog with sulfur substituted at C-4. It is a further object of this invention to use the C-4 substituted sulfur all-*trans* retinoic acid analog to treat cancer. It is a further object of this invention to use the C-4 substituted sulfur all-*trans* retinoic acid analog to treat melanoma, leukemia, lymphoma, breast, prostate, ovarian, lung or other types of cancers. It is a further object of this invention to use the C-4 substituted sulfur all-*trans* retinoic acid analog to treat dermatological diseases and dermatological conditions. It is a further object of this invention to use the C-4 substituted sulfur all-*trans* retinoic acid analog to psoriasis, and dermatological conditions ranging from acne to photoaged skin to wrinkling.

It is an object of this invention to synthesis an all-*trans* retinoic acid analog with oxygen substituted at C-4. It is a further object of this invention to use the C-4 substituted oxygen all-*trans* retinoic acid analog to treat cancer. It is a further object of this invention to use the C-4 substituted oxygen all-*trans* retinoic acid analog to treat melanoma, leukemia, lymphoma, breast, prostate, ovarian, lung or other types of cancers. It is a further object of this invention to use the C-4 substituted oxygen all-*trans* retinoic acid analog to treat dermatological diseases and dermatological conditions. It is a further object of this invention to use the C-4 substituted

oxygen all-*trans* retinoic acid analog to treat psoriasis, and dermatological conditions ranging from acne to photoaged skin to wrinkling.

It is an object of this invention to synthesis an all-*trans* retinoic acid analog with nitrogen substituted at C-4. It is a further object of this invention to use the C-4 substituted nitrogen all-*trans* retinoic acid analog to treat cancer. It is a further object of this invention to use the C-4 substituted nitrogen all-*trans* retinoic acid analog to treat melanoma, leukemia, lymphoma, breast, prostate, ovarian, lung or other types of cancers. It is a further object of this invention to use the C-4 substituted nitrogen all-*trans* retinoic acid analog to treat dermatological diseases and dermatological conditions. It is a further object of this invention to use the C-4 substituted nitrogen all-*trans* retinoic acid analog to treat psoriasis, and dermatological conditions ranging from acne to photoaged skin to wrinkling.

It is an object of this invention to synthesis an all-*trans* retinoic acid analog with pyridyl groups substituted at C-4. It is a further object of this invention to use the C-4 substituted pyridyl groups all-*trans* retinoic acid analog to treat cancer. It is a further object of this invention to use the C-4 substituted pyridyl groups all-*trans* retinoic acid analog to treat melanoma, leukemia, lymphoma, breast, prostate, ovarian, lung or other types of cancers. It is a further object of this invention to use the C-4 substituted pyridyl groups all-*trans* retinoic acid analog to treat dermatological diseases and dermatological conditions. It is a further object of this invention to use the C-4 substituted pyridyl groups all-*trans* retinoic acid analog to treat psoriasis, and dermatological conditions ranging from acne to photoaged skin to wrinkling.

Animals, including mammals and humans may be treated.

Preferably, the other compound used in combination with the novel C-4 substituted ATRA analog is ATRA.

Brief Description of the Drawings

Figure 1. Schematic pathway for synthesis of C-4 azole substituted ATRA analogs. $TMSCHN_2$ is an abbreviation for trimethylsilyldiazomethane; CDI is an abbreviation for carbonyldiimidazole; and CDT is an abbreviation for carbonylditriazole.

Figure 2. Schematic pathway for synthesis of C-4 sulfur substituted ATRA analogs. TMSI is an abbreviation for trimethylsulfonium iodide, TPS is an abbreviation for triphenylphosphine sulfide, and DDT is an abbreviation for dithiothreitol.

Figure 3. Schematic pathway for synthesis of C-4 oxygen substituted ATRA analogs.

Figure 4. Schematic pathway for synthesis of C-4 nitrogen substituted ATRA analogs.

Figure 5. Schematic pathway for synthesis of C-4 pyridyl substituted ATRA analogs.

Figure 6. Schematic pathway for synthesis of C-4 substituted ATRA analogs that are mechanism-based inhibitors.

Figure 7. VN/14-1RA inhibits ATRA metabolism in hamster liver microsomes. Figure 7A has no VN/14-1RA. Figure 7B has 1 μM of VN/14-1RA.

Figure 8. VN/14-1RA and ATRA inhibit growth of LNCaP cells.

Figure 9. VN/16-1RA and ATRA inhibit growth of LNCaP cells.

Figure 10. VN/17-1RA and ATRA inhibit growth of LNCaP cells.

Figure 11. Liarozole and ATRA inhibit growth of LNCaP cells.

Figure 12. VN/14-1RA inhibits growth of MCF-7Ca tumors in nude mice.

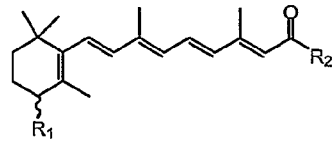
Detailed Description of the Invention

All-*trans* retinoic acid (ATRA) is a well known and characterized compound. Its catabolic pathway involves ATRA 4-hydroxylase. The iron oxene species (Fe^{V}=O) of ATRA 4-hydroxylase is responsible for molecular oxygen activation and thus, the break-down of ATRA.

The Fe^{V}=O group of ATRA 4-hydroxylase has access to the C-4 of ATRA in that C-4 is within bonding distance of the activated oxygen. Substitution of suitable groups at the C-4 of ATRA will generate ATRA analogs which both react with the retinoid-binding site of the enzyme and interacts with the heme iron and/or the protein residue with high specificity. Substitutions of suitable groups can increase the inhibitory affects of the new compounds with K_i values in the nanomolar range.

For ATRA analogs with C-4 substitutions with azole, sulfur, oxygen, or nitrogen, following binding at the active-site of the 4-hydroxylase enzyme, the lone pair of electrons coordinate to the prosthetic heme iron causing inhibition of the enzyme. Blockage of ATRA 4-hydroxylase activity increases the amount of ATRA.

In this invention, the term "novel compounds" or "C-4 substituted ATRA analogs" refers to ATRA analogs with various moieties substituted for hydrogen at C-4 and also hydroxyl or various moieties substituted for hydroxyl at C-15. The chemical structure of the novel compounds or C-4 substituted ATRA analogs is shown in Scheme I below where R_1 is azole, sulfur, oxygen, nitrogen, pyridyl, acetylinic, cyclopropyl-amine, esters, oxime, cyano, oxirane, or aziridine; and R_2 is hydroxyl, aminophenols, esters, or azoles.



(I)

The compound may be used in a pharmaceutical composition. The pharmaceutical composition may be formulated for oral administration, parentral administration or for injectable administration.

In making the compositions of the present invention, the novel compound can be mixed with a pharmaceutically acceptable carrier or an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier, or medium for the novel compound. Thus, the compositions can be in the form of tablets, pills, powers, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, soft and hard gelatin capsules, and other orally ingestible formulations.

The pharmaceutical compositions may be in the form of a solution, suspension, tablet, capsule or the like, prepared according to methods well known in the art. It is also contemplated that administration of such compositions may be by the oral, injectable and/or parenteral routes depending upon the needs of the artisan. The novel compound can be administered by nasal or oral inhalation, oral ingestion, injection (intramuscular, intravenous, and intraperitoneal), transdermally, or other forms of administration.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, and methyl cellulose. The formulations can additionally include lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propyl-hydroxybenzoates, sweetening agents; and flavoring agents. The compositions of the present invention can also be formulated so as to provide quick, sustained or

delayed release of the novel compound after administration to the patient by employing procedures known in the art.

The term "pharmaceutically acceptable carrier" refers to those components in the particular dosage form employed which are considered inert and are typically employed in the pharmaceutical arts to formulate a dosage form containing a particular active compound. This may include without limitation solids, liquids and gases, used to formulate the particular pharmaceutical product. Examples of carriers include diluents, flavoring agents, solubilizers, suspending agents, binders or tablet disintegrating agents, encapsulating materials, penetration enhancers, solvents, emolients, thickeners, dispersants, sustained release forms, such as matrices, transdermal delivery components, buffers, stabilizers, and the like. Each of these terms is understood by those of ordinary skill.

Aerosol formulations for use in this invention typically include propellants, such as a fluorinated alkane, surfactants and co-solvents and may be filled into aluminum or other conventional aerosol containers which are then closed by a suitable metering valve and pressurized with propellant, producing a metered dose inhaler. Aerosol preparations are typically suitable for nasal or oral inhalation, and may be in powder or solution form, in combination with a compressed gas, typically compressed air. Additionally, aerosols may be useful topically.

Generally, the amount of the novel compound used in the treatment methods is that amount which effectively achieves the desired therapeutic result in animals. Naturally, the dosages of the various novel compounds will vary somewhat depending upon the parent compound, rate of in vivo hydrolysis, etc. Those skilled in the art can determine the optimal dosing of the novel compound selected based on clinical experience and the treatment indication.

Preferably the amount of the novel compound is 0.1 to 100 mg/kg of body weight, more preferably, 5 to 40 mg/kg.

Suitable solid carriers are known, e.g., magnesium carbonate, magnesium stearate, talc, lactose and the like. These carriers are typically used in oral tablets and capsules.

Suitable carriers for oral liquids include, e.g., water, ethanol, propylene glycol and others.

Topical preparations useful herein include creams, ointments, solutions, suspensions and the like. These may be formulated to enable one to apply the appropriate dosage topically to the affected area once daily, up to 3-4 times daily as appropriate. Topical sprays may be included herein as well.

Depending upon the particular compound selected, transdermal delivery may be an option, providing a relatively steady state delivery of the medication which is preferred in some circumstances. Transdermal delivery typically involves the use of a compound in solution, with an alcoholic vehicle, optionally a penetration enhancer, such as a surfactant and other optional ingredients. Matrix and reservoir type transdermal delivery systems are examples of suitable transdermal systems. Transdermal delivery differs from conventional topical treatment in that the dosage form delivers a systemic dose of medication to the patient.

The novel compound can also be converted into a pharmaceutically acceptable salt or pharmaceutically acceptable solvate or other physical forms (e.g., polymorphs by way of example only and not limitation) via known in the art field methods.

General Methods for Synthesis of Novel Compounds

Melting points (mp) are determined with a Fischer-Johns melting point apparatus and are uncorrected. Proton magnetic resonance spectra (^{1}H NMR) are recorded in $CDCl_3$ on a Mac NMR 5.3 300 MHz spectrometer (internal standard Me_4Si, (= 0), and high resolution mass

spectra (HRMS) are determined on a Kratos Aspect Systems instrument, EI mode. Elemental analyses are performed by Chemisar Laboratories Inc., Guelph, Ontario, Canada. TLC is done on silica gel GHLF precoated plates (250 microns) purchased from Analtech, while flash column chromatography (FCC) is performed on silica gel (Merck grade 9385, 230 - 400 mesh, 60 Å) according to Still's method. (Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separation with Moderate Resolution. *J. Org. Chem.*, *43*, 2923-2925, 1978). "Pet. ether" refers to light petroleum, bp 40 - 60 °C. [11,12-^{3}H] All-*trans*-retinoic acid (ATRA, 51.8 (Ci/mmol) is purchased from New England Nuclear Life Science Products, Inc., while unlabeled ATRA is purchased from Sigma-Aldrich Chemical Co., St. Louis, MO. Liarozole fumarate is obtained from Janssen Pharmaceutica, Beerse, Belgium. (±)-4-Hydroxy-ATRA and 4-oxo-ATRA are prepared using a well-known technique (Samokyszyn, V. M.; Gall, W. E.; Zawada, G.; Freyaldenhoven, M. A.; Chen, G.; Mackenzie, P. I.; Tephly, T. R.; Radominska-Pandya, A. 4-Hydroxyretinoic Acid, a Novel Substrate for Human Liver Mcrosomal UDP-glucuronosyltransferase(s) and Recombinant UGT2B7. *J. Biol. Chem.* **2000**, *275*, 6908-6914.)

C-4 Azole Substituted ATRA Analogs

The pathway for synthesis of C-4 azole substituted ATRA analogs is shown in Figure 1. The starting point of the synthesis is ATRA, **1**. Methyl retinoate, **2**, is readily prepared in quantitative yield from ATRA, **1**, by reaction with trimethylsilyl diazomethane ($TMSCHN_2$) using a well-known technique (Hashimoto, N.; Aoyoma, T.; Shioiri, T. New Methods and Reagents in Organic Synthesis, A simple Efficient Preparation of Methyl Esters with Trimethylsilyldiazomethane ($TMSCHN_2$) and Its Application to Gas Chromatographic Analysis of Fatty Acids, *Chem. Pharm. Bull.*, *29*. 1475-1478, 1981). Then methyl retinoate, **2**, (1.04 g, 3.3

mmol) dissolved in dry CH_2Cl_2 (100 mL) is treated with excess activated MnO_2 (20 g, ex Fluka), and the reaction mixture is stirred at room temperature for 48 hours. The MnO_2 is removed by filtration, the filtrate is concentrated to afford an orange viscous oil which is purified by FCC [pet. ether/EtOAc, (8:1)] to give starting material (2.2 g) and 4-oxo-all-*trans*-methyl retinoate, **3**, (0.65 g, 60 %) as a viscous oil. 4-oxo-all-*trans*-methyl retinoate, **3**, is crystallized from pet. ether at room temperature, mp 94-95 °C. ^{1}H NMR δ 1.19 (6H, s, 16- and 17-Mes), 1.86 (3H, s, 18-Me), 2.04 (3H, s, 19-Me), 2.37 (3H, s, 20-Me), 3.72 (3H, s, OMe), 5.82 (1H, s, 14-H), 6.30 (4H, m, 7-, 8-, 10- and 12-Hs), 6.98 (1H, t, J = 11.4 Hz, 11-H). HRMS calculated for $C_{21}H_{28}O_3$ 328.2038 found 328.2030.

The next step is to synthesize (±)-4-hydroxy-all-*trans*-methyl retinoate, **4**. To a stirred solution of 4-oxo-all-*trans*-methyl retinoate, **3**, (500 mg, 1.52 mmol) in dry MeOH (15 mL) at room temperature is added $NaBH_4$ (53 mg, 1.40 mmol). After 30 minutes, the reaction is quenched with H_2O and concentrated. The residue is diluted with EtOAc, washed with H_2O, brine, dried (Na_2SO_4), and concentrated to give a yellow semi-solid which crystallized following tituration with hexane to give (±)-4-hydroxy-all-*trans*-methyl retinoate, **4**, yellow crystals (452 mg, 91 %), mp 102-104 °C. ^{1}H NMR δ 1.02, 1.05 (6H, 2s, 16- and 17-Mes), 1.84 (3H, s, 18-Me), 2.00 (3H, s, 19-Me), 2.36 (3H, s, 20-Me), 3.72, (3H, s, OMe), 4.00 (1H, brs, 4-H), 5.79 (1H, s, 14-H), 6.20 (4H, m, 7-, 8-, 10- and 12-Hs), 6.99 (1H, t, J = 11.7 Hz, 11-H). Anal. calculated for $C_{21}H_{30}O_3$: C, 76.31; H, 9.16. Found C, 76.39; H, 9.10. HRMS calculated for $C_{21}H_{30}O_3$ 330.2195 found 330.2191.

The next step is to synthesis (±)-4-(1*H*-imidazol-1-yl)-methyl retinoate, **5**. A solution of 4-hydroxy-all-*trans*-methyl retinoate, **4**, (300 mg, 0.9090 mmol) and carbonyldiimidazole (CDI, 195 mg, 1.2025 mmol) in dry CH_3CN (5.0 mL) is stirred at room temperature for 10 minutes.

The reaction mixture is diluted with water (20 mL) and extracted with 10 % MeOH in CH_3Cl (10 mL x 3). The combined extract is washed with brine (10 mL x 2), dried (Na_2SO_4) and evaporated to give a viscous yellow oil (350 mg). This is purified by FCC [CH_2Cl_2 /EtOAc /Et_3N, (7:3:0.3)] to give (±)-4-(1*H*-imidazol-1-yl)-methyl retinoate, **5**, as a yellow semi-solid (311 mg, 90%): ^1H NMR δ 1.09 and 1.12 (6H, 2s, 16- and 17-Hs), 1.60 (3H, s, 18-Me), 2.02 (3H, s, 19-Me), 2.36 (3H, 3, 20-Me), 3.72 (3H, s, OMe), 4.53 (1H, s, 4-H), 5.80 (1H, s, 14-H), 6.25 (4H, m, 7-, 8-, 10- and 12-Hs), 6.91 (1H, s, 4^1-H), 6.98 (1H, t, J = 14.7 Hz, 11-H), 7.07 (1H, s, 5^1-H), 7.50 (1H, s, 2^1-H). HRMS calculated for $C_{24}H_{32}O_2N_2$ 380.5331 found 380.5334.

The next step involves synthesis of (±)-4-(1*H*-imidazole-1-yl)retinoic acid, **6** (also referred to as "**VN/14-1RA**"). A solution of (±)-4-(1*H*-imidazol-1-yl)-methyl retinoate, **5**, (270 mg, 0.7077 mmol) in 12 mL of 1M KOH in a 1:9 mixture of water and MeOH is diluted with MeOH (14 mL), and is followed by refluxing under N_2 for 2 hour. The reaction mixture is concentrated to approximately 10 mL, diluted with cold water (25 mL) and is acidified with a few drops of 6N HCl. The resulting yellow precipitate is filtered, washed and dried to give pure (±)-4-(1*H*-imidazole-1-yl)retinoic acid, **6**, as a yellow solid (225 mg, 86.6 %): mp 128-130 °C, ^1H NMR δ 1.13 (6H, s, 16- and 17-Hs), 1.67 (3H, s, 18- Me), 2.02 (3H, s, 19-Me), 2.32 (3H, s, 20-Me), 4.84 (1H, s, 4-H), 5.85 (1H, s, 14-H), 6.21 (3H, s, 8-, 10- and 12-Hs), 6.33 (1H, d, J = 15.0 Hz, 8-H), 7.00 (1H, t, J = 14.0 Hz, 11-H), 7.16 (1H, s, 4^1-H), 7.26 (1H, s, 5^1-H), 7.46 (1H, s, 2^1-H), 8.75 (1H, brs, -COOH). Anal. calculated for $C_{23}H_{30}O_2N_2$: C, 75.38; H, 8.25; N, 7.64. Found: C, 75.72; H, 8.65; N, 7.67. HRMS calculated for $C_{23}H_{30}O_2N_2$ 366.3061 found 366.3056.

To synthesize triazole groups at C-4, one follows an alternate pathway. Beginning with (±)-4-hydroxy-all-*trans*-methyl retinoate, **4**, a solution of (±)-4-hydroxy-all-*trans*-methyl retinoate, **4**, (270 mg, 0.8182 mmol) and N,N^1-carbonyldi(1,2,4-triazole) (CDT) (187.1 mg,

1.1340 mmol) in dry CH_3CN (4.5 mL) is stirred at room temperature for 10 minutes. The reaction mixture is diluted with water (20 mL) as was processed as described above for (±)-4-(1*H*-imidazol-1-yl)-methyl retinoate, **5**, above to give a yellow solid (310 mg). Analytical TLC [CH_2Cl_2/EtOH, (20:1)] reveals the presence of two compounds, both more polar that (±)-4-hydroxy-all-*trans*-methyl retinoate, **4**. This crude product is subjected to FCC and on elution with CH_2Cl_2/EtOH (35:1), gives (±)-4-(1*H*-1,2,4-triazol-1-yl)methyl retinoate, **7**, (177mg, 57 %): mp. 105-108 °C; ^{1}H NMR δ 1.10 and 1.13 (6H, 2s, 16- and 17-Mes), 1.63 (3H, s, 18-Me), 2.02 (3H, s, 19-Me), 2.36 (3H, s, 20-Me), 3.72 (3H, s, OMe), 4.82 (1H, s, 4-H), 5.80 (1H, s, 14-H), 6.30 (4H, m, 7-, 8-, 10- and 12-Hs), 6.99 (1H, t, J = 14.1 Hz, 11-H), 7.99 (1H, s, 3^1-H), 8.02 (1H, s, 5^1-H). Anal. calculated for $C_{23}H_{31}O_2N_3$: C, 72.41; H, 8.19; N, 11.01. Found C, 72.45; H, 8.15; N, 10.97. HRMS calculated for $C_{23}H_{31}O_2N_3$ 381.5208 found 381.5211.

Further elution with CH_2Cl_2/EtOH (20:1) affords (±)-4-(4*H*-1,2,4-triazole-1-yl)methyl retinoate, **8**, (89 mg, 28.5 %): mp 62-65 °C; ^{1}H-NMR δ 1.10 and 1.13 (6H, 2s, 16- and 17-Mes), 1.64 (3H, s, 18-Me), 2.02 (3H, s, 19-Me), 2.36 (3H, s, 20-Me), 3.72 (3H, s, OMe), 4.64 (1H, s, 4-H), 5.81 (1H, s, 14-H), 6.25 (4H, m, 7-, 8-, 10- and 12-Hs), 6.98 (1H, t, J = 14.4 Hz, 11-H), 8.15 (2H, s, 3^1- and 5^1-H). Anal. calculated for $C_{23}H_{31}O_2N_3$: C, 72.41; H, 8.19; N, 11.01. Found C, 72.55; H, 8.10; N, 11.00. HRMS calculated for $C_{23}H_{31}O_2N_3$ 381.5208 found 381.5213.

To synthesize (±)-4-(1*H*-1,2,4-triazol-1-yl)retinoic acid, **9**, (also referred to as "VN/16-1RA"), use the method that describes the synthesis for **VN/14-1RA** (above) but use (±)-4-(1*H*-1,2,4-triazol-1-yl)methyl retinoate, **7**, (285 mg, 0.7470 mmol) to give **VN/16-1RA** (247 mg, 90 %): mp 95-97 °C; ^{1}H-NMR δ 1.10 and 1.13 (6H, 2s, 16- and 17-Mes), 1.65 (3H, s, 18-Me), 2.03 (3H, s, 19-Me), 2.36 (3H, s, 20-Me), 4.86 (1H, s, 4-H), 5.84 (1H, s, 14-H), 6.32 (4H, m, 7-, 8-, 10- and 12-Hs), 7.01 (1H, t, J = 14.5 Hz, 11-H), 8.10 (1H, s, 3^1-H), 8.31 (1H, s, 5^1-H). Anal.

calculated for $C_{22}H_{29}O_2N_3$: C, 71.90; H, 7.95; N, 11.43. Found C, 71.70; H, 8.11; N, 11.55. HRMS calculated for $C_{22}H_{29}O_2N_3$ 367.4938 found 367.4935.

To synthesize (±)-4-(4*H*-1,2,4-triazol-1-yl)retinoic acid, **10**, (also referred to as "**VN/17-1RA**") use the method that describes the synthesis for **VN/14-1RA** (above) but use (±)-4-(4*H*-1,2,4-triazole-1-yl)methyl retinoate, **8**, (134 mg, 0.3512 mmol) to give **VN/17-1RA** (110 mg, 85 %): mp 105-108 °C; ^{1}H-NMR δ 1.11 and 1.14 (6H, 2s, 16- and 17-Mes), 1.65 (3H, s, 18-Me), 2.03 (3H, s, 19-Me), 2.37 (3H, s, 20-Me), 4.78 (1H, s, 4-H), 5.85 (1H, s, 14-H), 6.19 (4H, m, 7-, 8-, 10- and 12-Hs), 7.01 (1H, t, J = 14.2 Hz, 11-H), 8.46 (2H, s, 3^1- and 5^1- H). Anal. calculated for $C_{22}H_{29}O_2N_3$: C, 71.90; H, 7.95; N, 11.43. Found C, 71.90; H, 7.79; N, 11.30. HRMS calculated for $C_{22}H_{29}O_2N_3$ 367.4938 found 367.4939.

C-4 Sulfur Substituted ATRA Analogs

Figure 2 shows the schematic pathway for synthesis of C-4 sulfur substituted ATRA analogs. The C-4 sulfur substituted ATRA analogs include 4-thiirane **12**, 4-thiol **14**, and 4-alkylthio derivatives **15**. Methyl retinoate **2** is transformed into 4-oxirane **11** via 4-oxomethylretinoate **3**. Treatment of 4-oxirane **11** with trimethylsulfonium iodide (TPI) in picric acid followed by hydrolysis affords 4-thiirane **12**. 4-thiol **14** is prepared from 4-mesylate **13** by treatment with dithiothreitol (DTT). Treatment of 4-thiol **14** with various alkyl halides affords the desired 4-alkyl derivatives **15**.

C-4 Oxygen Substituted ATRA Analogs

Figure 3 shows the synthesis pathway for C-4 oxygen substituted ATRA analogs. 4-oxirane **16** is synthesized from the 4-oxo compound **3** by treatment with TMSI followed by hydrolysis in methanolic KOH as shown in Figure 3.

C-4 Nitrogen Substituted ATRA Analogs

Figure 4 shows the synthesis pathway for C-4 nitrogen substituted ATRA analogs such as 4-oxime **17**, 4-azido **18**, 4-cyano **19**, 4-amine **20**, 4-aziridine **21**, and 4-allylic azoles **22**. Treatment of 4-oxo methylretinoate **3** with hydroxylamine hydrochloride followed by hydrolysis yields 4-oxime **17**. Treatment of 4-mesylate **13** with either NaN_3 or NaCN followed by hydrolysis yields 4-azido **18** and 4-cyano **19**, respectively. $NaBH_4$ reduction of 4-cyano **19** yields 4-amine **20**. Reaction of 4-oxirane **11** sequentially with NaN_3, LAH and methanolic KOH yields 4-allylic azoles **22**.

C-4 Pyridyl ATRA Analogs

Introduction of various pyridyl groups at C-4 yields potent inhibitors of ATRA 4-hydroxylase. These C-4 substituted pyridyl ATRA analogs interact with ATRA 4-hydroxylase's active site. The synthesis pathway of these C-4 substituted pyridyl ATRA analogs are shown in Figure 5. Thus 4-oxo methylretinoate, **2**, is transformed to the key intermediate vinyl iodide, **24**, via the hydrazone, **23**. Palladium catalyzed cross-coupling reaction of **24** with different diethyl(-pyridyl)borane reagents affords the desired C-4 substituted pyridyl ATRA analogs, **25**, **26** and **27**.

C-4 Alkylating Agent ATRA Analogs

C-4 alkylating agent ATRA analogs are mechanism based inhibitors which are substrate analogs of ATRA. These C-4 substituted ATRA analogs contain a latent electrophilic group which is activated by ATRA 4-hydroxylase resulting in irreversible enzyme inactivation because of covalent modification of the active site of ATRA 4-hydroxylase. Figure 6 shows the schematic pathway for synthesis of C-4 substituted ATRA analogs that are mechanism-based inhibitors. The alkylating agents are C-4 substituted ATRA analogs: acetylinic ATRA, **30**, cyclopropyl-amine ATRA, **32**, and cyclopropyl-ether-ATRA, **35**. Acetylinic ATRA, **30**,

undergoes oxygen insertion to yield the highly reactive oxirene species which covalently binds to the prosthetic heme via its α-ketocarbene tautomer. Similarly, cyclopropyl-amine ATRA, **32**, and cyclopropyl-ether-ATRA, **35**, each inhibit ATRA 4-hydroxylase following one-electron enzymatic oxidation.

Protection of the carboxylic acid moiety of 4-keto-ATRA as the 2-alkyl-1,3-oxazolidie, **28**, according to established procedure (Schow SR, Bloom JD, Thompson AS, Winzenberg KN and Smith III AB (1986) Milbemycin - Avemictic studies. 5. Total synthesis of milbemycin 3 and its C(12) epimers. *J Am Chem Soc* **108:** 2662-2674.) followed by treatment with lithium acetylide (Mauvais A, Burger A, Roussel PJ, Hetru C and Luu B (1994) Acetylenic inhibitors of C-22 hydroxylase of ecdysone biosynthesis. *Bioorg Chem* **22:** 36-50.) yields 4-hydroxy, 4-trimethylsilylacetylene **29**. Reduction of the latter with $SnCl_2$ in HCl yields acetylinic ATRA, **30** (Figure 6).

The cyclopropyl compounds may also be synthesized from 2-alkyl-1,3-oxazolidie, **28**, as shown in Figure 6. Condensation of 2-alkyl-1,3-oxazolidie, **28**, with cyclopropylamine, followed by reduction of the resulting imine with $NaBH_4$ gives cyclopropyl-amine ATRA, **32**. The cyclopropyl ether ATRA, **35**, is prepared by vinylation and subsequent cyclopropanation.

ATRA 4-hydroxylase Inhibition

C-4 substituted ATRA analogs inhibit ATRA 4-hydroxylase. Two types of assays demonstrate this inhibitory effect. One assay uses hamster liver microsomes. The other type of assay uses COS-1 cells transfected with hP450RAI, human ATRA 4-hydroxylase. The reason for the two types of assays is that hamster liver microsomes have several different cytochrome P450 enzymes, and one needs to see that the novel compounds specifically inhibit ATRA 4-hydroxylase.

Hamster Liver Microsome Assay Method

Washed hamster liver microsomes were prepared as follows: Livers are removed from sacrificed animals, rinsed in ice-cold 0.9 % NaCl solution and homogenized in a 3-fold volume of 0.25 M sucrose-0.05 M Tris-HCl (pH 7.4) using a blender. Microsomes are isolated by differential centrifugation (10,000 x g, 20 minutes; 100,000 x g, 60 minutes; 4°C) using a well-known technique (Van Wauwe, J.; Van Nyen, G.; Coene, M-C.; Stoppie, P.; Cols, W.; Goossens, J, Borghgraef, P.; Janssen, PAJ, Liarozole, an Inhibitor of Retinoic Acid Metabolism, Exerts Retinoid-Mimetic Effects, *In Vivo. J. Pharmacol. Expt. Ther. 261*, 773-779, 1992). The microsomes are suspended in PBS buffer (pH 7.4), in 1 ml aliquots and stored at -70°C until required.

The standard reaction mixture (total of 400 µl) is composed of assay buffer, 140 µl; microsomes, 100 µl (500 mg protein); NADPH, 100 µl (20 nM); and a C-4 substituted ATRA analog dissolved in DMSO, 40 µl. After a 3 minute preincubation at 37°C, the reaction is initiated by addition of 20 µl of [11,12-^{3}H]-ATRA (20 µCi/ml). The incubation is carried out for 30 minutes under oxygen with shaking in a water bath at 37°C. The reaction is stopped by acidification with 0.1 ml formic acid, and the samples are extracted (x 2) with EtOAc (2 ml) containing 0.05 % butylated hydroxyanisole. The combined organic extracts are evaporated in vacuo, dissolved in 200 µl of the mobile phase for HPLC. An aliquot is analyzed for tritium content by liquid scintillation spectrometry. Usually, > 95% of the added radioactivity is recovered. Most of the samples (150 µl) are analyzed on a 10 (m C_{18} Bondapak column (3.9 x 300 mm, Millipore), eluted with a multi-linear gradient solvent system: i, MeOH-H_2O-HCOOH (60:40:0.05) containing 10 mM ammonium acetate (100 (0%) and ii, MeOH (0 (100%) at 2 ml/min. The radioactivity is measured by an on-line radio-detector. The R_{ts} of ATRA, 4-

hydroxy-ATRA and 4-oxo-ATRA are determined by UV absorbance at 350 nm in separate cuvettes. Typically, 80 ± 5% of [11, 12-^{3}H]ATRA is converted into the metabolites.

The C-4 substituted ATRA analogs are tested at two concentrations, 500 and 1000 nM. IC_{50} and K_i values are determined.

hP450RAI-Transfected COS-1 Cells Method

An hP450RAI expression vector is transfected into COS-1 according to standard protocols, and the assay is conducted using standard protocols (White JA, Guo Y-D, Baetz K, Beckett-Jones B, Bonasoro J. Hsu KE, Dilworth FJ, Jones G and Petkovich M, Identification of the retinoic acid-inducible all-*trans*-retinoic acid 4-hydroxylase, *J Biol Chem* 271: 29922-29927 1996; White JA, Beckett-Jones B, Guo Y-D, Dilworth FJ, Bonasoro J. Jones G and Petkovich M, Cloning of the human retinoic acid-metabolizing enzymes (hP450RAI) identifies a novel family of cytochromes P450(CYP26), *J Biol Chem* 272: 18538-18541, 1997). Briefly, COS-1 cells are transfected with 3 g of hP450RAI in pTLI or the empty control pTLI together with 1 g of ferridoxin and ferridoxin reductase expression vectors. Media from transfected cells is incubated with 575 pM [11, 12-^{3}H]ATRA for 24 hours, and reactions are terminated by acidification with 0.1 % acetic acid. Metabolism of [11, 12-^{3}H]ATRA to polar metabolites is quantified as described above, and the novel compounds are assessed for their inhibitory potencies.

VN/14-1RA, VN/16-1RA, VN/16-1RA's Methyl Ester, VN/17-1RA

Incubation of [11,12-^{3}H]ATRA with hamster liver microsomes as described above results in the formation of polar metabolites, including 4-hydroxy-ATRA and 4-oxo-ATRA (see Figure 7A). However, **VN/14-1RA** (1 μM) significantly suppressed the microsomal conversion of

ATRA to polar metabolites (see Figure 7B). **VN/14-1RA** inhibits cytochrome P450-dependent metabolism of ATRA.

The IC_{50} values for **VN/14-1RA, VN/16-1RA**, its methyl ester, **VN/17-1RA**, liarozole, and ketoconazole (keto) are presented in Table 1 below and show that these four C-4 substituted azole ATRA analogs are excellent inhibitors of hamster liver microsomal ATRA 4-hydroxylase enzyme. These novel C-4 substituted azole ATRA analogs are more potent than either liarozole or ketoconazole. **VN/14-1RA** with an IC_{50} value of 100 nM is 60-fold more potent than liarozole (IC_{50} = 6000 nM).

Table 1

Compounds	IC_{50} Value (nM)
VN/14-1RA	100 ± 1.0
VN/16-1RA	880 ± 8.0
VN/16-1RA methyl ester	680 ± 3.0
VN/17-1RA	1,620 ± 8.0
Liarozole	6,000 ± 30.0
keto	34,000 ± 170.0

Enzyme Inactivation Activity Of Acetylinic ATRA,

Cyclopropyl-amine ATRA, and Cyclopropyl-ether-ATRA

Acetylinic ATRA, **30**, cyclopropyl-amine ATRA, **32**, and cyclopropyl-ether-ATRA, **35**, cause enzyme inactivation as a mechanism of their action. These three novel compounds are quite specific, because of the interaction with the enzyme's active site and their conversion to a form that binds to the enzyme either irreversibly or very tightly. These three novel compounds provide sustained enzymatic inhibition until new enzyme is synthesized. Thus, these three novel compounds have longer lasting effects and less side effects. To determine enzyme inactivation, hamster liver microsomes are preincubated with acetylinic ATRA, **30**, cyclopropyl-amine

ATRA, **32**, or cyclopropyl-ether-ATRA, **35**, as described above for 5, 10, 15, 20, or 60 minutes. The unbound acetylinic ATRA, **30**, cyclopropyl-amine ATRA, **32**, or cyclopropyl-ether-ATRA, **35**, is then removed by charcoal treatment. The enzyme activity is then measured as described above, and the K_i value of the inactivation reaction is calculated. The irreversible nature of the inhibition is demonstrated by incubating the pretreated enzyme preparation with a high concentration of substrate because if acetylinic ATRA, **30**, cyclopropyl-amine ATRA, **32**, or cyclopropyl-ether-ATRA, **35**, is not tightly bound to the enzyme and is reversible, it would possible to displace the inhibitor with excess amount of substrate. In addition, to demonstrate the irreversible nature of the inhibition, one dialyzes the pretreated enzyme preparation for various lengths of time to remove acetylinic ATRA, **30**, cyclopropyl-amine ATRA, **32**, or cyclopropyl-ether-ATRA, **35**, which may dissociate slowly from the enzyme and then performs standard enzyme activity evaluation.

High Specificity for ATRA 4-hydroxylase (CYP26A1)

The high specificity of the novel C-4 substituted ATRA analogs for ATRA 4-hydroxylase is demonstrated by testing for inhibition of aromatase, CYP17, and other cytochrome P450s found in hamster liver microsome preparations. To demonstrate the lack of inhibition of cytochrome P450s enzymes (other than ATRA 4-hydroxylase), one measures the conversion of antipyrine (a well-known probe substrate for cytochrome P450s) to norantipyrine, and 4-hydroxy- and 3-hydroxymethyl-antipyrine using well-known techniques (Engle G, Hofmann U, Heidemann H, Cosme J, Eichelbaum M, Antipyrine as a probe for human oxidative drug metabolism: identification of the cytochrome P450 enzymes catalyzing 4-hydroxyantipyrine, 3-hydroxymethylantipyrine, and norantipyrine formation, *Clin Pharm Thera* **59**: 613-623, 1996). To demonstrate the lack of inhibition of aromatase, one measures the conversion of [1β-

^{3}H]androstenedione to estrone and estradiol using well-known techniques (Brodie et al, The effect of an aromatase inhibitor, 4-hydroxy-4-androstene-3,17-dione, onestrogen-dependent processes in reproduction and breast cancer, *Endocrinology*, Jun;100(6):1684-95, 1977). To demonstrate the lack of inhibition of CYP17, one measures the conversion of [21-^{3}H]17α-hydroxypregnenolone to dehydroepiandrosterone and androst-5-ene-3β, 17β-diol using well-known techniques (Njar VCO, Kato K, Nnane IP, Grigoryev DN, Long BJ and Brodie AMH, Novel 17-Azolyl Steroids; Potent Inhibitors of Human Cytochrome 17-Hydroxylase-C-17,20-Lyase (P45017): Potential Agents for the Treatment of Prostate Cancer, *J Med Chem* **41:** 902-912, 1998).

Inhibition of *in vivo* Catabolism of ATRA

In order to demonstrate that the novel compounds increase the biological half-life of exogenously administered ATRA and enhance ATRA's endogenous plasma levels, one determines the effects of the novel compounds on the plasma elimination of exogenously administered ATRA and also on the endogenous levels of the retinoid, using a well-known procedure (Van Wauwe et al., 1990) . Rats weighing 200-220 g are treated p.o. with the novel compounds [e.g., 5, 10, 20, or 40 mg/kg prepared in polyethylene glycol 200 (PEG 200)] or vehicle (PEG 200) in a volume of 0.5 ml per 100 g body weight. One hour later, the animals are anesthetized with diethyl ether and injected i.v. with ATRA (0.1 mg/kg). At designated times (e.g., 10, 20, 30, 60, 90, 180, or 210 minutes) after injection, rats are sacrificed by decapitation and trunk blood collected on heparin (500 U/ml). After centrifugation (1000 x g, 15 min), plasma fraction is recovered and processed immediately. The extracts are subjected to HPLC using conditions described above, eluent monitored by UV absorbance detection at 350 nm and ATRA is quantified by peak-area integration.

For experiments designed to assess the effects on endogenous plasma levels of ATRA, rats are treated p.o. with the novel compounds [e.g., 5, 10, 20, or 40 mg/kg prepared in polyethylene glycol 200 (PEG 200)] or vehicle (PEG 200) in a volume of 0.5 ml per 100 g body weight. The animals are sacrificed at various times (e.g., 1 hour intervals, up to 6 hours). Plasma is collected, processed and the levels of ATRA is determined by HPLC.

Retinoid Receptor Binding

Because the novel compounds are retinoid-related analogs, they bind to the retinoid receptors *in vivo* and thus have increased therapeutic potentials. Similar to ATRA, these novel compounds act as a ligand for the retinoic acid receptors (RARs, α, β, and γ) but not the retinoid X receptors (RXRs, α, β, and γ). To demonstrate that these novel C-4 substituted ATRA analogs are ligands for RAR but not RXRs, one uses a well-known assay system involving recombinant RAR and RXR protein expressed in *E. coli.* (Nervi C, Grippo JF, Sherman MI, George MD, Jetten AM, Identification and characterization of nuclear retinoic acid-binding activity inhuman myeloblastic leukemia HL-60 cells, *Proc Natl Acad Sci USA* **86**: 5854- 5858, 1989) The dissociation constants for the inhibitors is determined by the well described charcoal absorption method (Yang N, Schule R, Mangelsdorf DJ, Evans RM, Characterization of DNA binding and retinoic acid binding properties of retinoic acid receptor, *Proc Natl Acad Sci USA* **88**: 3559-3663, 1991). Briefly, serial dilutions of the novel compounds (10^{-11} to 10^{-5} M) in dimethyl sulfoxide, 100 μL each is used. 12 mg of crude cytosolic extracts is prepared from pET15b (Novagen, Madison, WI)/hRAR-α, -β, and -γ prepared protein is used for each data point. All reactions are conducted in binding buffer (60 mM Na imidazole, 500 mM NaCl, 20 mM Tris, pH 7.9) for 14-16 hours at 4 °C in a final volume of 1 mL. Unbound [^{3}H]ATRA is removed by addition of 0.5 mL of equivalent-sized dextran-treated charcoal (final concentration 3% [wt/vol]) for 15 minutes

at 4 °C. Following centrifugation, 0.5 mL of supernatant is subjected to liquid scintillation counting. Binding in the presence of 100-fold excess of unlabeled ligand is defined as unspecific binding, while the total binding minus the nonspecific binding defines specific binding. The IC_{50} values are obtained from logarithmic plots.

Inhibition of Prostate Cancer Proliferation *In-vitro*

The novel C-4 substituted ATRA analogs inhibit proliferation of prostate cancer *in-vitro*. Experiments are conducted on two prostate cancer cell lines, LNCaP cells and PC-3 cells. LNCaP cells are androgen-dependent cell cultures. PC-3 cells are androgen independent cell culture.

Methods

LNCaP cells are transferred into ATRA-free medium 3 days prior to start of experiments. Medium consist of phenol red-free IMEM supplemented with 5% FBS and 1% P/S. Cell are then plated into 24-well culture plates (15000 cells per well) in 1 mL of same medium. After a 24-hour attachment period, the vehicle (ethanol) or ATRA (10^{-5} M) alone or ATRA in combination with a novel compound at a range of concentrations are added to triplicate wells. Medium/treatments are changed every 3 days. After 9 days of treatment, cells are removed from the wells with typsin/EDTA and counted in a Coulter counter. Using well-known methods (Wouters W, Van Dun J, Dillen A, Coene M.-C, Cools W and De Coster R, Effects of liarozole, a new antitumoral compound an retinoic acid-induced inhibition of cell growth and on retinoic acid metabolism in MCF-7 breast cancer cells, *Cancer Res* **52:** 2841-2846, 1992), one determines the inhibitory effect of the novel compounds on LNCaP cells grown with ATRA.

This method also is repeated using PC-3 cells.

VN/14-1RA, VN/16-1RA, VN/17-1RA

Figure 8 shows the inhibitory effects of **VN/14-1RA** in combination with ATRA on LNCaP cells. LNCaP cells growth curves in the presence of vehicle (no drug, ●), 10^{-5} M ATRA (■), 10^{-5} M ATRA combined with 10^{-8} M **VN/14-1RA** (✳), 10^{-5} M ATRA combined with 10^{-7} M **VN/14-1RA** (◆), 10^{-5} M ATRA combined with 10^{-6} M **VN/14-1RA** (▼), or 10^{-5} M ATRA combined with 10^{-5} M **VN/14-1RA** (▲). All dosages of **VN/14-1RA** in combination with ATRA inhibit the proliferation of LNCaP cells better than no drug or ATRA only. The two higher concentrations of **VN/14-1RA** (10^{-5} M and 10^{-6} M) prevent the cells from increasing in number.

Figure 9 shows the inhibitory effects of **VN/16-1RA** in combination with ATRA on LNCaP cells. LNCaP cells growth curves in the presence of vehicle (no drug, ●), 10^{-5} M ATRA (■), 10^{-5} M ATRA combined with 10^{-8} M **VN/16-1RA** (✳), 10^{-5} M ATRA combined with 10^{-7} M **VN/16-1RA** (◆),10^{-5} M ATRA combined with 10^{-6} M **VN/16-1RA** (▼), or 10^{-5} M ATRA combined with 10^{-5} M **VN/16-1RA** (▲). All dosages of **VN/16-1RA** in combination with ATRA inhibit the proliferation of LNCaP cells better than no drug or ATRA only. The highest concentration of **VN/16-1RA** (10^{-5} M) stop the cells from increasing in number.

Figure 10 shows the inhibitory effects of **VN/17-1RA** in combination with ATRA on LNCaP cells. LNCaP cells growth curves in the presence of vehicle (no drug, ●), 10^{-5} M ATRA (■), 10^{-5} M ATRA combined with 10^{-8} M **VN/17-1RA** (✳), 10^{-5} M ATRA combined with 10^{-7} M **VN/17-1RA** (◆),10^{-5} M ATRA combined with 10^{-6} M **VN/17-1RA** (▼), or 10^{-5} M ATRA combined with 10^{-5} M **VN/17-1RA** (▲). All dosages of **VN/17-1RA** in combination with ATRA inhibit the proliferation of LNCaP cells better than no drug or ATRA only. All concentrations of **VN/17-1RA** are effective in preventing the cells from increasing in number.

As a comparison, Figure 11 shows the inhibitory effects of liarozole in combination with ATRA on LNCaP cells. LNCaP cells growth curves in the presence of vehicle (no drug, ●), 10^{-5} M ATRA (■), 10^{-5} M ATRA combined with 10^{-8} M liarozole (✳), 10^{-5} M ATRA combined with 10^{-7} M liarozole (◆),10^{-5} M ATRA combined with 10^{-6} M liarozole (▼), or 10^{-5} M ATRA combined with 10^{-5} M liarozole (▲). Liarozole when combined with ATRA also inhibit the proliferation of LNCaP cells better than no drug or ATRA only. Only the highest concentration of liarozole (10^{-5} M) prevents the cells from increasing in number.

Inhibition of Prostate Cancer and Breast Cancer *in-vivo*

The anti-proliferative effects of the novel compounds on prostate cancer is demonstrated by administering the novel compounds to SCID mice which have been implanted with LNCaP cells or PC-3 cells. These LNCaP cells and PC-3 cells develop into tumors in SCID mice. Because the mechanism of growth simulations are different, but together display many of the properties of clinical prostate cancer, the models provide an indication of efficacy in humans. In addition, one can demonstrate the anti-proliferative effects of the novel compounds on breast cancer by administering the novel compounds to SCID mice which have been implanted with MCF-7Ca cells which develop into tumors in SCID mice.

Methods

LNCaP cells are cultured as described above. Subconfluent cell are scraped into DPBS, counted and suspended in Matrigel (3 x 10^7 cells/mL). Male SCID mice 4-6 weeks old are obtained from NCI, Frederick, MD. Each mouse is inoculated s.c. with 0.1 mL of the cell suspension at two sites. Growth rate are determined from tumor volumes using calipers using well-known techniques (Yue W, Wang J, Savinov A, Brodie A, Effect of aromatase inhibitors on growth of mammary tumors in a nude mouse model, *Cancer Res,* Jul 15;55(14):3073-7 (1995).

Tumor volumes are calculated according to the equation: $V = 4/3 \times \pi \times r_1^2 \times r_2$ ($r_1 < r_2$). Tumors are allowed to grow for 4-5 weeks following cell inoculation. Mice are then grouped (6 mice per group) for castration or treatment with vehicle, liarozole (as a comparison), or one of the C-4 substituted ATRA analogs (at various concentration based on IC_{50} and/or K_i values for each novel compound, mg/kg/day). The route of administration of C-4 substituted ATRA analogs include s.c., i.m., i.p., and oral. Tumors are measured weekly for 4-5 weeks of treatment and tumor volumes calculated. Blood is collected from the euthanized animals that are autopsied 1 hour after the last injection. Tumors are excised, weighed and stored at -80 °C until required. This standard well-known test for evaluating compounds for antitumor efficacy in LNCaP tumors in SCID mice is described in Grigoryev D N, Kato K, Njar VCO, Long BJ, Ling Y, Wang X, Mohler J and Brodie AMH, Cytochrome P450c17 expressing E. Coli as a first-step Screening System for 17-hydroxylase-C17,20-Lyase Inhibitors, *Anal Biochem* 267: 319-330, 1999a, and in Grigoryev DN, Long BJ, Njar VCO, Liu Y, Nnane IP and Brodie AMH, Effects of New 17-Hydroxylase/C17,20-Lyase Inhibitors on LNCaP Prostate Cancer Cell Growth, *In Vitro* and *In Vivo. Br j Cancer Cancer* 81: 622-630, 1999b.

For PC-3 tumors, one utilizes the same methods.

For MCF-7Ca tumors, one utilizes the same methods, except the SCID mice are female, receive ovariectomies, and receive androstenedione (0.1 mg/day).

VN/14-1RA

As shown in Figure 12, when 10 mg/kg of **VN/14-1RA** is administered to mice with MCF-7Ca tumors, the tumors do not increase in size as fast as tumors in the control group. After twenty-eight days, **VN/14-1RA** given at 10 mg/kg slows the proliferation of the tumors to one-

half the size of the tumors in the control group. VN/14-1RA is shown as ■; control is shown as ●.

U.S. Provisional Patent Application 2003/0162823 is herein incorporated by reference.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one of ordinary skill in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof. The artisan will further acknowledge that the Examples recited herein are demonstrative only and are not meant to be limiting.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers or steps but not the exclusion of any other integer or group of integers or steps.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that the prior art forms part of the common general knowledge in Australia.

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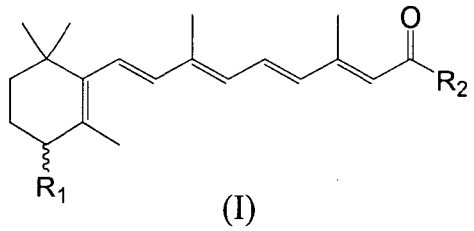
The claims defining the invention are as follows:

1. A chemical compound having the formula (I)



(I)

wherein,

R_1 is selected from the group consisting of a sulfur containing group, an oxygen containing group that forms, together with the 4-position carbon, an oxirane group, $-OR_4$, where R_4 is hydrogen or an alkyl group, a nitrogen containing group, acetylinic, an ester group, oxime and aziridine; and

R_2 is selected from the group consisting of a hydroxyl, aminophenol, $-OR_3$ and azole group, wherein R_3 is selected from the group consisting of an alkyl, aryl and heterocyclic group, or a pharmaceutically acceptable salt thereof.

2. The chemical compound as in Claim 1, wherein R_2 is hydroxyl.

3. The chemical compound as in Claim 1, wherein R_2 is $-OCH_3$.

4. The chemical compound as in Claim 1, wherein R_1 is a sulfur containing group selected from the group consisting of thiol and alkylthiols, or R_1 is a sulfur containing group that forms, together with the 4-position carbon, a thiirane.

5. The chemical compound as in Claim 1, wherein R_1 is an $-OR_4$ group, where R_4 is hydrogen or an alkyl group.

6. The chemical compound as in Claim 1, wherein R_1 is cyclopropylether or an oxygen containing group that forms, together with the 4-position carbon, an oxirane group.

7. The chemical compound as in Claim 1, wherein R_1 is a nitrogen containing group and said nitrogen containing group has the formula $-NR_5R_6$ group, where R_5 and R_6 are independently

selected from the group consisting of hydrogen and alkyl groups, or R_5 and R_6 may together form a ring.

8. The chemical compound as in Claim 7, wherein R_5 and R_6 form a imidazolyl ring or a triazole ring.

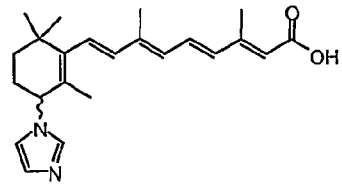
9. The chemical compound as in Claim 1, wherein R_1 is a nitrogen containing group and said nitrogen containing group is selected from the group consisting of cyano, amino, azido, cyclopropylamino, or R_1 is a nitrogen containing group that forms, together with the 4-position carbon, an aziridine group or an oxime group.

10. The chemical compound as in Claim 1, wherein R_1 is a nitrogen containing group and said nitrogen containing group is selected from the group consisting of pyridyl groups.

11. The chemical compound as in Claim 1, wherein R_1 is a nitrogen containing group and said nitrogen containing group is selected from the group consisting of allylic azoles.

12. The chemical compound as in Claim 11, wherein R_1 is a methyleneazolyl.

13. The chemical compound as in Claim 1, wherein the compound is formula (II)



(II),

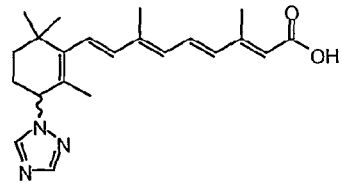
or a pharmaceutically acceptable salt thereof.

14. The method of synthesizing the chemical compound as in Claim 13, comprising the steps of:

contacting (±)-4-hydroxymethyl retinoate with carbonyldiimidazole in CH_3CN at room temperature to obtain (±)-4-(1*H*-imidazol-1-yl)methyl retinoate; and

hydrolysizing (±)-4-(1*H*-imidazol-1-yl)methyl retinoate in refluxing methanolic KOH to obtain (±)-4-(1*H*-imidazol-1-yl)retinoic acid.

15. The chemical compound as in Claim 1, wherein the compound is formula (III)



(III),

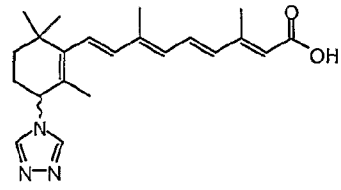
or a pharmaceutically acceptable salt thereof.

16. The method of synthesizing the chemical compound as in Claim 15, comprising the steps of:

contacting (±)-4-hydroxymethyl retinoate with carbonylditriazole in CH_3CN at room temperature to obtain (±)-4-(1*H*-1,2,4-triazol-1-yl)methyl retinoate; and

hydrolysizing of (±)-4-(1*H*-1,2,4-triazol-1-yl)methyl retinoate in refluxing methanolic KOH to obtain (±)-4-(1H-1,2,4-triazol-1-yl)retinoic acid.

17. The chemical compound as in Claim 1, wherein the compound is formula (IV)



(IV),

or a pharmaceutically acceptable salt thereof.

18. The method of synthesizing the chemical compound as in Claim 17, comprising the steps of:

contacting (±)-4-hydroxymethyl retinoate with carbonylditriazole in CH_3CN at room temperature to obtain (±)-4-(1*H*-1,2,4-triazol-1-yl)methyl retinoate; and

hydrolysizing (±)-4-(1*H*-1,2,4-triazol-1-yl)methyl retinoate in refluxing methanolic KOH to obtain (±)-4-(1H-1,2,4-triazol-1-yl)retinoic acid.

19. A method of treating an animal having cancer comprising administering a therapeutically effective amount of at least one compound according to claim 1.

20. The method of Claim 19, wherein said animal has a cancer selected from the group consisting of prostate, breast, ovarian, lung, melanoma, leukemia and lymphoma.

21. A method of treating an animal having a dermatological condition comprising administering a therapeutically effective amount of at least one compound according to claim 1.

22. The method of Claim 21, wherein said animal has a dermatological condition selected from the group consisting of old age, wrinkling, and skin photodamage.

23. A method of treating an animal having a dermatological disease comprising administering a therapeutically effective amount of at least one compound according to claim 1.

24. The method of Claim 23, wherein said animal has a dermatological disease selected from the group consisting of psoriasis and acne.

25. A method for inhibiting all-*trans* retinoic acid 4-hydroxylase in an animal, comprising administering an effective amount of at least one compound according to claim 1.

26. The method of Claims 19, 20, 21, 22, 23, 24 or 25, wherein said animal is a mammal.

27. The method of Claims 19, 20, 21, 22, 23, 24 or 25, wherein said animal is a human.

28. A pharmaceutical composition comprising the compound (±)-4-(1*H*-imidazole-1-yl)retinoic acid or a pharmaceutically acceptable salt thereof.

29. The pharmaceutical composition of Claim 28, further comprising pharmaceutically acceptable inactive ingredients selected from the group consisting of diluents, carriers, solvents, disintregrants, lubricants, stabilizers, and coatings.

30. The pharmaceutical composition of Claims 28 or 29, wherein the compositions is formulated for oral administration.

31. The pharmaceutical composition of Claims 28 or 29, wherein the compositions is formulated for parentral administration.

32. The pharmaceutical composition of Claims 28 or 29, wherein the compositions is formulated for injectable administration.

33. A pharmaceutical composition comprising the compound (±)-4-(1*H*-1,2,4-triazol-1-yl)retinoic acid or a pharmaceutically acceptable salt thereof.

34. The pharmaceutical composition of Claim 33, further comprising pharmaceutically acceptable inactive ingredients selected from the group consisting of diluents, carriers, solvents, disintregrants, lubricants, stabilizers, and coatings.

35. The pharmaceutical composition of Claims 33 or 34, wherein the compositions is formulated for oral administration.

36. The pharmaceutical composition of Claims 33 or 34, wherein the compositions is formulated for parentral administration.

37. The pharmaceutical composition of Claims 33 or 34, wherein the compositions is formulated for injectable administration.

38. A pharmaceutical composition comprising the compound (±)-4-(4*H*-1,2,4-triazol-1-yl)retinoic acid or a pharmaceutically acceptable salt thereof.

39. The pharmaceutical composition of Claim 38, further comprising pharmaceutically acceptable inactive ingredients selected from the group consisting of diluents, carriers, solvents, disintregrants, lubricants, stabilizers, and coatings.

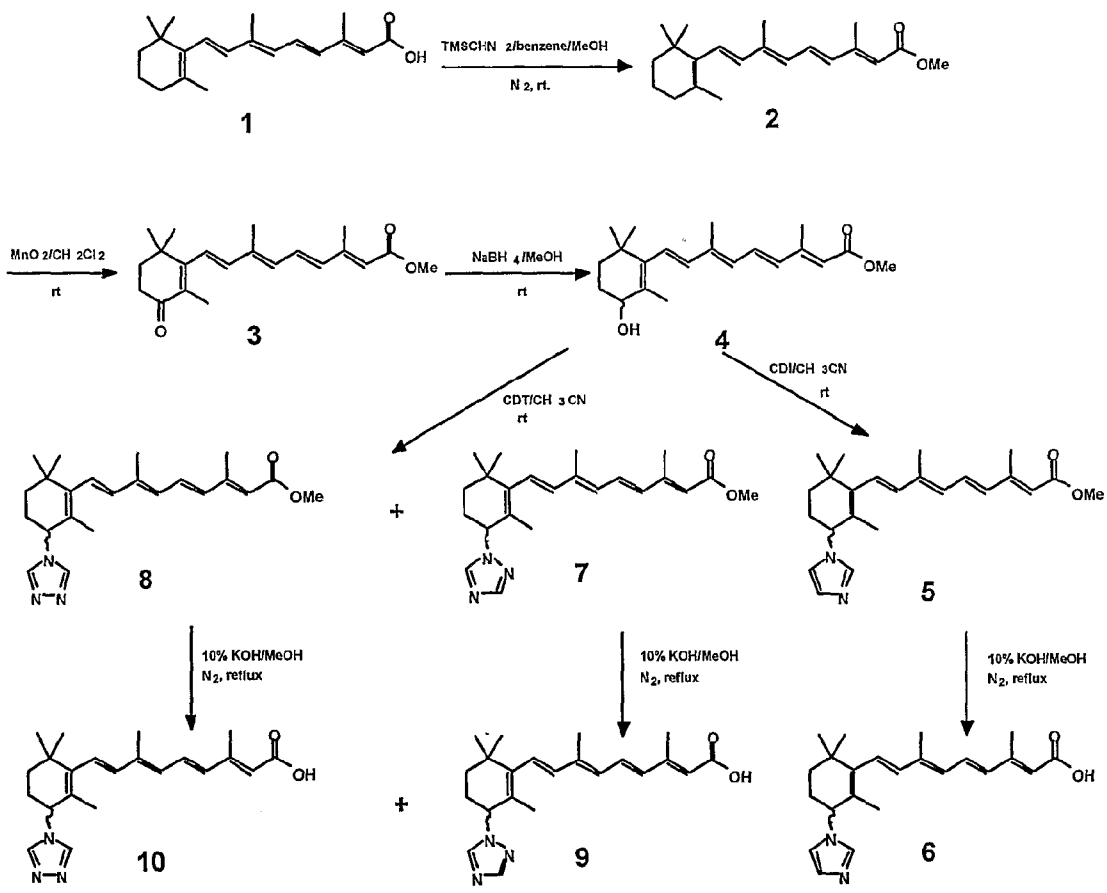
40. The pharmaceutical composition of Claims 38 or 39, wherein the compositions is formulated for oral administration.

41. The pharmaceutical composition of Claims 38 or 39, wherein the compositions is formulated for parentral administration.

42. The pharmaceutical composition of Claims 38 or 39, wherein the compositions is formulated for injectable administration.

43. A pharmaceutical composition comprising the compound of Claim 1.

44. The pharmaceutical composition as claimed in claim 28, 33, 38 or 43, further comprising all-*trans* retinoic acid (ATRA).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OMe
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2
MnO 2/CH 2Cl 2
rt
3
NaBH 4/MeOH
rt
4
CDI/CH 3CN
rt
CDT/CH 3CN
rt
8
7
5
10% KOH/MeOH
N2, reflux
10
9
6

Figure 1

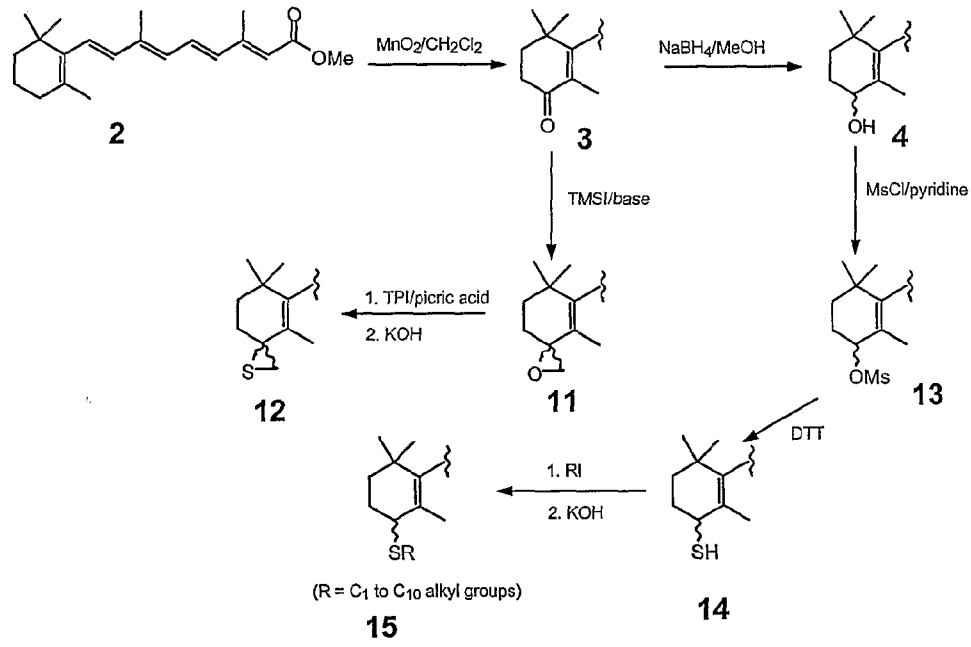


Figure 2

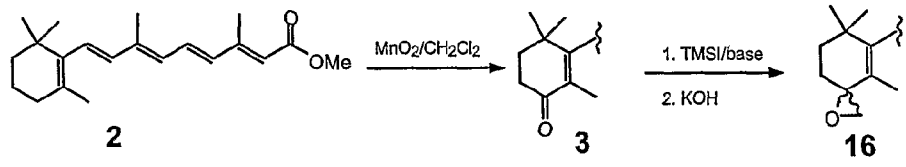


Figure 3

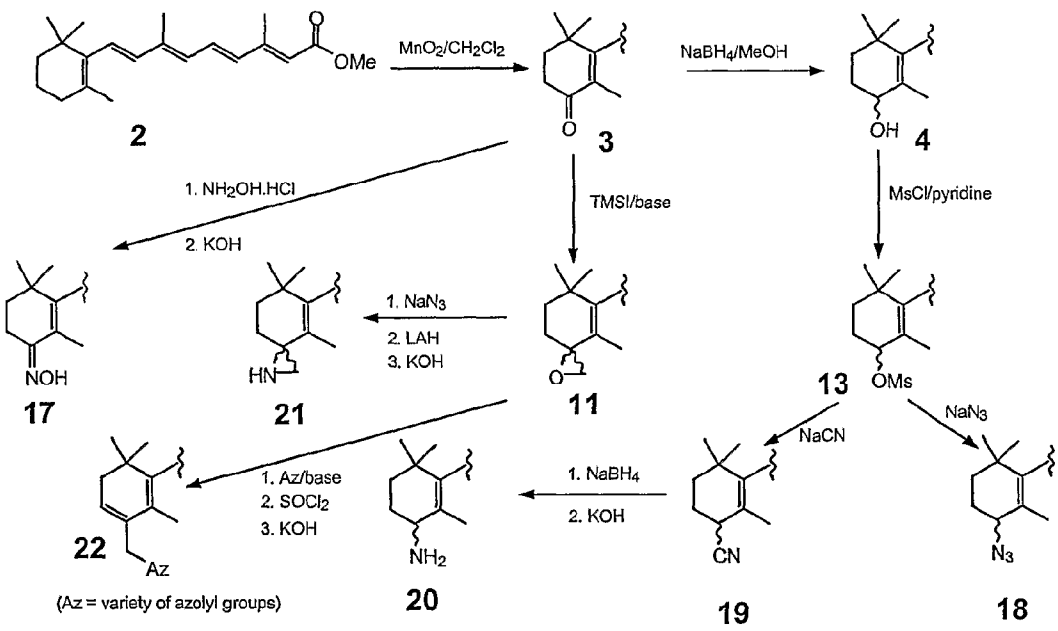


Figure 4

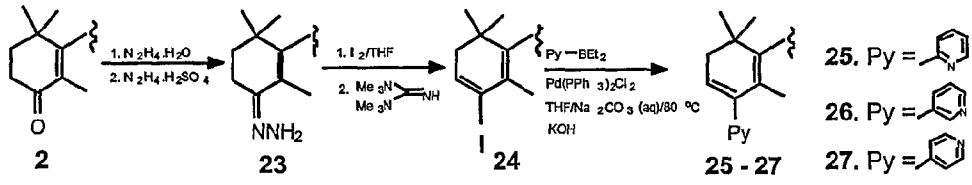
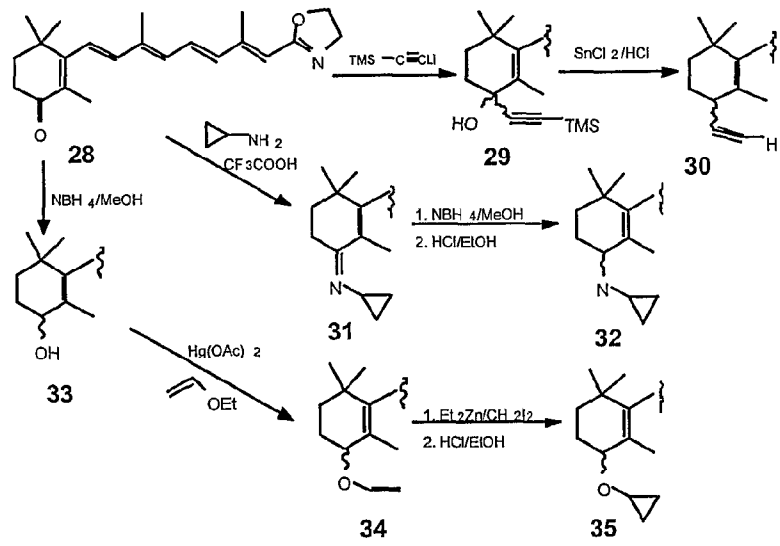


Figure 5


TMS —C≡CLi
SnCl 2/HCl
28
29
30
NH 2
CF3COOH
NBH 4/MeOH
1. NBH 4/MeOH
2. HCl/EtOH
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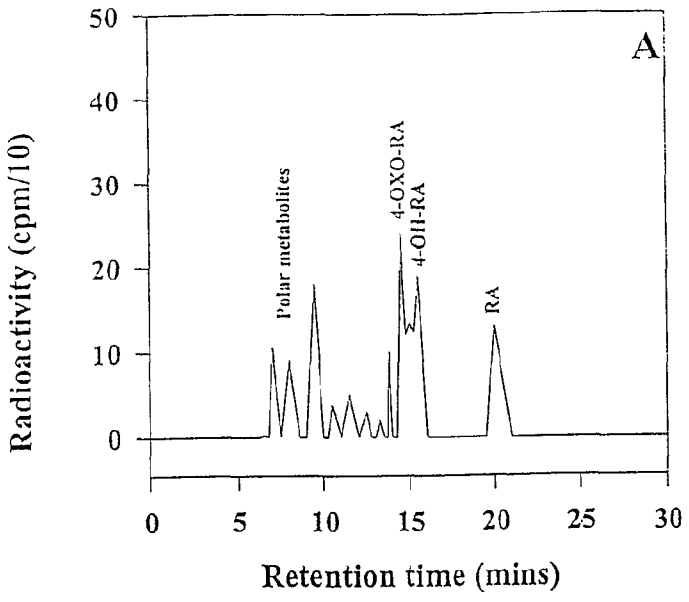
32
33
Hg(OAc) 2
OEt
1. Et2Zn/CH 2I2
2. HCl/EtOH
34
35

Figure 6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Radioactivity (cpm/10)
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40
30
20
10
0
Polar metabolites
4-OXO-RA
4-OH-RA
RA
0
5
10
15
20
25
30
Retention time (mins)

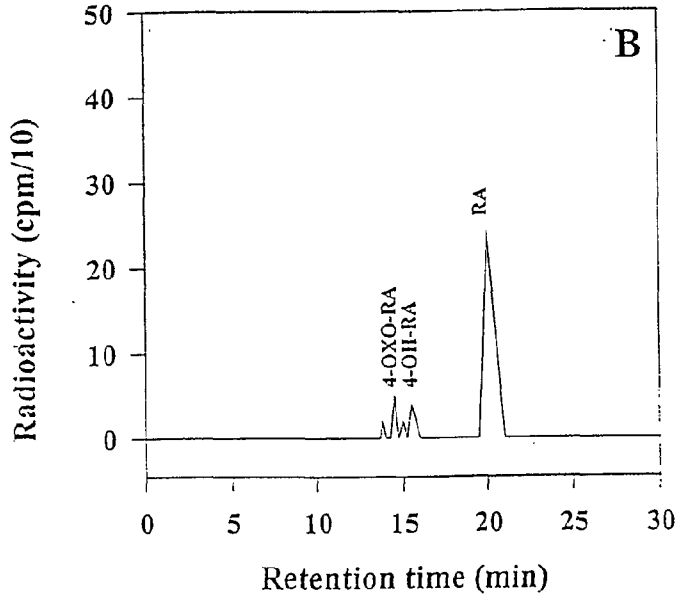
B
Radioactivity (cpm/10)
50
40
30
20
10
0
4-OXO-RA
4-OH-RA
RA
0
5
10
15
20
25
30
Retention time (min)

Figure 7

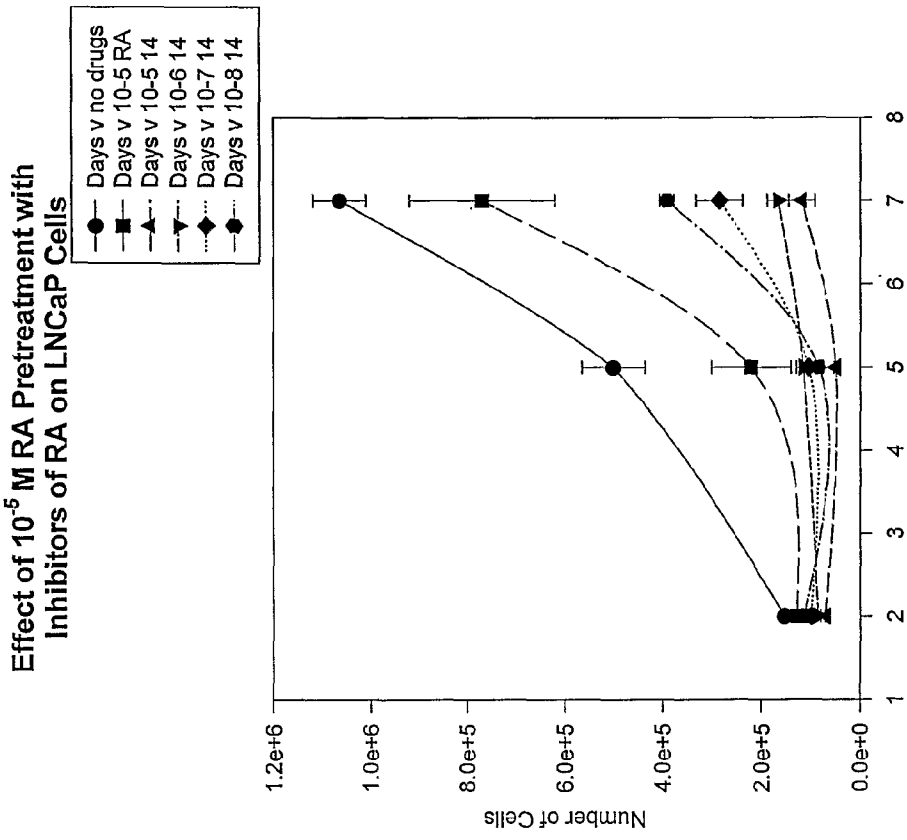
Effect of 10-5 M RA Pretreatment with Inhibitors of RA on LNCaP Cells
Days v no drugs
Days v 10-5 RA
Days v 10-5 14
Days v 10-6 14
Days v 10-7 14
Days v 10-8 14
Number of Cells
1.2e+6
1.0e+6
8.0e+5
6.0e+5
4.0e+5
2.0e+5
0.0e+0
1
2
3
4
5
6
7
8
Days

Figure 8

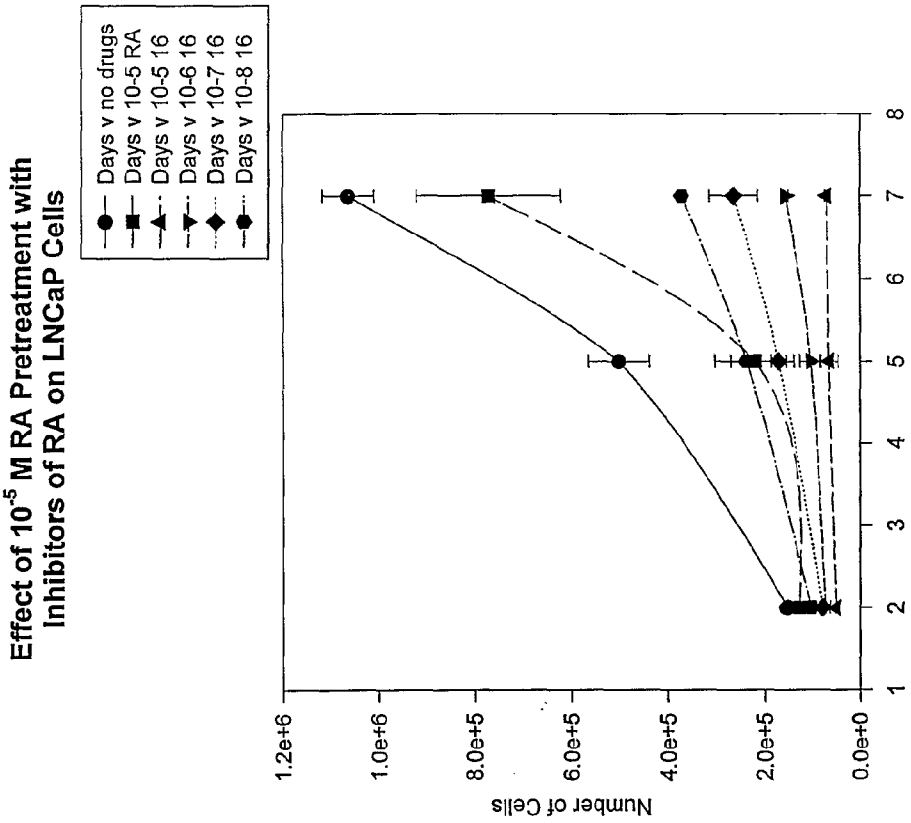
Effect of 10-5 M RA Pretreatment with Inhibitors of RA on LNCaP Cells
Days v no drugs
Days v 10-5 RA
Days v 10-5 16
Days v 10-6 16
Days v 10-7 16
Days v 10-8 16
Number of Cells
1.2e+6
1.0e+6
8.0e+5
6.0e+5
4.0e+5
2.0e+5
0.0e+0
1
2
3
4
5
6
7
8
Days

Figure 9

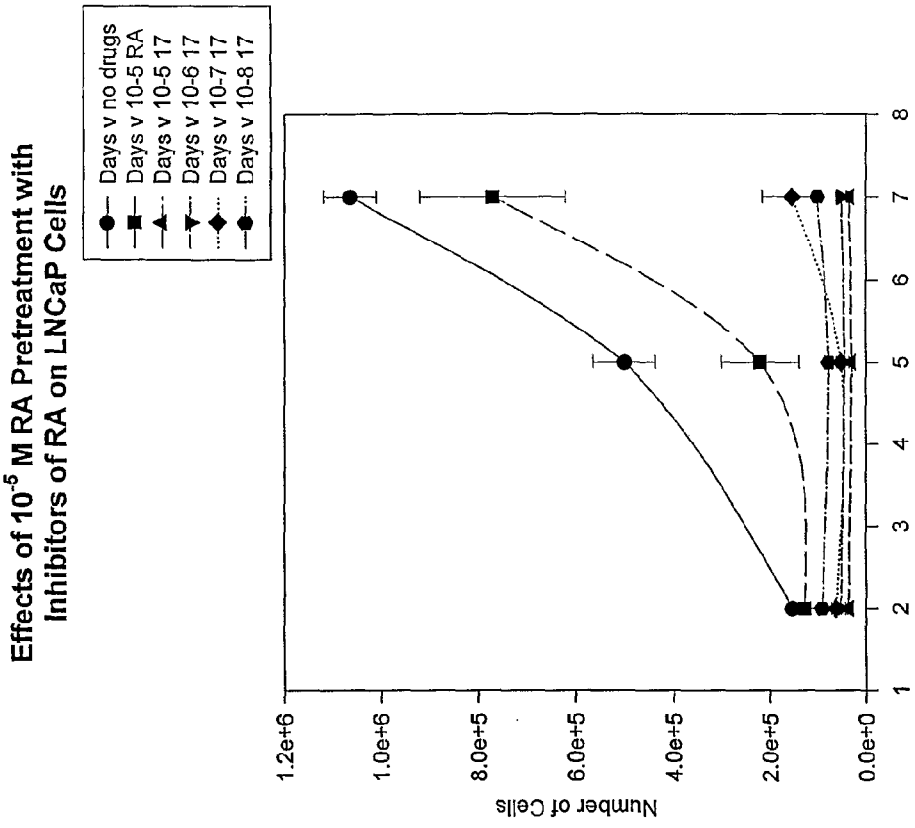
Effects of 10^{-5} M RA Pretreatment with Inhibitors of RA on LNCaP Cells
Days v no drugs
Days v 10-5 RA
Days v 10-5 17
Days v 10-6 17
Days v 10-7 17
Days v 10-8 17
Number of Cells
1.2e+6
1.0e+6
8.0e+5
6.0e+5
4.0e+5
2.0e+5
0.0e+0
1
2
3
4
5
6
7
8
Days

Figure 10

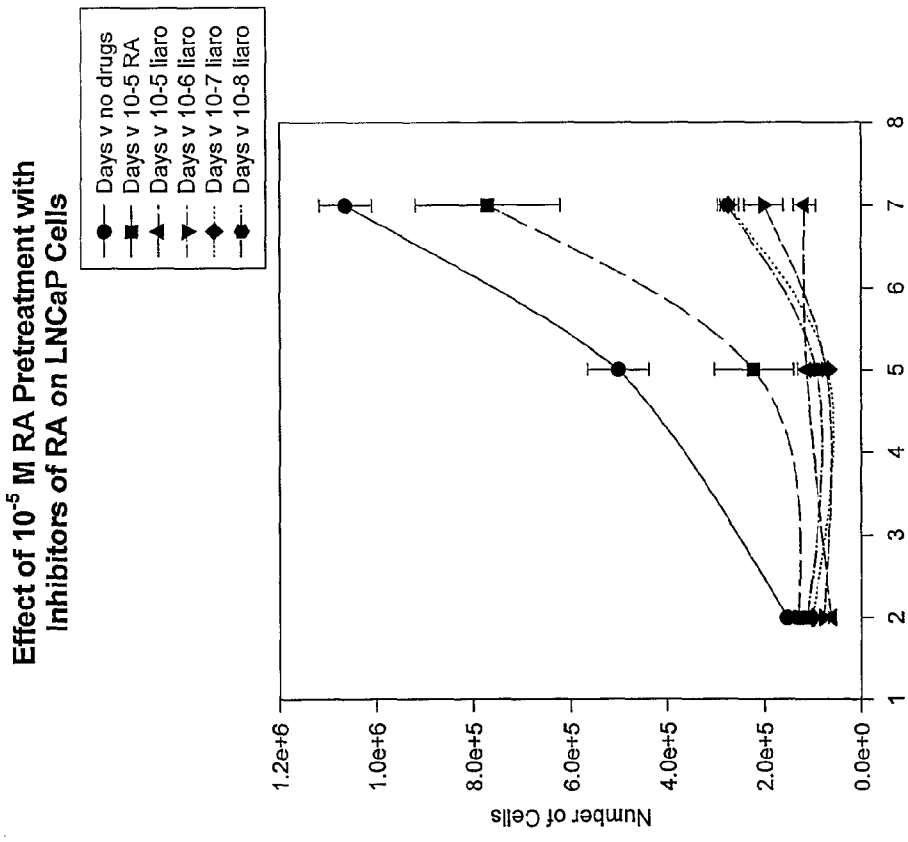
Effect of 10-5 M RA Pretreatment with Inhibitors of RA on LNCaP Cells
Days v no drugs
Days v 10-5 RA
Days v 10-5 liaro
Days v 10-6 liaro
Days v 10-7 liaro
Days v 10-8 liaro
Number of Cells
1.2e+6
1.0e+6
8.0e+5
6.0e+5
4.0e+5
2.0e+5
0.0e+0
1
2
3
4
5
6
7
8
Days

Figure 11

THE EFFECT OF VN/14-1 ON THE GROWTH OF MCF-7Ca HUMAN BREAST TUMORS IN OVARIECTOMIZED FEMALE ATHYMIC NUDE MICE SUPPLEMENTED WITH ANDROSTENEDIONE (0.1 mg/day).

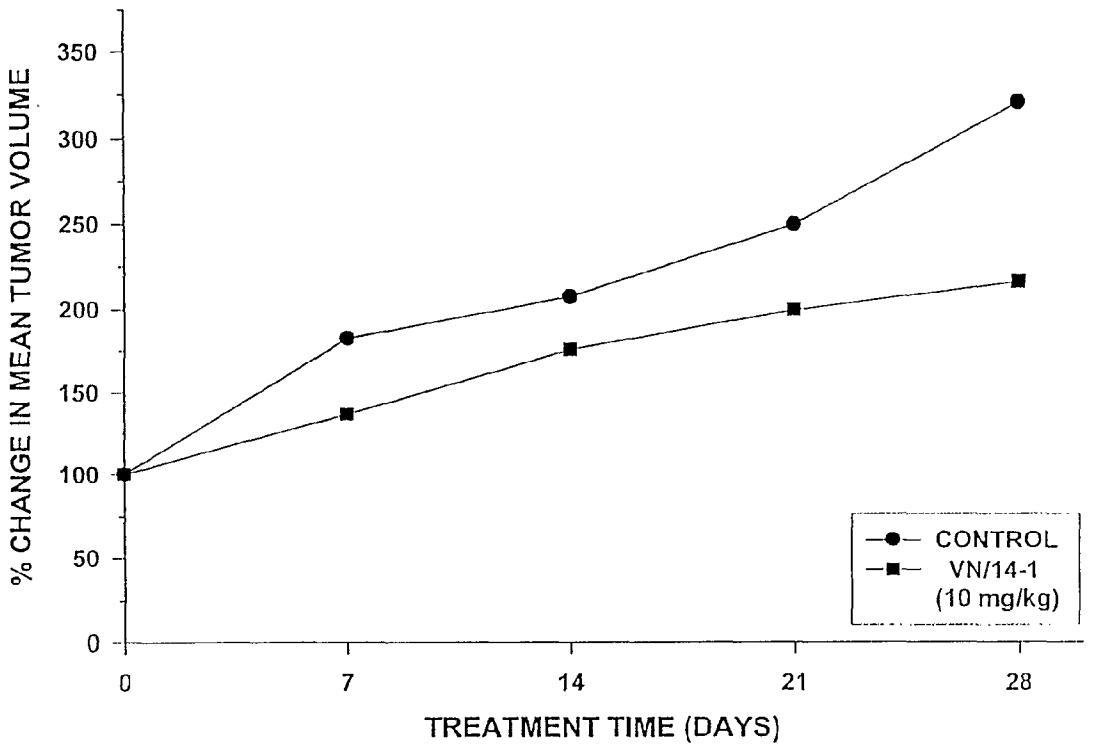

Figure 12