(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 January 2014 (30.01.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/018671 A1

(51) International Patent Classification:
C07D 493/08 (2006.01) *A61P 35/00* (2006.01)
A61K 31/7048 (2006.01)

(21) International Application Number:
PCT/US2013/051885

(22) International Filing Date:
24 July 2013 (24.07.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/675,232 24 July 2012 (24.07.2012) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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(54) Title: THERAPEUTIC COMPOUNDS FOR THE TREATMENT OF CANCER

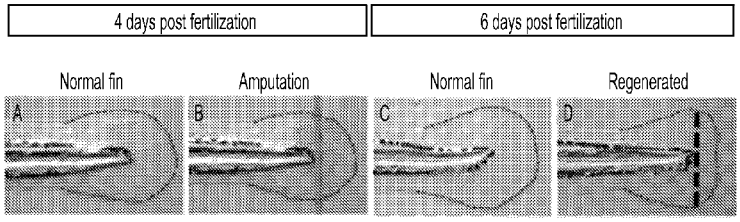
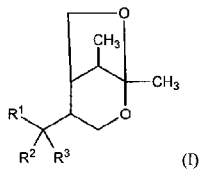


FIG. 1



(I)

(57) Abstract: The invention provides a compound of formula (I) wherein R^1-R^3 have any of the values defined in the specification, or a salt thereof. The compounds modulate the activity of the FGF1R pathway and are useful for treating cancer.

WO 2014/018671 A1

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

THERAPEUTIC COMPOUNDS FOR THE TREATMENT OF CANCER

Priority of Invention

This application claims priority to United States Provisional Application Number 61/675,232 that was filed on 24 July 2012. The entire content of this provisional application is hereby incorporated herein by reference.

Background of the Invention

One of the hallmarks of cancer is enhanced growth capacity. A major mechanism contributing to rapid cellular proliferation is hyperactivation of receptor tyrosine kinase (RTK) signaling pathways. For instance, aberrant *F*ibroblast *G*rowth *F*actor *R*eceptor (FGFR) signaling, a receptor tyrosine kinase pathway is observed in multiple cancers including breast, prostate, ovarian and pancreatic carcinomas. Among breast cancers, up to 10% exhibit increased FGFR signaling and are strongly associated with resistance to standard therapies and poor prognosis (see Turner, N., et al., *Cancer Res.* **2010**, *70*, 2085-2094). Multiple studies have demonstrated dependencies of tumor cells on FGFR for survival, suggesting that proteins within this signaling pathway are validated therapeutic targets in many cancers.

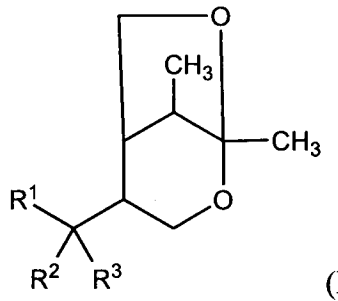
However, known small molecule receptor tyrosine kinase (RTK) inhibitors targeting FGFR activity are inadequate due to broad specificity and inhibition of multiple RTKs which may be associated with cytotoxicity and increased side effects in patients. Therefore, there is a critical need to identify new compounds with high specificity for the FGFR signaling pathway that are tolerated by normal cells without side effects. Natural products represent a validated source of structurally diverse and biologically active compounds that have been historically successful as anti-cancer agents and cellular tools: for example, rapamycin, an immunosuppressant obtained from *Streptomyces hygroscopicus* is also a potent anticancer agent which acts by specifically inhibiting mammalian target of rapamycin (mTOR) kinase. There is a need for new therapeutics for FGF dependent carcinomas.

Summary of the Invention

A whole animal *in vivo* screen for FGF signaling modulators using a larval zebrafish tail regeneration system has been developed. Using this assay, a novel polyketide natural product, colomitide C (**1**) that inhibited regeneration of tail fins (an FGFR-dependent process) and was nontoxic towards the larvae was identified. The compound is produced in high concentrations through fermentation of the fungus (*Cadophora luteo-olivacea*) and gram quantities can be easily isolated. Colomitide C was also tested in a 3-D "mammosphere assay" with mammary epithelial

cells isolated from a transgenic mouse in which FGFR1 activation can be induced. In this experiment, treatment with colomitide C prevented the transformation of normal cells into a "tumorsphere." Additionally, treatment of two-week old tumorspheres with colomitide C resulted in the reversal of the transformation process and return to the smaller, hollow "normal" mammosphere state within one day. Staining studies with labeled cellular markers showed that colomitide C treatment leads to upregulation of apoptosis in FGFR activated cells. Pharmacokinetic studies demonstrated that colomitide C is stable in both blood plasma and liver microscomes.

Accordingly the invention provides a compound of formula I:



(I)

wherein:

R^1 is $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, or $(C_1\text{-}C_6)$alkynyl, wherein each $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, and $(C_1\text{-}C_6)$alkynyl is optionally substituted with one or more groups independently selected from halo, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkoxycarbonyl, and $(C_1\text{-}C_6)$alkanoyloxy;

R^2 is H and R^3 is $-OR^a$ or $-NR^bR^c$; or R^2 and R^3 taken together are oxo (=O), or $=NR^d$;

R^a is H or $(C_1\text{-}C_6)$alkanoyl;

R^b and R^c are each independently selected from H, $(C_1\text{-}C_6)$alkyl, and $(C_1\text{-}C_6)$alkanoyl; and

R^d is hydroxy, $(C_1\text{-}C_6)$alkoxy, $-NR^bR^c$, or $(C_1\text{-}C_6)$alkanoyloxy, wherein the $(C_1\text{-}C_6)$alkoxy and $(C_1\text{-}C_6)$alkanoyloxy are each optionally substituted with one or more groups independently selected from halo, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkoxycarbonyl, aryl, and $(C_1\text{-}C_6)$alkanoyloxy, and wherein each aryl is optionally substituted with one or more groups independently selected from halo, nitro, cyano, hydroxy, trifluoromethyl, trifluoromethoxy, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkoxycarbonyl, and $(C_1\text{-}C_6)$alkanoyloxy;

or a salt thereof.

The invention also provides a pharmaceutical composition comprising a compound of formula I or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

The invention also provides a method for treating or preventing cancer in an animal comprising administering a compound of formula I or a pharmaceutically acceptable salt thereof to

the animal.

The invention also provides a method for modulating the FGF1R pathway in a cell comprising contacting the cell with a compound of formula I or a salt thereof.

The invention also provides a method for treating a pathological condition associated with the FGF1R pathway in an animal comprising administering a compound of formula I or a pharmaceutically acceptable salt thereof to the animal.

The invention also provides a compound of formula I or a pharmaceutically acceptable salt thereof for use in medical therapy.

The invention also provides a compound of formula I or a pharmaceutically acceptable salt thereof for the prophylactic or therapeutic treatment of cancer.

The invention also provides a compound of formula I or a pharmaceutically acceptable salt thereof for prophylactic or therapeutic treatment of a condition associated with the FGF1R pathway.

The invention also provides the use of a compound of formula I or a pharmaceutically acceptable salt thereof to prepare a medicament for treating cancer in an animal (e.g. a mammal such as a human).

The invention also provides the use of a compound of formula I or a pharmaceutically acceptable salt thereof to prepare a medicament for treating a pathological condition associated with the FGF1R pathway in an animal (e.g. a mammal such as a human).

The invention also provides a whole animal *in vivo* screen for FGF signaling modulators using a larval zebrafish tail regeneration system as described herein.

The invention also provides processes and intermediates disclosed herein that are useful for preparing a compound of formula I or a salt thereof.

Brief Description of the Figures

Figure 1: Larvae fin amputation and regeneration (A) Normal larvae fin at 4 days post fertilization. (B) Amputation is performed at the bold vertical line red line. (C) Normal larvae fin at 6 days post fertilization. (D) Regenerated larvae fin at 6 days post fertilization, 2 days after amputation. Vertical dotted line indicates the amputated level. In A-D, the shape of the fin is outlined by a curved dotted line.

Figure 2: Images of regenerating fins after treating with DMSO (A, Control) and BIO (B). Dotted and solid vertical lines represent the amputation plane and the distal edge of the regenerating fin, respectively. Horizontal arrows represent the length to be measured.

Figure 3: Colomitide C can reverse the proliferative phenotype in iFGFR1 mammary epithelial cells. A) The iFGFR1 transgenic mouse model in which FGFR1 can be induced by a dimerizer AP20187. This results in the FGFR1 kinase domains to dimerize and activates its downstream signal transduction which results into enhance proliferation of the mammary epithelial cells. B) In presence of the dimerizer, mammary epithelial cells from iFGFR1 transgenic mice undergo uncontrolled proliferation. Colomitide C can effectively prevent the uncontrolled proliferation as seen in this mammosphere assay.

Figure 4: shows that Colomitide C can inhibit proliferation and induce apoptosis in iFGFR1 mammary epithelial cells. The top panel shows the total number of iFGFR1cells seen by using a using a dye (Hoechst33342) that has been untreated or treated with Colomitide C. While all four untreated iFGFR1cells can take up EdU, which suggests that they are proliferating, only 2 out of 20 iFGFR1cells treated with Colomitide C are shown to take up EdU, suggesting that majority of these cells are not proliferating following the treatment of Colomitide C (middle panel). Also, most of the Colomitide C treated iFGFR1cells are shown to undergo apoptosis as shown by TUNEL assay, while none of the control untreated cells are undergoing apoptosis.

Figure 5: Colomitide C inhibits phosphorylation of AKT and affects the downstream targets of AKT signaling pathway. Western analysis of phospho ERK, ERK , phospho-AKT, AKT, phospho-GSK3 β and GSK3 β in iFGFR1 mammary epithelial cells.

Figure 6: Colomitide C can inhibit proliferation and induce apoptosis in human breast cancer cell lines. Three breast cancer cell lines, MDA-MB-361, CAMA1 and HCC38 were treated with or without Colomitide C and tested for proliferation (EdU incorporation) and apoptosis (TUNEL). For each cell line, there is a significant loss of proliferation (as shown by almost 100% reduction in EdU uptake) and increased apoptosis (as shown by significant increase in TUNEL staining) following Colomitide C treatment.

Figure 7: Colomitide C targets phosphorylation of AKT in HCC38 human breast cancer cells. A known inhibitor SU5402 is used as a control.

Detailed Description

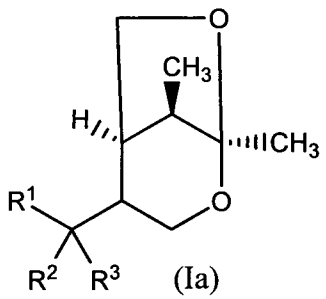
The following definitions are used, unless otherwise described: halo is fluoro, chloro, bromo, or iodo. Alkyl, alkoxy, alkenyl, alkynyl, etc. denote both straight and branched groups; but reference to an individual radical such as propyl embraces only the straight chain radical, a branched chain isomer such as isopropyl being specifically referred to. Aryl denotes a phenyl radical or an ortho-

fused bicyclic carbocyclic radical having about nine to ten ring atoms in which at least one ring is aromatic.

Specific values listed below for radicals, substituents, and ranges, are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents.

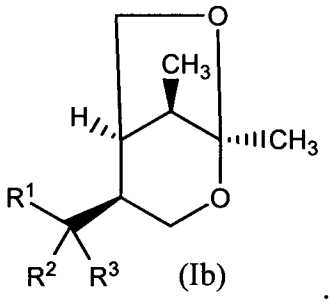
Specifically, (C_1-C_6)alkyl can be methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, sec-butyl, pentyl, 3-pentyl, or hexyl; (C_1-C_6)alkoxy can be methoxy, ethoxy, propoxy, isopropoxy, butoxy, iso-butoxy, sec-butoxy, pentoxy, 3-pentoxy, or hexyloxy; (C_2-C_6)alkenyl can be vinyl, allyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1,-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, or 5-hexenyl; (C_2-C_6)alkynyl can be ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl, 3-butynyl, 1-pentynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, or 5-hexynyl; (C_1-C_6)alkanoyl can be acetyl, propanoyl or butanoyl; (C_1-C_6)alkoxy-carbonyl can be methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, pentoxycarbonyl, or hexyloxycarbonyl; (C_2-C_6)alkanoyloxy can be acetoxy, propanoyloxy, butanoyloxy, isobutanoyloxy, pentanoyloxy, or hexanoyloxy; and aryl can be phenyl, indenyl, or naphthyl.

In one embodiment, the compound of formula I is a compound of formula (Ia):



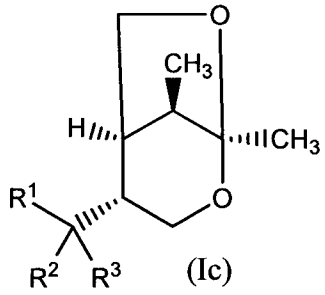
(Ia)

In one embodiment, the compound of formula I is a compound of formula (Ib):



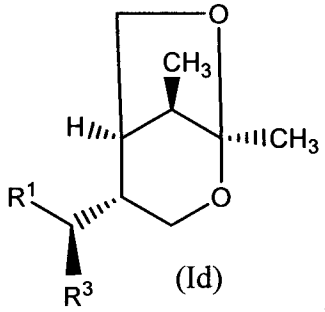
(Ib)

In one embodiment, the compound of formula I is a compound of formula (Ic):



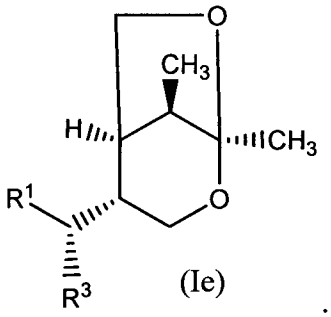
(Ic)

In one embodiment, the compound of formula I is a compound of formula (Id):



(Id)

In one embodiment, the compound of formula I is a compound of formula (Ie):



(Ie)

In one embodiment, R^1 is $(C_1\text{-}C_6)$alkyl.

In one embodiment, R^1 is 1-methylpropyl.

In one embodiment, R^2 is H and R^3 is –OH or NH_2.

In one embodiment, R^2 and R^3 taken together are oxo (=O).

In one embodiment, R^2 and R^3 taken together are $=NR^d$.

In one embodiment, R^d is hydroxy.

In one embodiment, R^d is $(C_1\text{-}C_6)$alkoxy optionally substituted with one or more groups independently selected from halo, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkoxycarbonyl, aryl, and $(C_1\text{-}$

C_6)alkanoyloxy, wherein each aryl is optionally substituted with one or more groups independently selected from halo.

In one embodiment, R^d is (C_1-C_6)alkanoyloxy, optionally substituted with one or more groups independently selected from halo, (C_1-C_6)alkoxy, (C_1-C_6)alkoxycarbonyl, aryl, and (C_1-C_6)alkanoyloxy, wherein each aryl is optionally substituted with one or more groups independently selected from halo.

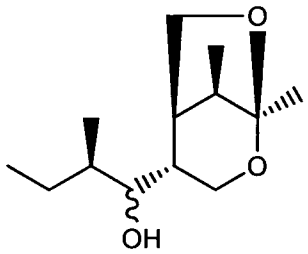
In one embodiment, R^d is benzyloxy, phenylcarbonyloxy, amino, or α-trifluoromethyl-α-methoxybenzyl.

In one embodiment the compound of formula I is not colomitide C. In another embodiment the invention provides colomotide C in isolated or purified form (e.g. at least about 80, 90, 95, or 99% pure).

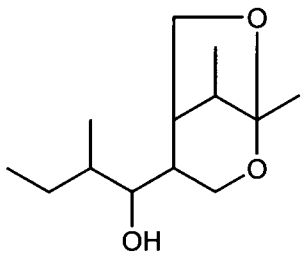
In one embodiment the compound of formula I is not colomitide A. In another embodiment the compound of formula I is not colomitide B. In one embodiment the compound of formula I is not colomitide A or colomitide B. In one embodiment the compound of formula I is not colomitide A, colomitide B or colomitide C.

Processes and intermediates useful for preparing compounds of formula I are provided as further embodiments of the invention and are illustrated by the following procedures in which the meanings of the generic radicals are as given above unless otherwise qualified.

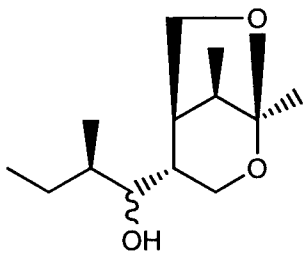
An intermediate useful for preparing a compound of formula I, is a compound of the following formula



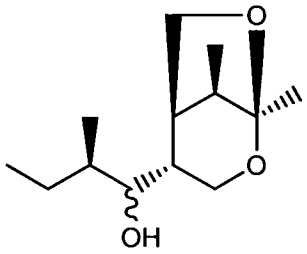
Another intermediate useful for preparing a compound of formula I, is a compound of the following formula



An intermediate useful for preparing a compound of formula I(b), is a compound of the following formula



An intermediate useful for preparing a compound of formula I(c), I(d) or I(e), is a compound of the following formula



It will be appreciated by those skilled in the art that compounds of the invention having a chiral center may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein, it being well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase. When a compound is shown with a wedged (up) or dashed (back) bond the compound may be enriched by about 60%, 80%, 90%, 95%, 98%, or 99% in the absolute stereoisomer represented.

In cases where compounds are sufficiently basic or acidic, a salt of a compound of formula I can be useful as an intermediate for isolating or purifying a compound of formula I. Additionally, administration of a compound of formula I as a pharmaceutically acceptable acid or base salt may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α-ketoglutarate, and α-glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

The compounds of formula I can be formulated as pharmaceutical compositions and administered to a mammalian host, such as a human patient in a variety of forms adapted to the chosen route of administration, i.e., orally or parenterally, by intravenous, intramuscular, topical or subcutaneous routes.

Thus, the present compounds may be systemically administered, e.g., orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid

carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

The active compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying

techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the present compounds may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the present compounds can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Examples of useful dermatological compositions which can be used to deliver the compounds of formula I to the skin are known to the art; for example, see Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

Useful dosages of the compounds of formula I can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

The amount of the compound, or an active salt or derivative thereof, required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

The biological activity of a compound of the invention may be determined using pharmacological models which are well known to the art, or using Test A described below.

Test A Fin Regeneration Assay

Regeneration of the tail fin of zebrafish larvae was used as one of biological assays. The assay was a modification of a procedure reported by Kawakami A, et al., *Dev Dyn.* **2004,** 231(4): 693-699.

Zebrafish housing, mating, embryo collection, and raising larvae are performed by following standard procedures in the Zebrafish Book (Westerfield, M. (2000). The zebrafish book. A guide for the laboratory use of zebrafish (Danio rerio). 4th ed., Univ. of Oregon Press, Eugene). Zebrafish larvae used in the assay were not given any food during the assay period (up to 6 days post fertilization). Embryos and larvae were developed at 28°C in the embryo media. For anesthetizing, approximately 1% volume of tricaine (MS-222) solution (40 mg/ml) was added to larvae.

After anesthetizing larvae at four-day post fertilization, a larva was placed on a transparent plastic dish, and associated liquid was removed by absorbing it using Kimwipe, so that the larva attached to the dish. Under a stereomicroscope, the tail was cut with a razor blade at the immediately caudal level of the neural tube (Fig. 1). The cutting was made perpendicular to the neural tube.

Five larvae were placed in one well of a 24-well tissue culture plate. The media in a well was removed without disturbing larvae, and 1 ml of new embryo media was added to the well. A compound, which was dissolved in dimethyl sulfoxide (DMSO), was added to the 1 ml media with five larvae. Depending on the concentration of the stock solution of the compound, and depending on the final concentration to be tested, the volume of compound added to the 1ml media was changed. In a standard assay, the volume of compound solutions or DMSO was 10 ul, and the maximum volume of the compound solution in some assays was 20 ul. Ten ul of 500 nM BIO in DMSO was added to a well as a control (BIO: 6-Bromoindirubin-3′-oxime, CAS Number: 667463-62-9). The solution was mixed by pipeting without disturbing larvae.

After one-day treatment, media was removed and new media (1 ml) was added to each well. The same compound was added at the same concentration. One day later (total two days of treatment, larvae are now at 6 days post fertilization), larvae were anesthetized by adding tricaine solution into the well. A picture of the larva fin was taken by using Zeiss V8 Discovery stereomicroscope with Plan S 1.0x objective lens, Jenoptik Progress C3 camera and iSolution light software. The magnification of the Zeiss V8 Discovery stereomicroscope was x8.

The longest length of regenerated area was determined by iSolution software by measuring the pixel number of the line that was drawn perpendicular to the amputation plane (Fig. 2). The average of regenerated lengths of the control group was set as 1.00, and regenerated length of each larva was calculated. The regenerated length by treating larvae with a compound was evaluated in comparison to a control group, and statistical significance was determined by Student's t-test. If the p value was smaller than 0.05, it was considered that the compound has significant activity of either enhancing or inhibiting regeneration. If more than three larvae of a control group (treated by DMSO) were dead, it was considered that the quality of the larvae of the specific batch was bad, and the entire data of the assay was not used to evaluate any compound in the assay.

BIO has been shown to impair larvae fin regeneration in a similar, but not identical assay (Lijoy K. Mathew, et al., FASEB J. 2008 August; 22(8): 3087–3096). Thus, if larvae treated by BIO did not show statistically significant inhibition of fin regeneration, it was considered that the quality of the larvae of the specific batch was bad, and the entire data of the assay was not used to evaluate any compound in the assay. If more than three larvae of a treated group were dead, the compound in the assay was not evaluated.

The biological activity of a compound of the invention may also be determined using pharmacological models which are well known to the art, or using Test B described below.

Test B Activity of colomitide C on larvae fin regeneration

Colomitide C was tested for the fin regeneration assay. As shown in Table 1, at 10 ug/ml, colomitide C inhibited fin regeneration. The degree of regenerated lengths was 74.8±1.9%, compared to DMSO treated control larvae.

Table 1 Assay results for different concentrations of Colomitide C

	Measurement value (pixel)							Relative length of the regenerated area							p
	#1	#2	#3	#4	#5	ave.	SD	#1	#2	#3	#4	#5	ave.	SD	value
1% DMSO	60.8	63	63.1	57	0	60.98	2.85	0.997	1.033	1.035	0.935		1.000	0.047	
BIO (0.5 nM)	40	51.6	41	47	0	44.90	5.43	0.656	0.846	0.672	0.771		0.736	0.089	0.004
Colomitide C (1 ug/ml)	61.4	57.2	63	69.1	0	62.68	4.93	1.007	0.938	1.033	1.133		1.028	0.081	0.578
Colomitide C (5 ug/ml)	55	54	52	62.2	0	55.80	4.45	0.902	0.886	0.853	1.020		0.915	0.073	0.106
Colomitide C (10 ug/ml)	38	58	49	44.3	44	46.66	7.44	0.623	0.951	0.804	0.727	0.722	0.765	0.122	0.009

value 0 indicates that the animal was dead. This value is not integrated in calculation

Activity of benzyl-colomitide C on larvae fin regeneration

Benzyl-colomitide C was tested in the larvae fin regeneration assay. As shown in Table 2, it showed toxicity at 10 ug/ml. However, as shown in Table 3, at lower concentrations, it exhibited an inhibitory activity on fin regeneration.

Table 2 Comparison of activity of Colomitide C and benzyl-colonitide C at 10 ug/ml

	Measurement value (pixel)							Relative length of the regenerated area							
	#1	#2	#3	#4	#5	ave.	SD	#1	#2	#3	#4	#5	ave.	SD	p-value
DMSO	45.10	61.20	47.54	57.31	65.00	55.23	8.62	0.817	1.108	0.861	1.038	1.177	1.000	0.156	
BIO	29.50	39.00	29.12	28.84	0.00	25.29	14.77	0.534	0.706	0.527	0.522		0.572	0.089	0.002
Colomitide C. (10 ug/ml)	39.56	38.28	40.00	18.97	42.01	35.76	9.48	0.716	0.693	0.724	0.344	0.761	0.648	0.172	0.009
Benzyl colomitide (10 ug/ml)	0.00	0.00	0.00	0.00	0.00										

value 0 indicates that the animal was dead. This value is not integrated in calculation

Table 3 Activity of benzyl-Colonitide C at concentrations lower than 10 ug/ml

	Measurement value (pixel)							Relative length of the regenerated area							
	#1	#2	#3	#4	#5	ave.	SD	#1	#2	#3	#4	#5	ave.	SD	p-value
DMSO	44.01	42.05	41.44	0.00	0.00	42.50	1.35	1.036	0.989	0.975			1.000	0.032	
BIO	34.44	41.63	33.62	32.02	0.00	35.42	4.26	0.810	0.980	0.791	0.753		0.834	0.100	0.042
Benzyl colomitide (1 ug/ml)	37.20	38.64	38.60	0.00	0.00	38.15	0.82	0.875	0.909	0.908			0.898	0.019	0.009
Benzyl colomitide (3 ug/ml)	36.24	38.12	38.95	36.14	0.00	37.36	1.40	0.853	0.897	0.916	0.850		0.879	0.033	0.005
Benzyl colomitide (5 ug/ml)	32.25	31.95	35.44	31.78	33.00	32.88	1.50	0.759	0.752	0.834	0.748	0.777	0.774	0.035	0.000

value 0 indicates that the animal was dead. This value is not integrated in calculation

Mammary Mammosphere Assay:

To test whether Colomitide C can have any effect on specific signaling pathways which are found to be upregulated in cancer, a mouse model system where the FGFR1 signaling pathway can be induced via a FGFR1 dimerizer, AP20187 was used. Upregulation of FGFR1 in the mammary epithelial cells results into uncontrolled proliferation of these cells which can be assayed by transformed acinar structure in the matrigel (Figure 3A). As shown in the figure 3, Colomitide C can prevent the transformation of these cells upon FGFR1 activation. Additionally, it can reverse the phenotype when applied to the FGFR1 mediated transformed acinar structures. These data suggest that Colomitide can potentially inhibit RTK pathways, like FGFR1 upregulation.

Colomitide C can inhibit proliferation and induce apoptosis in iFGFR1 mammary epithelial cells

To understand how Colomitide C can inhibit transformation of iFGFR1 mammary epithelial cells, proliferation and apoptosis assays of these cells in presence and absence of Colomitide C was carried out. As shown in Figure 4, Colomitide C can inhibit the iFGFR1 mammary epithelial cells to incorporate 5-ethynyl-2'-deoxyuridine (EdU), which suggest that Colomitide C inhibits proliferation of these cells. More strikingly, TUNEL assay shows that iFGFR1 mammary epithelial cells undergo apoptosis in presence of Colomitide C when FGFR1 is upregulated (Figure 4).

Colomitide C affects AKT phosphorylation in iFGFR1 mammary epithelial cells

To identify which arm of the FGFR1 pathway is affected by Colomitide C, analysis of the downstream targets of FGFR1 was carried out.

GFR1 can mediate activation of several sinaling pathways, including induction of ERK1/2 and AKT signaling pathways. These are marked by phosphorylation of the ERK1/2 and/or AKT. Aberrant phosphorylation of these two proteins are found in many cancers. Western analysis of these two proteins was carried out. Whereas phosphorylation of ERK1/2 remains unaffected, phosphorylation of AKT is reduced in presence of Colomitide C, suggesting that Colomitide C acts specifically in the AKT pathway in the iFGFR1 mammary epithelial cells (Figure 5). This is further evident from the fact that phosphorylation of GSK3β, a direct target of AKT, is markedly reduced in these iFGFR1 cells (Figure 5).

Colomitide C can inhibit proliferation and induce apoptosis in human breast cancer cells

To examine whether Colomitide C can have therapeutic potential in human cancers, its effect on the proliferation in the human breast cancer cell lines, MDA-MB 361, CAMA 1, and HCC 38 was examined. All these cell lines are known to have aberrant FGFR signaling. Following 24 hours treatment with the Colomitide, all the three breast cancer cell lines have dramatic reduction in proliferation and induction of apoptosis, as seen previously in the iFGFR1 mammary epithelial cells (Figure 6). This is a proof of principle that Colomitide C can have potent anti-cancer therapeutic properties. Additionally, the AKT phosphorylation was reduced in the Colomitide C treated HCC38 cells, suggesting that for this breast cancer cell line, Colomitide C is likely affecting the AKT signaling pathway (Figure 7). The above experiments provide strong proof of principle that Colomitide C can have anti-cancer activity and can be of therapeutic value.

The invention will now be illustrated by the following non-limiting Examples.

Examples

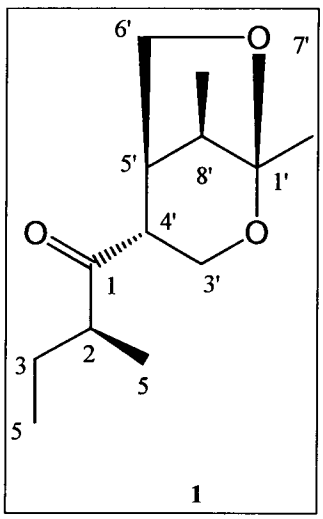
Example 1.

A. Fungal Fermentation and Extraction

The strain was cultured in 20 erlenmeyer flask containing 100 g rice media at room temperature. After 30 days, the culture was extracted with two times 4 liters of methanol and two times 4 liters of ethylacetate, consecutively. The extracts were combined and dried in the vacuum. Dried extract was suspensed in 500 ml of water and then partitioned with 500 ml of ethylacetete. Dried ethylacetate extract was then dissolved in 500 ml of 90 % methanol (v/v in water) and extracted with 250 ml of n-hexane. Methanol extracts (later called as ethylacetate extracts) was dried and further processed for the isolation of colomitide C (**1**)

B. Isolation of Colomitide C (**1**)

7.8 g of crude ethylacetate extract was separated using ISCO® flash chromatography with 50 g silica as stationary phase and linear gradient elution of 100 % to 0 % of CH_2Cl_2/MeOH. Fraction 24 (5.4 g) was then further chromatographed using ISCO® flash chromatography with 50 g silica as mobile oil phase and linear gradient elution of 100 % to 0 % of n-hexane/ethylacetate. Colomitide C (**1**) (fraction 37 ; 1802.8 mg) was isolated as yellowish clear oil. The next fractions (called fraction 24.53 ; 3324.5 mg)was re-separated using 50 g silica with linear gradient elution of 100 % to 0 % of n-hexane/ethylacetate to obtain more colomitide C (**1**) (970.2 mg). Total colomitide C (**1**) isolated from this culture was 2773 mg.



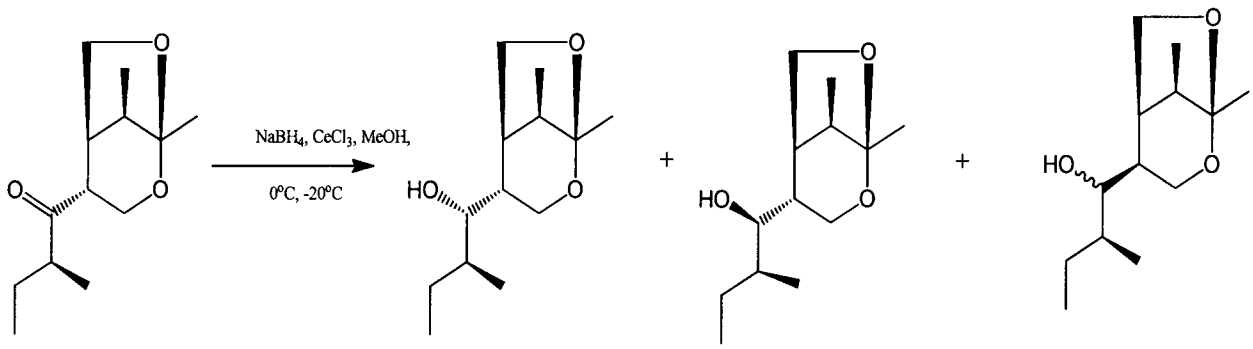
1

Table 1. NMR data of **1** (CD_3OD, 600 MHz)

	δ_H (*J* in Hz)	δ_C.	COSY H to H	HMBC H to C	ROESY H to H
1		214.8			
2	2.83 q (6.6)	41.2	3A, 3B, 5	1, 3, 4, 5	5', 4
3	A 1,65 m	26.5	2, 13B, 4	1, 2, 4, 5	4', 5
	B 1.37 m		2, 3A, 4	1, 2, 4, 5	4',5
4	0.86 t (7.2)	10.5	3A/B	2, 3	8', 2
5	1.04 d (6.6)	15.3	2	1, 2, 3	5', 4', 3A/B
1'		108.6,			
3'	A 4.04 d (12.0)	58.6	4',3'B	1', 5', 4', 1	
	B 3.83 dd (12.0, 4.8)		4', 3'A	1	
4'	2.78 dd (4.8, 3.5)	52.5	5', 3'A/B	8', 5'	6'B, 3A, 3B, 5
5'	2.71 dd (4.2, 3.5)	41.9	4', 6'A/B	1'(weak), 3' (weak)	6'A, 2, 8', 5, 8'-Me
6'	A 4.17 dd (8.4, 4.2)	71.0	5', 6B	6', 4'	5', 8'-Me
	B 4.00 d (8.4)		5', 6A	1' (weak), 8', 4'	4'
8'	1.81 q (7.2)	42.4	8'-Me	1 (weak), 5',4', 6', 8'-Me	5', 1'-Me, 5, 8'-Me
1'-Me	1.27 s	19.0		1', 8'	
8'-Me	0.90 d (7.2)	12.0	8'	1', 8', 5'	

C. Reduction of colomitide C

Colomitide C (**1**) (28 mg ; 0.12 mmol) and cerium chloride heptahydrate (92 mg ; 0.32 mmol) were dissolved in 1 ml methanol. While stirring at 0°C, sodium borohydride (9.3 mg ; 0.24 mmol) was added into solution and the reaction was continued at 0°C for 1 hr. After the mixture was kept at -20° C for 24 hr, it was then dried out and portioned between water and EtOAC. Ethylacetate fraction (25.6 mg) was then subjected to a separation using preparative HPLC with gradient elution 35% - 100 % of acetonitrile/H_2O. Three isomers of products **2** (70 %), **3** (15%) and **4** (8%) were isolated as white solid.



1 2 3 4

Structure of **2**, **3** and **4**

Table 2. NMR data of **2** (CD_3OD, 600 MHz)

Position	δ_H (*J* in Hz)	δ_C, mult.	COSY (H to H)	HMBC (H to C)	NOESY
1					
1	3.74 d (9.4)	71.3	2	4', 2' 5	8', 3'B, 2, 3A
2	1.48 m *	37.1	3A/B, 5	3	1, 4', 5
3	A. 1.48 m *	27.1	3B, 2, 4	2, 5	10, 6B, 13, 14*
	B. .35 m		3A, 2, 4	1 2, 5	3A
4	0.95 t (7.0)	21.6	3A/B	2, 3	1, 4'
5	0.80 d (6.3)	11.2	2	1, 2, 3A/B	4', 2
1'		108.3			
3'	A. 3.87 dd (12.5 ; 5.1)	60.7	4', 3B'	5', 1'	4'
	B. 3.45 d (12.5)		4', 3'A	1', 5', 4', 1	4', 1, 3A
4'	1.72 br	43.1	5', 3'A/B, 1	1	3'A/B, 1, 5', 2, 4, 5
5'	2.60 br	41.8	6A, 4'	3'	6'A, 8', 4', 8'-Me
6'	A. 4.13 dd (8.0 ; 5.0)	71.9	5', 6B	4'	5', 8'-Me
	B. 3.89 d (8.0)		6A	1', 4'	
8'	2.23 q (7.0)	40.4	8'-Me	5', 8'-Me	1, 5", 1'Me, 8'-Me
8'-Me	1.30 s	18.9		1', 8'	1', 3'
1'-Me	0.93 d (7.0)	13.0	7	1', 8', 5'	6A, 8', 5'

Table 3. NMR data of **3** (CD_3OD, 600 MHz)

Position	δ_H (*J* in Hz)	δ_C,mult.	COSY (H to H)	HMBC (H to C)	NOESY
1	3.64 d (9.7)	74.2	4'	3', 3, 5	8', 3', 2, 5
2	1.53 m	36.2	5		8', 5', 5
3	A. 1.42 m	20.4	3B, 4	2, 4, 5	5'
	B. 1.11 m		3A, 4	2, 4, 5	
4	0.93 t (7.5)	11.1	3 A/B	2, 3	3A/B
5	1.01 d (6.6)	15.8	12	1, 2, 3	1, 3A
1'		108.3			
3'	A. 3.87 bs	61.0	4', 3'B	4'	4'
	B. 3.87 bs		4', 3'A	1', 5', 4', 1	4'
4'	1.62 m	43.6	5', 3'A/B, 1		3A
5'	2,17 (7.0 ; 4.0 ; 3.1)	42.5	4', 6'A	1, 8', 4', 8'-Me, 3'	(3'A/B, 6'B)**, 6'A, 8'-Me, 2, 3A
6'	A. 4.12 dd (8.4 ; 4.4)	71.8	4', 5', 6'B	1', 8', 5', 4'	5', 8'-Me
	B. 3.90 d (8.4)		6'A	1', 8', 4', 5'	4', 5'
7'	1.98 q (7.0)	41.1	8	1', 5', 8'-Me, 6'	8'-Me, 1'-Me, 1
1'-Me	1.30 s	19.2		1'-Me, 8'	8', 8'-Me
8'-Me	0.89 d (7.0)	13.3	3	1', 8', 5'	8'. 5', 6'A

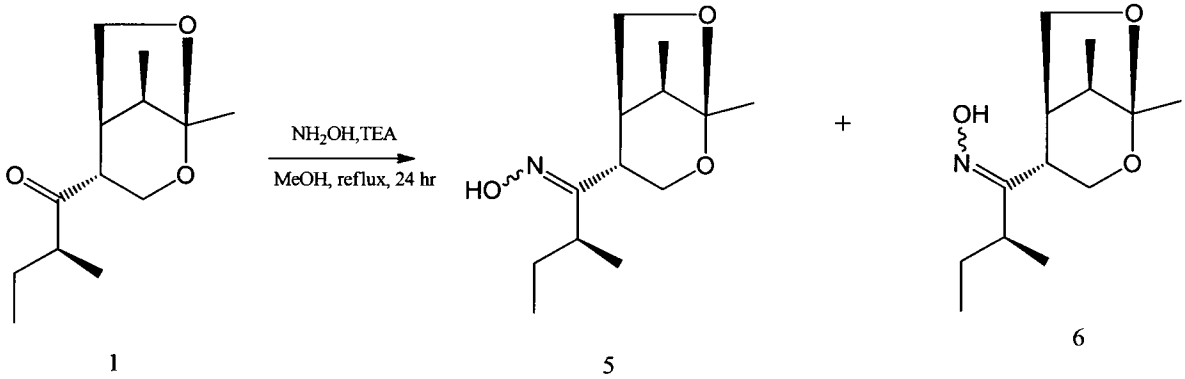
Table 4. NMR data of **4** (CD_3OD, 600 MHz)

Position	δ_H (*J* in Hz)	δ_C,mult.	COSY (H to H)	HMBC (H to C)	NOESY
1	3.51*	74.4	4'	5', 3, 2, 3	6'A
2	1.49 q (7.5)	35.6	5	3, 5	1/3'A,, 5
3	A. 1.43 m	26.7	3B, 4	1,, 2, 4, 5	5, 9A, 11
	B. 1.33 m		3A, 4	1,, 2, 4, 5	8'-Me, 5
4	0.89 t (7.0)	12.4		2, 3	
5	0.86 d (7.5)	13.0	2	1, 2, 3	4'
1'		107.5			
3'	A. 3.51*	62.2	3'B, 4'	5', 1	4', 6'A, 2
	B. 3.31**		3'A, 4'	5'	
4'	1.91 br	45.4	3'A/B, 1	8', 3'	8', 5', 3'A/1, 5
5'	2.39 br	43.5		1', 8', 4', 1	4', 8'-Me, 6'A/B
6'	A. 4.15 d (8.2)	68.1	6'B	1, 8', 4', 5'	1, 3'B
	B. 3.87 dd (8.2 ; 4.7)		5', 6'A	4'	5', 8'-Me
8'	1.77 q (7.0)	48.0	8		4', 5', 1'-Me, 8'-Me
1'-Me	1.28 s	20.3		1', 8'	8'
8'-Me	0.95 d (7.0)	14.2	8,	1', 8', 5'	

D. Preparation of oxime derivates of colomitide C

A mixture of 500 mg (2.2 mmol) of colomitide C (**1**), 2 ml (7.5 mmol) of triethanolamine and 0.2 ml (3.66 mmol) NH_2OH (50 % w/w in water) were dissolved in 15 ml MeOH. The mixture was refluxed for 24 hours. The reaction mixture was then dried and partitioned between water and EtOAc. Ethylacetate fraction were then subjected to separation using ISCO® flash chromatography with 40 g silica as mobile

phase and linear gradient elution of 100 % to 0 % of CH_2Cl_2/MeOH. Two isomers, 5 (80 %) and 6 (12%) were purified as white solids.



1 5 6

Structure of **5** and **6**

Table 5. NMR data of **5** (CD_3CN, 600 MHz)

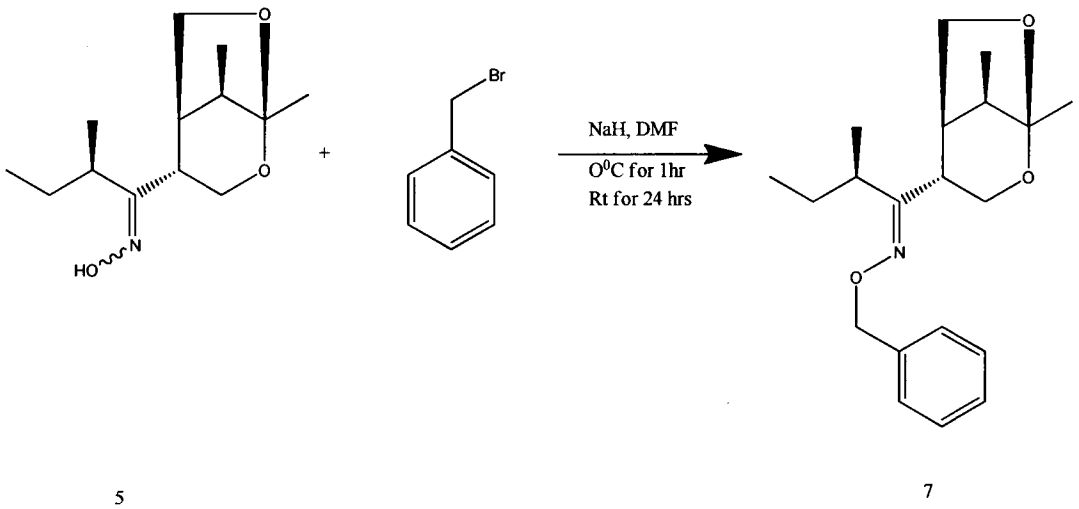
Position	δ_H (*J* in Hz)	δ_C,mult.	COSY (H to H)	HMBC (H to C)	NOESY
1		163.6			
2	2.76 m	37.7	5	1, 3, 4, 5, 4'	5, 4
3	A. 1.77 m	27.6	4	1, 2,, 4, 5	
	B. 1.47 m		4	1, 2, 4, 5	
4	0.90 t (7.0)	13.4	3A/B	2, 3	2
5	1.14 d (7.1)	16.7	2	2, 3	2, 4', 5'
1'		109.8			
2'					
3'	A. 3.88 d (11.7)	62.0	3'B. 4	1, 1', 4', 5'	
	B. 3.87 dd (11.7 ; 6.0)		3'A, 4	1, 1'	
4'	2.52 br	45.6	3'A/B, 5'	1', 3', 5', 6'	5
5'	2.42 br	44.4	4', 6'A	1', 3', 4', 8'-Me	5, 8'-Me
6'	A. 4.11 dd (8.2 ; 4.7)	73.0	5', 6'B	1', 4', 5'	8'-Me, 4', 5'
	B. 3.92 d (8.2)		6'A	1', 4', 5',	5'
7'					
8'	2.20 m	41.9	8'-Me	1', 4', 5', 6', 8'-Me	8'-Me
1'-Me	1.26 s	21.0		1', 8'	
8'-Me	0.87 d (7.0)	14.5	8'	1', 5', 8'	
1-NOH	8.40 s				

Table 6. NMR data of **6** (CD_3CN, 600 MHz)

Position	δ_H (*J* in Hz)	δ_C, mult.	COSY (H to H)	HMBC (H to C)	NOESY
1		163.6, C			
2	3.00 q (7.0)	35.7, CH	5	1, 3, 4, 5, 4',	5
3	A. 1.53 m	27.6, CH_2	3B, 4	1, 2, 4, 5	
	B. 1.43 m		3A, 4	1, 2, 4, 5	
4	0.82 t (7.0)	13.1, CH_3	3A/B	2, 3	
5	1.05 d (7.0)	17.0, CH_3	2	1, 2, 3	2, 4'
1'		108.6, C			
2'					
3'	A. 3.82, t (11.1)	63.9, CH_2	3'A	1, 1', 4', 5'	4'
	B. 3.60, dd (11.1 ; 5.3)		3'B, 4'	1', 4', 5'	
4'	2.71, dd (11.1 ; 5.3)	43.1, CH	3'A/B	1, 2, 3', 5', 6'	5, 3'A/B, 5', 8',
5'	2.27, d (3.5)	45.7, CH	6'A/B	1, 1', 3', 4',8', Me-8'	5, 6'A/B, 8', Me-8'
6'	A. 4.21, d (8.2)	68.9, CH_2	5', 6'B	1', 4', 5', 8'	5'
	B. 3.84, dd (8.2 ; 3.5)		5', 6'A	4',	5', Me-8'
7'					
8'	1.90 q (7.0)	48.4, CH	Me-8'	1', 4', 5', 6', Me-8'	4'
Me-1'	1.26 s	20.6, CH_3		1', 8'	
Me-8'	0.91, d (7.0)	15.1, CH_3	8'	1', 5', 8'	4',5', 8', 6'B
NOH-1	8.33 s				

Preparation of benzyl oxime of colomitide C

A mixture of 16.2 mg (0.067 mmol) of oxime derivate (**5**) of colomitide C and 10 mg (0.4 mmol) of NaH were dissolved in DMF and cooled in ice bath for 30 minutes. To the mixture was added 40 µl (0.33 mmol) benzylbromide and kept at 0°C for 1 hour. The reaction was continued at room temperature for 24 hours. The reaction mixture was then dried out under vacuum and portioned between EtOAc and water. EtOAc fraction was subjected to separation using silica column with mobile phase 98 : 2 (CH_2Cl_2/MeOH). Benzyl oxime derivate (**7**) of colomitide was isolated as clear oily compound (60%).



5 7

Structure of 7

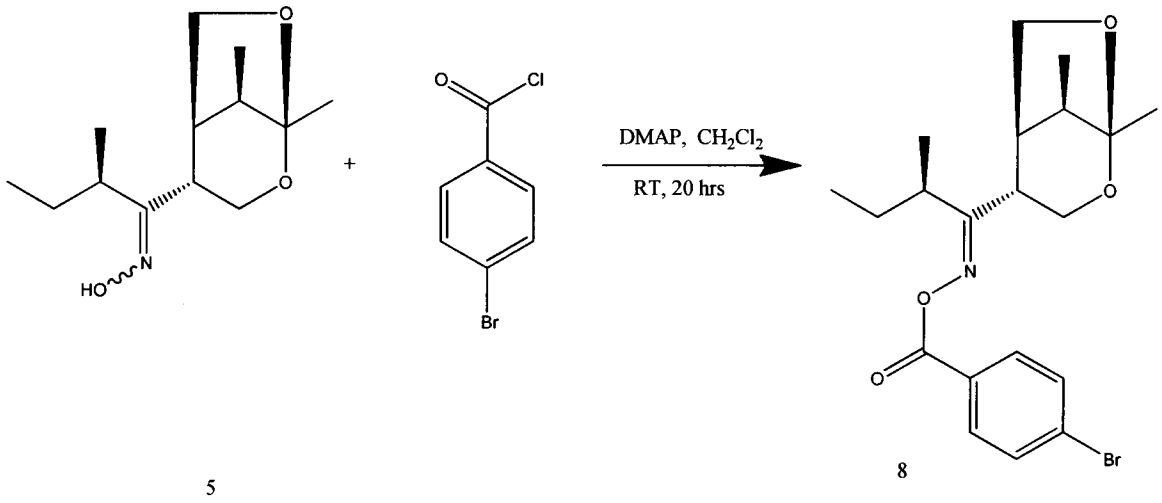
Table 7. NMR data of **7** (CD_3OD, 600 MHz)

Position	δ_H (*J* in Hz)	δ_C,mult.	COSY (H to H)	HMBC (H to C)	NOESY
1		163.6			
2	2.64 q (7.0)	38.8	3A/B, 5	1,3, 4, 5, 4'	5, 5'
3	A. 1.74 m	27.6	2, 3B, 4	1, 2, 4, 5	4
	B. 1.44 m		2, 3A, 4	1, 2, 4, 5	4
4	0.84 t (7.1)	13.9	3A/B	2, 3	3A/B
5	1.10 d (7.0)	16.5	2	1, 2, 3	
1'		110.2			
2'					
3'	A. 3.95 d (11.2)	62.1	3'B, 4	1, 1', 5'	
	B. 3.83 dd (11.2 ; 5.3)		3'A, 4	1, 1',	
4'	2.49 bs	46.0	3'A/B, 5'	1, 5', 6', 8'	5, 6'A
5'	2.35 bs	44.3	4', 6'A/B	1', 3', 8'-Me	5, 8'-Me
6'	A. 4.11 dd (8.3 ; 4.7)	72.8	5', 6'B	1', 4', 5'	4', 8'-Me
	B. 3.94 d (8.3)		5;, 6'A	1', 4', 5', 8'	
7'					
8'	2.15 q (7.0)	41.6	8'-Me	1', 4', 5', 6', 8'-Me	8'-Me
1'-Me	1.28 s	20.2		1', 8'	
8'-Me	0.79 d (7.0)	12.8	8'	1', 5', 8'	6'A
1"		140.0, C			
2"/6"	7.37 d (7.0)	129.6, CH	3"/5"	2"/6". 4", 7"	7"
3"/5"	7.32 dd (7.1 , 7.0)	128.9, CH	2", 4"/ 6", 4"	1", 3"/5"	
4"	7.27 t (7.1)	128.7, CH	3", 5"	2"/6"	
7"	A/B 5.04 d (4.7)	76.6, CH_2		1", 2"/6"	6'A/B, 7"

E. Preparation of p-bromobenzoyl oxime of colomitide

To a stirring solution of 7 mg (0.03 mmol) of oxime form (**5**) in 4 ml CH_2Cl_2 in ice bath, 7.3 mg (0.06 mmol) of dimethylaminopyridine and 4.8 µl (0.06 mmol) of pyridine were consecutively added. The

reaction mixture was kept at 0°C for 30 minutes after addition of 13 mg (0.06 mmol) of p-bromobenzoyl chloride. The reaction was continued at room temperature for 24 hours before evaporation under vacuum and partition between water and EtOAc. EtOAc fraction was then subjected to separation using preparative HPLC (C18 column) with gradient elution of 50 % to 100 % acetonitrile/H_2O. Benzoyl oxime derivate (**8**) was isolated as white solids (yield 72 %).



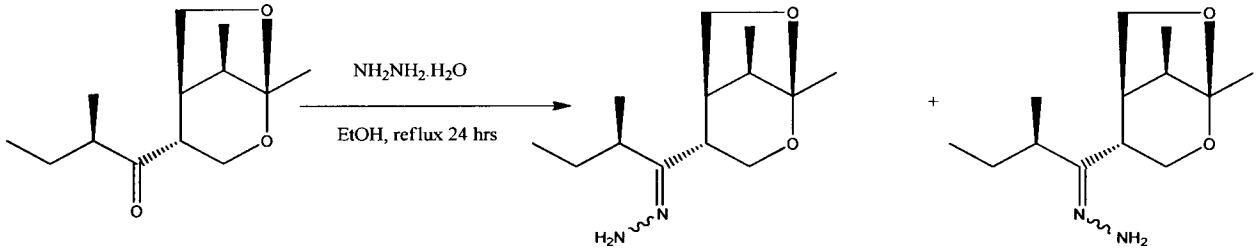
Structure of **8**

Table 8. NMR data of **8** (CD_3OD, 600 MHz)

Position	δ_H (*J* in Hz)	δ_C,mult.	COSY (H to H)	HMBC (H to C)	NOESY
1		174.4,			
2	2.83 m	39.6,	3A/B, 5	1, 3, 4, 5, 4'	4', 5', 5
3	A. 1.78 m	27.4,	3B, 2, 4	1, 2, 4, 5	4
	B. 1.58 m		3A, 2, 4	1, 2, 4, 5	4
4	0.93 t (7.2)	13.9,	3A/B	2, 3	5
5	1.25 d (7.3)	16.7,	2	1, 2, 3	
1'		109.5			
3'	A. 4.02 d (11.9)	61.0	3'B, 4'	1, 1'. 4', 5'	4'
	B. 3.87 dd (11.9 ; 5.3)		3'A, 4'	1, 1'. 4', 5'	4'
4'	2.75 bs	46.2,	3'A/B, 5'	1, 3', 5', 8', 6'	
5'	2.51 bs	43.6,	4', 6'A/B	1', 6', 4'. 8'. 8'-Me	5, 8', 8'-Me
6'	A. 4.15 dd (8.6 ; 4.6)	72.2	6'B, 5'	1', 8', 4', 5'	5', 8'-Me
	B. 3.98 d (8.6)		6'A, 5'	1', 8', 4', 5'	5', 4'
8'	2.32 q (7.3)	41.5,	8'Me	1', 8'-Me, 6',4', 5', 1'-Me	8'-Me
Me-1'	1.28 s	20.4,		1', 8'	
Me-8'	0.89 d (7.3)	12.5,	8'	1', 8', 5'	
1"		129.4,			
2"/6"	7.93 d(7.9)	132.0,	3"/5"	7", 4", 6"/2'	
3"/5"	7.71 d(7.9)	132.8,	2", 4"/ 6", 4"	1", 5"/3"	
4"		128.7,			
7"		163.8,			

F. Preparation of hydrazone derivate of colomitide C

A solution of 22.5 mg (0.1 mmol) of colomitide C and 20 mg (0.4 mmol) of $NH_2.NH_2.H_2O$ in 1.5 ml ethanol was refluxed for 24 hours. The mixture was then dried under vacuum and partitioned with water and ethylacete. Ehylacetate fraction was then separated using silica column with mobile phase 97.5/2.5 = CH_2Cl_2/MeOH to give **10** (70 %) and **11** (15 %), were obtained in this reaction.



1 9 10

Structures of **9** and **10**

Table 9. NMR data of **9** (CD_3OD, 600 MHz)

Position	δ_H (*J* in Hz)	δ_C,mult.	COSY (H to H)	HMBC (H to C)	NOESY*)
1		159,5			
2	2.70 m	35.8		1, 3, 4, 5, 4'	
3	A. 1.67 m	26.8		1, 2, 4, 5	
	B. 1.41 m			1, 2, 4, 5	
4	0,71 t (7.0)	12.3		2, 3	
5	1.1 d (6.6)	15.4		1, 2, 3	
1'		111.3			
3'	A. 3.86 d (11.7)	62.6		1, 1', 4', 5'	
	B. 3.85 dd (11.7 ; 6.0)			1, 1', 4', 5'	
4'	2.60 bs	44.0		1	3'A/B, 4, 5
5'	2,40 bs	44,7		1', 6, 4', 8'-Me	6'A/B,
6'	A. 4.12 dd (8.4 ; 4.7)	72.2		1', 4', 5'	5
	B. 3.94 d (8.4)			1', 4', 5'	
8'	2.35 q (7.2)	40.8		1', 6', 8'-Me, 1'-Me	
1'-Me	1.30 s	19.8		1', 8'	
8'-Me	0.86 d (7.2)	13.7		1', 5', 8'	

Table 10. NMR data of **10** (CD_3OD, 600 MHz)

Position	δ_H (*J* in Hz)	δ_C, mult.	COSY (H to H)	HMBC (H to C)	NOESY*)
1		159.4			
2	2.45 m	41.0		1, 3, 4, 5	
3	A. 1.64 m	30.0		1, 2, 4, 5	
	B. 1.45 m			1, 2, 4, 5	
4	1.09 d (7.0)	21.8		2, 3	
5	0.91 t (7.3)	13.0		1, 2, 3	
1'		110.2			
3'	A. 3.99 d (11.7)	61.0		1, 5'	4'
	B. 3.95 dd (11.7 ; 6.0)			1, 1', 5'	4'
4'	2.72 dd (3.3 ; 4.4)	44.1			
5'	2.67 bs	43.8			
6'	A. 4.14 dd (8.2 ; 4.8)	73.0		1', 4'	4'
	B. 3.97 d (8.2)			1', 4'	4'
8'	1.94 q (6.7)	44.2		6', 4'	
1'-Me	1.33 s	21.0		1', 1'-Me	
8'-Me	0.88 d (6.7)	14.8		1', 5'	

Example 2. The following illustrate representative pharmaceutical dosage forms, containing a compound of formula I ('Compound X'), for therapeutic or prophylactic use in humans.

(i) Tablet 1	mg/tablet
Compound X=	100.0
Lactose	77.5
Povidone	15.0
Croscarmellose sodium	12.0
Microcrystalline cellulose	92.5
Magnesium stearate	3.0
	300.0

(ii) Tablet 2	mg/tablet
Compound X=	20.0
Microcrystalline cellulose	410.0
Starch	50.0
Sodium starch glycolate	15.0
Magnesium stearate	5.0
	500.0

(iii) Capsule	mg/capsule
Compound X=	10.0
Colloidal silicon dioxide	1.5
Lactose	465.5
Pregelatinized starch	120.0
Magnesium stearate	3.0
	600.0

(iv) Injection 1 (1 mg/ml)	mg/ml
Compound X= (free acid form)	1.0
Dibasic sodium phosphate	12.0
Monobasic sodium phosphate	0.7
Sodium chloride	4.5
1.0 N Sodium hydroxide solution (pH adjustment to 7.0-7.5)	q.s.
Water for injection	q.s. ad 1 mL

(v) Injection 2 (10 mg/ml)	mg/ml
Compound X= (free acid form)	10.0
Monobasic sodium phosphate	0.3
Dibasic sodium phosphate	1.1
Polyethylene glycol 400	200.0
1.0 N Sodium hydroxide solution (pH adjustment to 7.0-7.5)	q.s.
Water for injection	q.s. ad 1 mL

(vi) Aerosol	mg/can
Compound X=	20.0
Oleic acid	10.0
Trichloromonofluoromethane	5,000.0
Dichlorodifluoromethane	10,000.0
Dichlorotetrafluoroethane	5,000.0

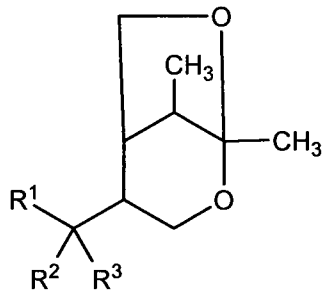
The above formulations may be obtained by conventional procedures well known in the pharmaceutical art.

All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

CLAIMS

What is claimed is:

1. A compound of formula I:



(I)

wherein:

R^1 is $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkyl, or $(C_1\text{-}C_6)$alkenyl, wherein each $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkyl, or $(C_1\text{-}C_6)$alkenyl is optionally substituted with one or more groups independently selected from halo, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkoxycarbonyl, and $(C_1\text{-}C_6)$alkanoyloxy;

R^2 is H and R^3 is $-OR^a$ or $-NR^bR^c$; or R^2 and R^3 taken together are oxo (=O), or $=NR^d$;

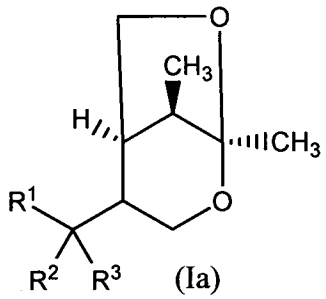
R^a is H or $(C_1\text{-}C_6)$alkanoyl;

R^b and R^c are each independently selected from H, $(C_1\text{-}C_6)$alkyl, and $(C_1\text{-}C_6)$alkanoyl; and

R^d is hydroxy, $(C_1\text{-}C_6)$alkoxy, $-NR^bR^c$, or $(C_1\text{-}C_6)$alkanoyloxy, wherein the $(C_1\text{-}C_6)$alkoxy and $(C_1\text{-}C_6)$alkanoyloxy are each optionally substituted with one or more groups independently selected from halo, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkoxycarbonyl, aryl, and $(C_1\text{-}C_6)$alkanoyloxy, and wherein each aryl is optionally substituted with one or more groups independently selected from halo, nitro, cyano, hydroxy, trifluoromethyl, trifluoromethoxy, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkoxycarbonyl, and $(C_1\text{-}C_6)$alkanoyloxy;

or a salt thereof.

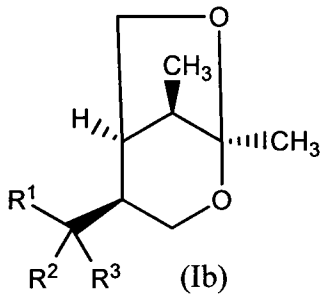
2. The compound of claim 1 which is a compound of formula (Ia):



(Ia)

or a salt thereof.

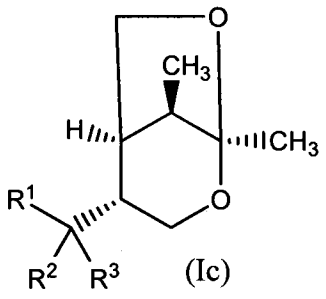
3. The compound of claim 1 which is a compound of formula (Ib):



(Ib)

or a salt thereof.

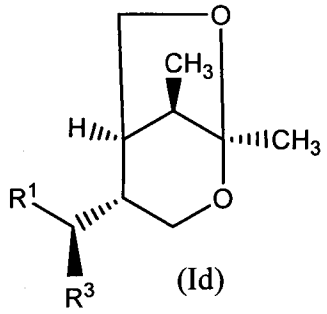
4. The compound of claim 1 which is a compound of formula (Ic):



(Ic)

or a salt thereof.

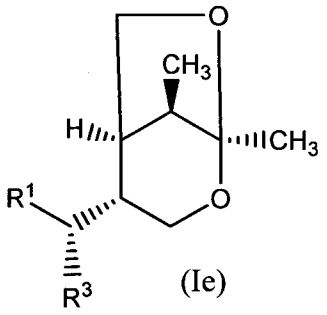
5. The compound of claim 1 which is a compound of formula (Id):



(Id)

or a salt thereof.

6. The compound of claim 1 which is a compound of formula (Ie):



(Ie)

or a salt thereof.

7. The compound of any one of claims 1-6 wherein R^1 is $(C_1\text{-}C_6)$alkyl.

8. The compound of any one of claims 1-6 wherein R^1 is 1-methylpropyl.

9. The compound of any one of claims 1-8 wherein R^2 is H and R^3 is –OH or NH_2.

10. The compound of any one of claims 1-4 and 7-8 wherein R^2 and R^3 taken together are oxo (=O).

11. The compound of any one of claims 1-4 and 7-8 wherein R^2 and R^3 taken together are $=NR^d$.

12. The compound of claim 11 wherein R^d is hydroxy.

13. The compound of claim 11 wherein R^d is $(C_1\text{-}C_6)$alkoxy optionally substituted with one or more groups independently selected from halo, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkoxycarbonyl, aryl, and $(C_1\text{-}C_6)$alkanoyloxy, wherein each aryl is optionally substituted with one or more groups independently selected from halo.

14. The compound of claim 11 wherein R^d is $(C_1\text{-}C_6)$alkanoyloxy, optionally substituted with one or more groups independently selected from halo, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkoxycarbonyl, aryl, and $(C_1\text{-}C_6)$alkanoyloxy, wherein each aryl is optionally substituted with one or more groups independently selected from halo.

15. The compound of claim 11 wherein R^d is benzyloxy, phenylcarbonyloxy, amino, or α-trifluoromethyl-α-methoxybenzyl.

16. A pharmaceutical composition comprising a compound as described in any one of claims 1-15 or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

17. A method for treating cancer in an animal comprising administering a compound as described in any one of claims 1-15 or a pharmaceutically acceptable salt thereof to the animal.

18. The method of claim 17 wherein the cancer is breast cancer.

19. A method for modulating the FGF1R pathway in a cell comprising contacting the call with a compound as described in any one of claims 1-15 or a salt thereof.

20. A method for treating a pathological condition associated with the FGF1R pathway in an animal comprising administering a compound as described in any one of claims 1-15 or a pharmaceutically acceptable salt thereof to the animal.

21. A compound as described in any one of claims 1-15 or a pharmaceutically acceptable salt thereof for use in medical therapy.

22. A compound as described in any one of claims 1-15 or a pharmaceutically acceptable salt thereof for the prophylactic or therapeutic treatment of cancer.

23. A compound as described in any one of claims 1-15 or a pharmaceutically acceptable salt thereof for the prophylactic or therapeutic treatment of a pathological condition associated with the FGF1R pathway.

24. The use of a compound as described in any one of claims 1-15 or a pharmaceutically acceptable salt thereof to prepare a medicament for treating cancer in an animal (e.g. a mammal such as a human).

25. The use of a compound as described in any one of claims 1-10 or a pharmaceutically acceptable salt thereof to prepare a medicament for treating a pathological condition associated with the FGF1R pathway in an animal (e.g. a mammal such as a human).

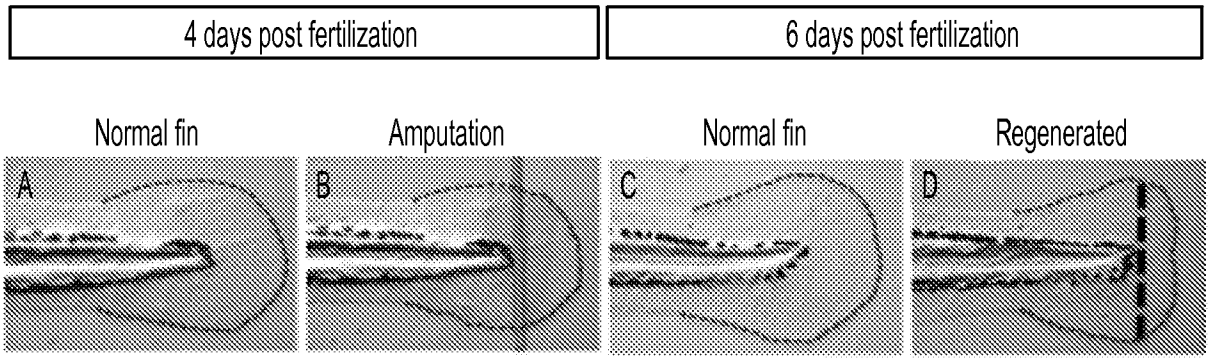

4 days post fertilization
6 days post fertilization
Normal fin
Amputation
Normal fin
Regenerated
A
B
C
D

FIG. 1

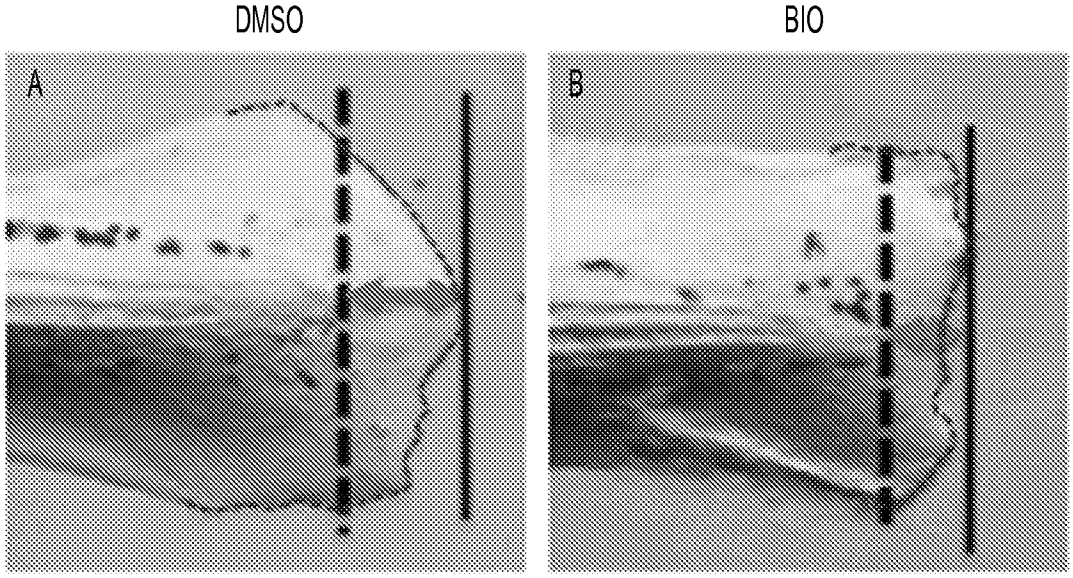


FIG. 2

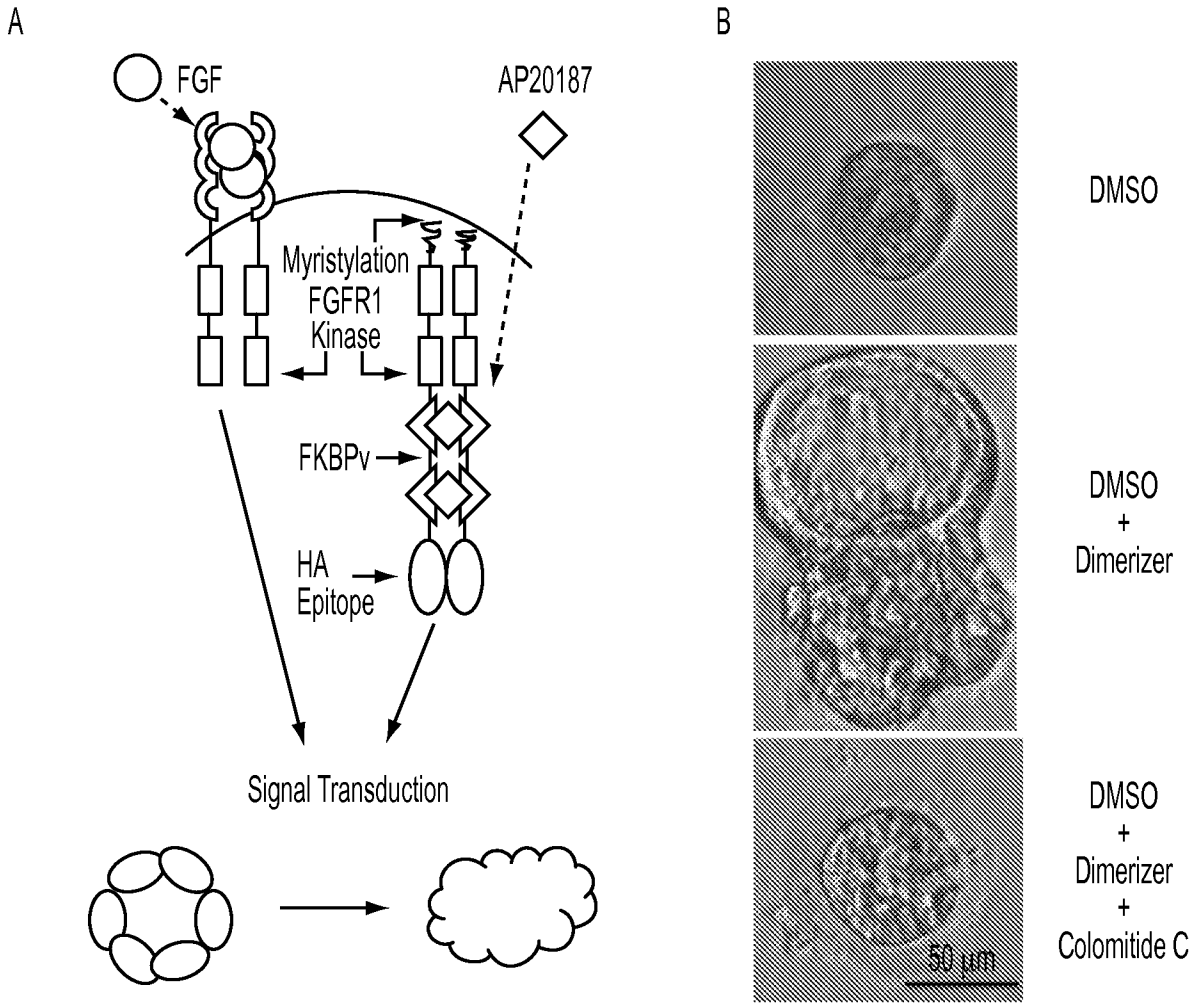

A
FGF
AP20187
Myristylation
FGFR1
Kinase
FKBPv
HA
Epitope
Signal Transduction
B
DMSO
DMSO
+
Dimerizer
DMSO
+
Dimerizer
+
Colomitide C
50 µm

FIG. 3

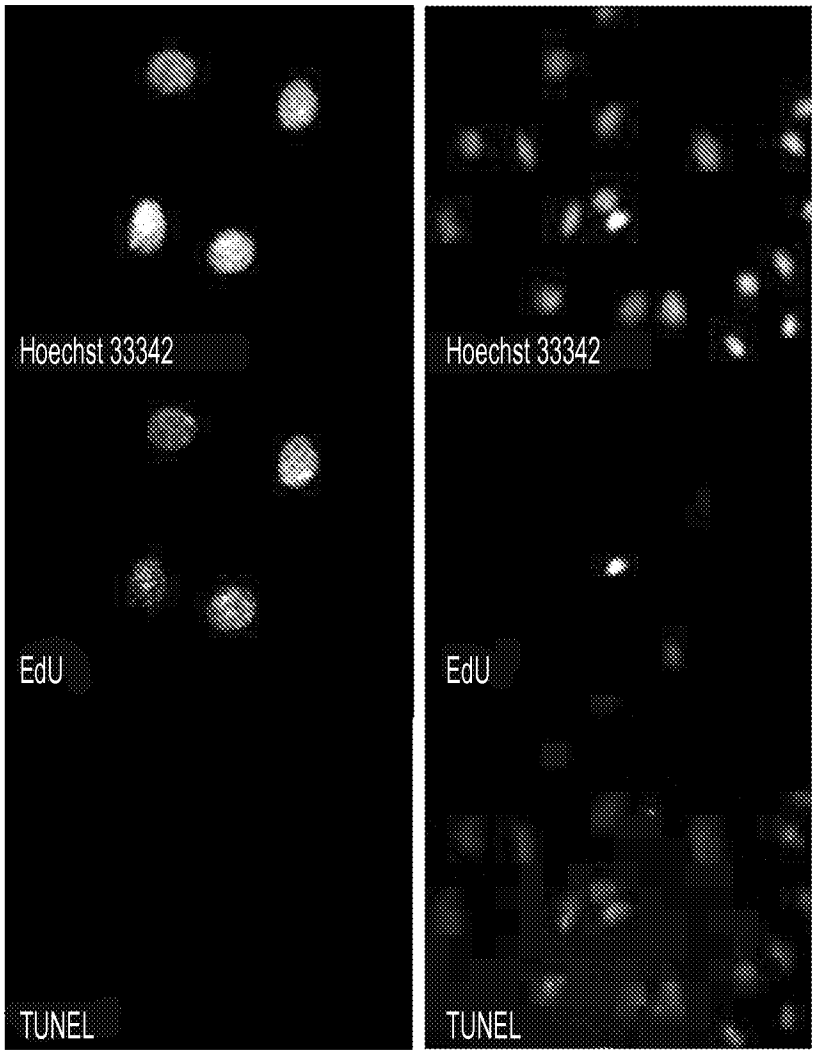

Hoechst 33342
EdU
TUNEL
Hoechst 33342
EdU
TUNEL
Dimerizer
Dimerizer + Colomitide C

FIG. 4

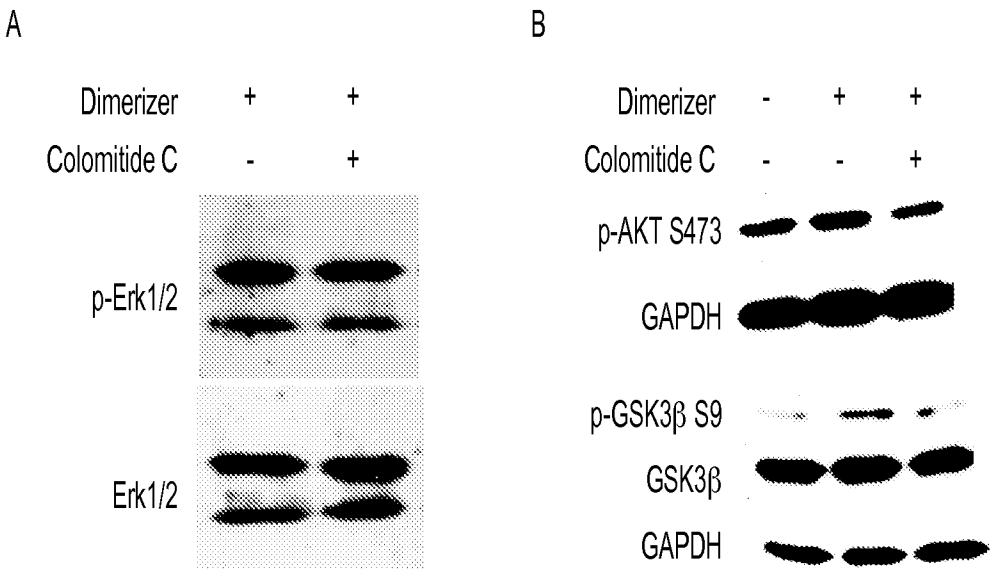

A
Dimerizer + +
Colomitide C - +
p-Erk1/2
Erk1/2
B
Dimerizer - + +
Colomitide C - - +
p-AKT S473
GAPDH
p-GSK3β S9
GSK3β
GAPDH

FIG. 5

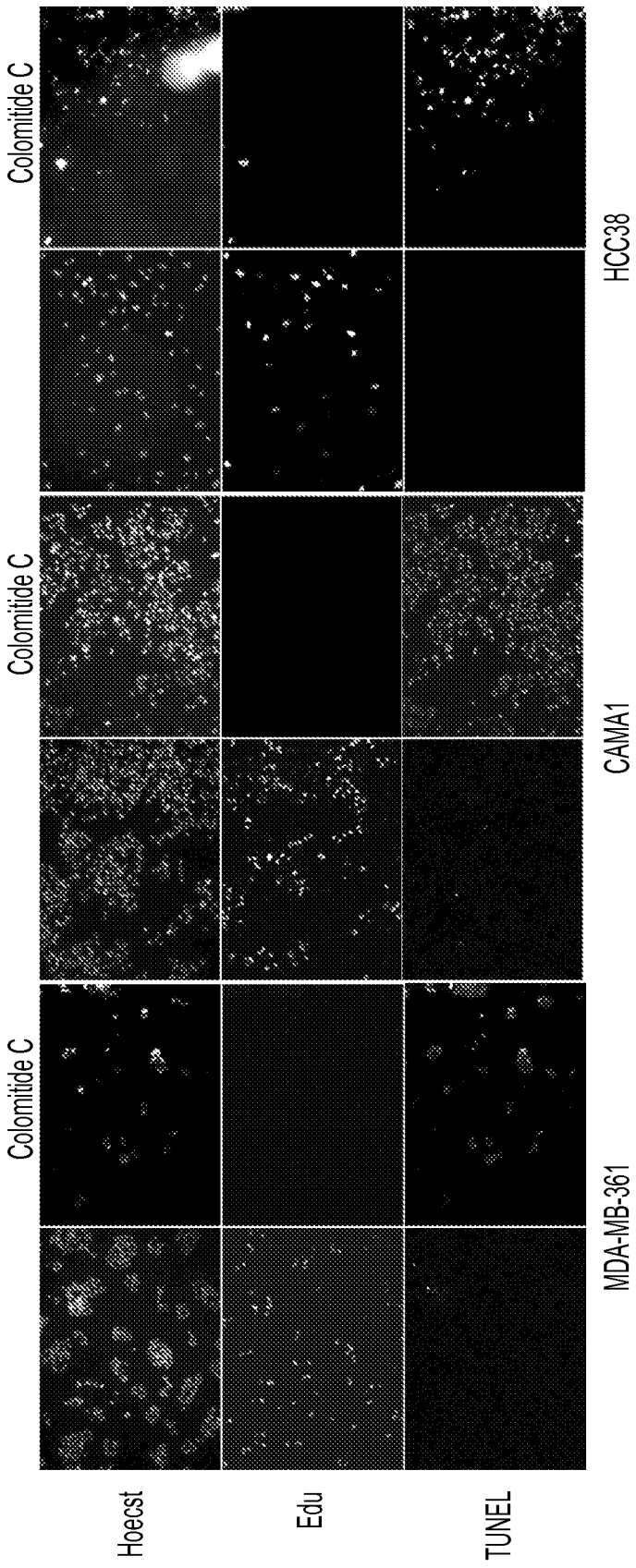

Colomitide C
Colomitide C
Colomitide C
MDA-MB-361
CAMA1
HCC38
Hoecst
Edu
TUNEL

FIG. 6

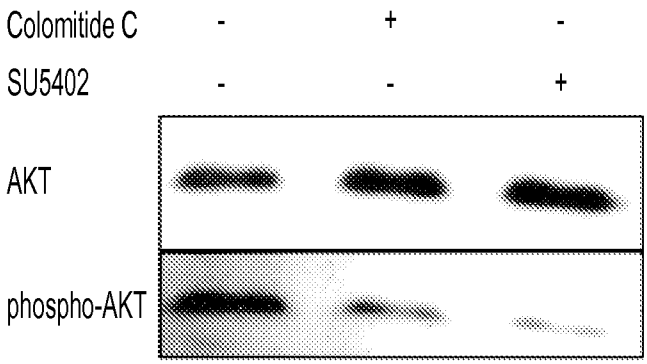


FIG. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/051885

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D493/08 A61K31/7048 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DONG, J-YAN ET AL: "Colomitides A and B: novel Ketals with an Unusual 2,-Dioxybicyclo[3.2.1]octance Ring System from the Aquatic Fungus YMF 1.01029", CHEMISTRY & BIODIVERSITY, vol. 6, no. 8, 2009, pages 1216-1223, XP002712225, abstract, last sentence; table 3; compounds 1,2 -----	1-25

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 2013	20/09/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Schmid, Arnold