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(71) **Applicants**: **CANCER PREVENTION PHARMACEUTICALS, INC.** [US/US]; 1760 E. River Road, Suite 250, Tucson, AZ 85718 (US). **SANOFI WINTHROP INDUSTRIE** [FR/FR]; 20 Avenue Raymond Aron, 92160 Antony (FR).

(72) **Inventors**: **SHANNON, Patrick**; c/o Cancer Prevention Pharmaceuticals, Inc., 1760 E. River Road, Suite 250, Tucson, AZ 85718 (US). **BRAVO GONZALEZ, Roberto, Carlos**; Im Klosteracker 57, 4102 Binningen (CH). **DUCASSOU, Jean**; 6 Allee du Gabot, 33610 Cestas (FR).

(74) **Agent**: **VOGES, Mark, H.**; Parker Highlander PLLC, 1120 S. Capital of Texas Highway, Building One, Suite 200, Austin, TX 78746 (US).

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(54) **Title**: EFLORNITHINE AND SULINDAC, FIXED DOSE COMBINATION FORMULATION

(57) **Abstract**: Provided herein are fixed-dose combination formulations of a pharmaceutically effective amount of eflornithine together with a pharmaceutically effective amount of sulindac. Also provided are methods of use and of methods of manufacture of these formulations.

DESCRIPTION

EFLORNITHINE AND SULINDAC, FIXED DOSE COMBINATION FORMULATION

[0001] The present application claims the priority benefit of United States provisional application No. 62/248,810, filed October 30, 2015, United States provisional application No. 62/358,698, filed July 6, 2016, European application No. 16306429.8, filed October 28, 2016, and European application No. 16306430.6, filed October 28, 2016, the entire contents of each of which are specifically incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0002] The present invention relates generally to the field of cancer biology and medicine. More particularly, it concerns compositions for the prevention and treatment of carcinomas.

2. Description of Related Art

[0003] Cancer cells have the ability to co-opt multiple pathways to fulfill their increased requirement for specific metabolites (Vander Heiden, 2011). The nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin, ibuprofen, piroxicam (Reddy *et al.*, 1990; Singh *et al.*, 1994), indomethacin (Narisawa, 1981), and sulindac (Piazza *et al.*, 1997; Rao *et al.*, 1995), effectively inhibit colon carcinogenesis in the AOM-treated rat model. Sulindac sulfone, a metabolite of the NSAID sulindac, lacks COX-inhibitory activity yet induces apoptosis in tumor cells (Piazza *et al.*, 1995; Piazza *et al.*, 1997b) and inhibits tumor development in several rodent models of carcinogenesis (Thompson *et al.*, 1995; Piazza *et al.*, 1995, 1997a).

[0004] α-Difluoromethylornithine (DFMO) is an enzyme-activated, irreversible inhibitor of ornithine decarboxylase (ODC) and causes depletion in the intracellular concentrations of putrescine and its derivative, spermidine (Pegg, 1988). In experimental animal models, DFMO is a potent inhibitor of carcinogenesis that is especially active in preventing carcinogen-induced epithelial cancers of many organs, including those of the colon (Weeks *et al.*, 1982; Thompson *et al.*, 1985; Nowels *et al.*, 1986; Nigro *et al.*, 1987).

[0005] A major impediment to the translation of cancer chemoprevention research into clinical practice has been marginal agent efficacy and toxicities that exceed benefit (Psaty and Potter, 2006; Lippman, 2006). For example, the demonstrated marked efficacy of polyamine-inhibitory combination of long-term daily oral *D,L*-α-difluoromethylornithine (DFMO, eflornithine) and sulindac among colorectal adenoma (CRA) patients has been demonstrated (Meyskens *et al.*, 2008), however, treatment was associated with modest, subclinical ototoxicity (McLaren *et al.*, 2008), and a greater number of cardiovascular events among patients with high baseline cardiovascular risk (Zell *et al.*, 2009).

[0006] The convenience of co-administering two or more active pharmaceutical ingredients in a unit dosage form, as opposed to the administration of a number of separate doses of two or more pharmaceuticals at regular intervals, has been recognized in the pharmaceutical arts and is described in U.S. Patent Nos. 6,428,809 and 6,702,683. Potential advantages to the patient and clinician include (1) minimization or elimination of local and/or systemic side effects; (2) more effective treatment of co-morbid conditions; (3) improved polypharmacy; and (4) better patient compliance with overall disease management, which in turn may lead to reduced costs due to fewer trips to the physician, reduced hospitalization, and improved patient well-being. Fixed dose combination products, with two or more formulations combined or co-formulated in a single dosage form, may be useful in multiple drug regimens where improved clinical effectiveness, enhanced patient adherence and simplified dosing are desired. However, pharmaceutical drug product development of solid oral dosage forms is complicated at both the research and development level and at the commercial manufacturing level even for single active pharmaceutical ingredient (API) formulation. For more-than-one API, additional complicating factors are expected, including (1) drug-drug interaction, (2) drug-excipient interaction, (3) simultaneous release profiles, (4) differential release profiles, and (5) blend uniformity of each drug component. In view of these hurdles, developing fixed-dose combinations with the same or similar release profiles as the single entity drug products typically represents a significant challenge. Fixed dose combinations of eflornithine and sulindac that overcome some or all of these challenges would have a significant potential impact for the effective treatment and/or prevention of a wide range of diseases or disorders, including familial adenoma polyposis (FAP).

SUMMARY OF THE INVENTION

[0007] In one aspect, the present invention provides compositions comprising a fixed dose combination of a pharmaceutically effective amount of eflornithine and a pharmaceutically effective amount of a nonsteroidal anti-inflammatory drug (NSAID) or a metabolite thereof. In some embodiments, the fixed dose combination is a pharmaceutically effective amount of eflornithine and a pharmaceutically effective amount of sulindac.

[0008] In some embodiments, the eflornithine is eflornithine hydrochloride monohydrate. In some embodiments, the eflornithine is eflornithine hydrochloride monohydrate racemate. In some embodiments, the eflornithine hydrochloride monohydrate is a racemic mixture of its two enantiomers. In some embodiments, the eflornithine hydrochloride monohydrate is a substantially optically pure preparation. In some embodiments, the eflornithine hydrochloride monohydrate is L-eflornithine hydrochloride monohydrate or D-eflornithine hydrochloride monohydrate. In some embodiments, the eflornithine is anhydrous free base eflornithine.

[0009] In some embodiments, the eflornithine is present in an amount of about 10 to about 1000 mg. In some embodiments, the eflornithine is present in an amount of about 250 to about 500 mg. In some embodiments, the eflornithine is present in an amount of about 300 to about 450 mg. In some embodiments, the eflornithine is present in an amount of about 350 to about 400 mg. In some embodiments, the eflornithine is present in an amount of about 35 to about 60 weight percent. In some embodiments, the eflornithine is present in an amount of about 40 to about 55 weight percent. In some embodiments, the eflornithine is present in an amount of about 50 to about 55 weight percent. In some embodiments, the eflornithine is present in an amount of about 52 to about 54 weight percent. In some embodiments, the amount of eflornithine hydrochloride monohydrate racemate is from 52 to 54 weight percent. In some embodiments, the eflornithine is present in an amount of about 375 mg. In some embodiments, the amount of eflornithine hydrochloride monohydrate racemate is 375 mg.

[0010] In some embodiments, the sulindac is present in an amount from about 10 to about 1500 mg. In some embodiments, the sulindac is present in an amount of about 50 to about 100 mg. In some embodiments, the sulindac is present in an amount of about 70 to about 80 mg. In some embodiments, the sulindac is present in an amount of about 75 mg. In some embodiments, the amount of sulindac is 75 mg. In some embodiments, the sulindac is present

in an amount of about 5 to about 20 weight percent. In some embodiments, the sulindac is present in an amount of about 8 to about 15 weight percent. In some embodiments, the sulindac is present in an amount of about 10 to about 12 weight percent. In some embodiments, the amount of sulindac is from 10 to 11 weight percent.

[0011] In some embodiments, the eflornithine is present in an amount of about 375 mg and the sulindac is present in an amount of about 75 mg.

[0012] In some embodiments, the formulation further comprises an excipient. In some embodiments, the excipient is starch, colloidal silicon dioxide, or silicified microcrystalline cellulose. In some embodiments, the excipient is colloidal silicon dioxide. In some embodiments, the formulation further comprises a second excipient. In some embodiments, the second excipient is silicified microcrystalline cellulose.

[0013] In some embodiments, the formulation further comprises a lubricant. In some embodiments, the lubricant is magnesium stearate, calcium stearate, sodium stearate, glyceryl monostearate, aluminum stearate, polyethylene glycol, boric acid or sodium benzoate. In some embodiments, the lubricant is magnesium stearate. In some embodiments, magnesium stearate is present in an amount of about 0.25 to about 2 weight percent. In some embodiments, the amount of magnesium stearate is from about 0.75 to about 2 weight percent. In some embodiments, the amount of magnesium stearate is from about 1 to about 1.5 weight percent. In some embodiments, the amount of magnesium stearate is about 1.1 weight percent. In some embodiments, magnesium stearate is present in an amount of about 1.5 weight percent.

[0014] In some embodiments, the compositions are in the form of a capsule, tablet, mini tablets, granules, pellets, solution, gel, cream, foam or patch. In some embodiments, the composition is in the form of a tablet, for example, a monolayer tablet.

[0015] In some embodiments, the weight of the tablet is from about 10 mg to about 2,500 mg. In some embodiments, the weight of the tablet is from about 250 mg to about 1,500 mg. In some embodiments, the weight of the tablet is from about 650 mg to about 1,000 mg. In some embodiments, the weight of the tablet is from about 675 mg to about 725 mg. In some embodiments, the weight of the tablet is about 700 mg.

[0016] In some embodiments, the weight of the capsule, mini tablet, granules, or pellets is from about 10 mg to about 2,500 mg. In some embodiments, the weight of the capsule, mini

tablet, granules, or pellets is from about 250 mg to about 1,500 mg. In some embodiments, the weight of the capsule, mini tablet, granules, or pellets is from about 650 mg to about 1,000 mg. In some embodiments, the weight of the capsule, mini tablet, granules, or pellets is from about 675 mg to about 725 mg. In some embodiments, the weight of the capsule, mini tablet, granules, or pellets is about 700 mg.

[0017] In some embodiments, the tablet further comprises a coating. In some embodiments, the coating is a modified release coating or an enteric coating. In some embodiments, the coating is a pH-responsive coating. In some embodiments, the coating comprises cellulose acetate phthalate (CAP), cellulose acetate trimelletate (CAT), poly (vinyl acetate) phthalate (PVAP), hydroxypropylmethylcellulose phthalate (HP), poly(methacrylate ethylacrylate) (1:1) copolymer (MA-EA), poly(methacrylate methylmethacrylate) (1:1) copolymer (MA MMA), poly(methacrylate methylmethacrylate) (1:2) copolymer, or hydroxypropylmethylcellulose acetate succinate (HPMCAS). In some embodiments, the coating masks the taste of eflornithine. In some embodiments, the coating comprises hydroxypropyl methylcellulose, titanium dioxide, polyethylene glycol, and iron oxide yellow.

[0018] In some embodiments, the amount of coating is from about 1 to about 5 weight percent. In some embodiments, the amount of coating is from about 2 to about 4 weight percent. In some embodiments, the amount of coating is about 3 weight percent. In some embodiments, the amount of coating is from about 5 mg to about 30 mg. In some embodiments, the amount of coating is from about 15 mg to about 25 mg. In some embodiments, the amount of coating is about 21 mg.

[0019] In some embodiments, the weight of the tablet comprising a coating is from about 675 mg to about 750 mg. In some embodiments, the weight of the tablet comprising a coating is from about 700 mg to about 725 mg. In some embodiments, the weight of the tablet comprising a coating is about 721 mg.

[0020] In one aspect, there is provided a method of preventing and/or treating a disease or condition in a patient in need thereof, comprising administering to the patient the fixed dose combination of a pharmaceutically effective amount of eflornithine and a pharmaceutically effective amount of sulindac provided herein.

[0021] In some embodiments, the method further comprises administering to the patient a second composition comprising the fixed dose combination of a pharmaceutically

effective amount of eflornithine and a pharmaceutically effective amount of sulindac provided herein. In some embodiments, the first and the second compositions comprise the same fixed dose combinations. In some embodiments, the first and the second administration occurs simultaneously. In some embodiments, the second administration follows the first administration by an interval of 1 second to 1 hour. In some embodiments, the first and the second compositions are both formulated as tablets and contain the same amounts of eflornithine and sulindac.

[0022] In some embodiments, the disease is cancer. In some embodiments, the cancer is colon cancer, breast cancer, pancreatic cancer, brain cancer, lung cancer, stomach cancer, a blood cancer, skin cancer, testicular cancer, prostate cancer, ovarian cancer, liver cancer, or esophageal cancer. In some embodiments, the colon cancer is familial adenomatous polyposis. In some embodiments, the cancer is a neuroendocrine tumor. In some embodiments, the neuroendocrine tumor is neuroblastoma.

[0023] In some embodiments, the condition is a skin condition. In some embodiments, the skin condition is facial hirsutism.

[0024] In some embodiments, the composition is administered orally, intraarterially, intravenously, or topically. In some embodiments, the composition is administered orally.

[0025] In some embodiments, the composition is administered orally. In some embodiments, the composition is administered every 12 hours. In some embodiments, the composition is administered every 24 hours. In some embodiments, the composition is administered at least a second time.

[0026] In another aspect, there is provided a method of producing a tablet comprising about 375 mg eflornithine hydrochloride and about 75 mg of sulindac comprising: (a) pre-mixing sulindac and an excipient to form a first mixture; (b) mixing the first mixture with a second mixture comprising eflornithine and an excipient to form a blend; (c) screening the blend to form a granulated blend; (d) adding a lubricant to the granulated blend to obtain a final blend; and (e) applying a compression force to the final blend to form a tablet. In some embodiments, the method further comprises mixing the granulated blend prior to step (d) and mixing the final blend prior to step (e).

[0027] In some embodiments, there are two excipients in the first mixture, wherein the first excipient is colloidal silicon dioxide, and the second excipient is silicified microcrystalline cellulose. In some embodiments, the excipient of the second mixture is silicified microcrystalline cellulose.

[0028] In some embodiments, the pre-mixing is performed in a polyethylene-coated container. In some embodiments, the mixing is performed in a diffusion blender.

[0029] In some embodiments, the lubricant is magnesium stearate. In some embodiments, the magnesium stearate is sieved through a screen prior to step (d). In some embodiments, the screen is a 500 μm screen.

[0030] In some embodiments, screening comprises applying the blend to a rotative calibrator. In some embodiments, the rotative calibrator comprises a 1.0 mm screen.

[0031] In some embodiments, the method further comprises a pre-compression step after step (d) and prior to step (e), wherein the blend is compressed with a force lower than the force of step (e) to form a pre-compressed blend, further wherein the compression force of step (e) then acts on the pre-compressed blend to form the tablet. In some embodiments, the pre-compression step prevents tablet capping. In some embodiments, a compression force of the pre-compression step is applied at about 5 to about 15 percent of the compression force applied in step (e). In some embodiments, the compression force of the pre-compression step is from 2.5 to 3.5 kN. In some embodiments, the compression force of the pre-compression step is about 3 kN. In some embodiments, the compression force of step (e) is from 20 to 35 kN. In some embodiments, the compression force of step (e) is about 25 kN.

[0032] In some embodiments, the method further comprises coating the tablet. In some embodiments, the coating comprises hydroxypropyl methylcellulose, titanium dioxide, polyethylene glycol, and iron oxide yellow.

[0033] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0035] FIG. 1: Stability analysis of prototype Lot 7107/04 of 700 mg tablets of eflornithine HCl monohydrate (375 mg) and sulindac (75 mg). Tablets have a 3% w/w coating. Samples were analyzed at time zero (T0) and at 6 months (T6) using a validated Karl Fischer titration method for determination of water content. Samples were stored in HDPE bottles with and without caps in verified stability chambers. Values represent the percentage of water in each tablet at the specified conditions.

[0036] FIGS. 2A-2B: Results of dissolution analysis of coated tablet Lots 7107/04 and 6A001. Reference tablets of 250 mg eflornithine HCl monohydrate and commercial 150 mg sulindac are included for comparison. Co-formulated tablets contain 375 mg of eflornithine HCl monohydrate and 75 mg of sulindac with a 3% w/w coating.

[0037] FIG. 3: Simplified scheme depicting a manufacturing process for tablets containing both eflornithine HCl monohydrate and sulindac.

[0038] FIGS. 4A-4C: (A) A typical HPLC chromatograph of eflornithine HCl monohydrate and sulindac co-formulated tablet demonstrating the ability to measure selected impurities. **(B-C)** X-ray powder diffraction (XRPD) patterns of eflornithine HCl monohydrate and sulindac active ingredients mixed with tablet excipients at time zero, 2 weeks, and 4 weeks. The lack of change supports both excipient compatibility and polymorph stability.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0039] In several aspects, compositions are provided for a fixed dose combination (FDC) of eflornithine and sulindac. Methods are also provided for the manufacture of the fixed dose combinations of the present invention which overcome problems associated with current methods. The methods of manufacture have been designed to solve problems including drug-drug interactions, drug-excipient interactions, and blend uniformity of each drug component. Accordingly, the fixed dose combination of the present invention may be used to minimize

local and/or systemic side effects, provide more effective treatments, improve polypharmacy, and provide better patient compliance.

I. Familial Adenomatous Polyposis

[0040] Excess polyamine formation has long been implicated in epithelial carcinogenesis, particularly colorectal carcinogenesis. Polyamines are small ubiquitous molecules involved in various processes, including, for example, transcription, RNA stabilization, and ion channel gating (Wallace, 2000). Ornithine decarboxylase (ODC), the first enzyme in polyamine synthesis, is essential for normal development and tissue repair in mammals but is down-regulated in most adult tissues (Gerner and Meyskens, 2004). Multiple abnormalities in the control of polyamine metabolism and transport result in increased polyamine levels that can promote tumorigenesis in several tissues (Thomas and Thomas, 2003).

[0041] Familial adenomatous polyposis (FAP) is a syndrome associated with high risk of colon and other cancers. FAP is caused by mutations in the adenomatous polyposis coli (*APC*) tumor suppressor gene, and APC signaling has been shown to regulate ODC expression in both human cells (Fultz and Gerner, 2002) and in a mouse model of FAP (Erdman *et al.*, 1999). Polyamine metabolism is up-regulated in intestinal epithelial tissues of humans with (Giardiello *et al.*, 1997) FAP.

[0042] Wild type *APC* expression leads to decreased expression of ODC, while mutant APC leads to increased expression of ODC. The mechanism of APC-dependent regulation of ODC involves E-box transcription factors, including the transcriptional activator *c-MYC* and the transcriptional repressor *MAD1* (Fultz and Gerner, 2002; Martinez *et al.*, 2003). *c-MYC* was shown by others to regulate ODC transcription (Bellofernandez *et al.*, 1993). Several genes involved in polyamine metabolism are essential genes for optimal growth in most organisms, and are down-regulated in non-proliferating and/or adult cells and tissues (Gerner and Meyskens, 2004). The polyamines influence specific cellular phenotypes, in part, by affecting patterns of gene expression, as reviewed elsewhere (Childs *et al.*, 2003).

[0043] Familial Adenomatous Polyposis (FAP), an inherited polyposis syndrome, is the result of germ-line mutation of the adenomatous polyposis coli (APC) tumor suppressor gene (Su *et al.*, 1992). This autosomal-dominant condition with variable expression is associated with the development of hundreds of colonic adenomas, which uniformly progress

to adenocarcinoma by forty years of age, two decades earlier than the mean age diagnosis for sporadic colon cancer (Bussey, 1990). In prior studies of pre-symptomatic individuals with FAP, increased levels of the polyamines spermidine and spermine, and their diamine precursor putrescine, have been detected in normal-appearing colorectal biopsies when compared to normal family member controls (Giardiello *et al.*, 1997). The activity of ornithine decarboxylase (ODC), the first and rate-limiting enzyme in mammalian polyamine synthesis, also is elevated in apparently normal colonic mucosal biopsies from FAP patients (Giardiello *et al.*, 1997; Luk and Baylin, 1984). These findings are of interest as the polyamines are necessary for optimal cell proliferation (Pegg, 1986). Further, suppression of ODC activity, using the enzyme-activated irreversible inhibitor DFMO, inhibits colon carcinogenesis in carcinogen-treated rodents (Kingsnorth *et al.*, 1983; Tempero *et al.*, 1989).

[0044] The Min (multiple intestinal neoplasia) mouse, which shares a mutated *APC/apc* genotype with FAP, serves as a useful experimental animal model for human FAP patients (Lipkin, 1997). The Min mouse can develop greater than 100 gastrointestinal adenomas/adenocarcinomas throughout the gastrointestinal tract by 120 days of life leading to GI bleeding, obstruction and death. A combination therapy of DFMO and sulindac was shown to be effective in reducing adenomas in these mice. See U.S. Patent No. 6,258,845 and Gerner and Meyskens, 2004, which are incorporated herein by reference.

II. Eflornithine

[0045] The term "eflornithine" when used by itself and free of context refers to 2,5-diamino-2-(difluoromethyl)pentanoic acid in any of its forms, including non-salt and salt forms (*e.g.*, eflornithine HCl), anhydrous and hydrate forms of non-salt and salt forms (*e.g.*, eflornithine hydrochloride monohydrate), solvates of non-salt and salts forms, its enantiomers (*R* and *S* forms, which may also by identified as *d* and *l* forms), and mixtures of these enantiomers (*e.g.*, racemic mixture). By "substantially optically pure preparation" is meant a preparation of a first enantiomer which contains about 5% wt. or less of the opposite enantiomer. Specific forms of eflornithine include eflornithine hydrochloride monohydrate (*i.e.*, CAS ID: 96020-91-6; MW: 236.65), eflornithine hydrochloride (*i.e.*, CAS ID: 68278-23-9; MW: 218.63), and anhydrous free base eflornithine (*i.e.*, CAS ID: 70052-12-9; MW: 182.17). Where necessary, the specific form of eflornithine has been further specified. In some embodiments, the eflornithine of the present disclosure is eflornithine hydrochloride monohydrate (*i.e.*, CAS ID: 96020-91-6). The terms "eflornithine" and "DFMO" are used

interchangeably herein. DFMO is an abbreviation for difluoromethylornithine. Other synonyms of eflornithine and DFMO include: α-difluoromethylornithine, 2-(difluoromethyl)-DL-ornithine, 2-(difluoromethyl)-*dl*-ornithine, 2-(Difluoromethyl)ornithine, DL-α-difluoromethylornithine, *N*-Difluoromethylornithine, αδ-diamino-α-(difluoromethyl)valeric acid, and 2,5-diamino-2-(difluoromethyl)pentanoic acid.

[0046] Eflornithine is an enzyme-activated irreversible inhibitor of ornithine decarboxylase (ODC), the rate-limiting enzyme of the polyamine biosynthetic pathway. As a result of this inhibition of polyamine synthesis, the compound is effective in preventing cancer formation in many organ systems, inhibiting cancer growth, and reducing tumor size. It also has synergistic action with other antineoplastic agents.

[0047] Eflornithine has been shown to decrease APC-dependent intestinal tumorigenesis in mice (Erdman *et al.*, 1999). Oral eflornithine administered daily to humans inhibits ODC enzyme activity and polyamine contents in a number of epithelial tissues (Love *et al.*, 1993; Gerner *et al.*, 1994; Meyskens *et al.*, 1994; Meyskens *et al.*, 1998; Simoneau *et al.*, 2001; Simoneau *et al.*, 2008). Eflornithine in combination with the non-steroidal anti-inflammatory drug (NSAID) sulindac, has been reported to markedly lower the adenoma recurrence rate among individuals with colonic adenomas when compared to placebos in a randomized clinical trial (Meyskens *et al.*, 2008).

[0048] Eflornithine was originally synthesized by Centre de Recherche Merrell, Strasbourg. Current U.S. Food and Drug Administration (FDA) approvals include:

- African sleeping sickness. High dose systemic IV dosage form—not marketed (Sanofi/WHO)
- Hirsutis (androgen-induced excess hair growth) topical dosage form

While no oral formulations of eflornithine have yet been approved by the FDA, topical and injectable forms have been approved. Vaniqa® is a cream, which contains 15% w/w eflornithine hydrochloride monohydrate, corresponding to 11.5% w/w anhydrous eflornithine (EU), respectively 13.9% w/w anhydrous eflornithine hydrochloride (U.S.), in a cream for topical administration. Ornidyl® is an eflornithine HCl solution suitable for injection or infusion. It is supplied in the strength of 200 mg eflornithine hydrochloride monohydrate per ml (20 g/100 mL).

[0049] Eflornithine and its use in the treatment of benign prostatic hypertrophy are described in U.S. Patents 4,413,141, and 4,330,559. The '141 Patent describes eflornithine as being a powerful inhibitor of ODC, both *in vitro* and *in vivo*. Administration of eflornithine is reported to cause a decrease in putrescine and spermidine concentrations in cells in which these polyamines are normally actively produced. Additionally, eflornithine has been shown to be capable of slowing neoplastic cell proliferation when tested in standard tumor models. The '559 Patent describes the use of eflornithine and eflornithine derivatives for the treatment of benign prostatic hypertrophy. Benign prostatic hypertrophy, like many disease states characterized by rapid cell proliferation, is accompanied by abnormal elevation of polyamine concentrations.

[0050] Eflornithine can potentially be given continuously with significant anti-tumor effects. This drug is relatively non-toxic at low doses of 0.4 g/m^2/day to humans while producing inhibition of putrescine synthesis in tumors. Studies in a rat-tumor model demonstrate that eflornithine infusion can produce a 90% decrease in tumor putrescine levels without suppressing peripheral platelet counts.

[0051] Side effects observed with eflornithine include effects on hearing at high doses of 4 g/m^2/day that resolve when it is discontinued. These effects on hearing are not observed at lower doses of 0.4 g/m^2/day when administered for up to one year (Meyskens *et al.*, 1994). In addition, a few cases of dizziness/vertigo are seen that resolve when the drug is stopped. Thrombocytopenia has been reported predominantly in studies using high "therapeutic" doses of eflornithine (>1.0 g/m^2/day) and primarily in cancer patients who had previously undergone chemotherapy or patients with compromised bone marrow. Although the toxicity associated with eflornithine therapy is not, in general, as severe as other types of chemotherapy, in limited clinical trials it has been found to promote a dose-related thrombocytopenia. Moreover, studies in rats have shown that continuous infusion of eflornithine for 12 days significantly reduces platelet counts compared with controls. Other investigations have made similar observations in which thrombocytopenia is the major toxicity of continuous intravenous eflornithine therapy. These findings suggest that eflornithine may significantly inhibit ODC activity of the bone marrow precursors of megakaryocytes. Eflornithine may inhibit proliferative repair processes, such as epithelial wound healing.

[0052] A phase III clinical trial assessed the recurrence of adenomatous polyps after treatment for 36 months with DFMO plus sulindac or matched placebos. Temporary hearing

loss is a known toxicity of treatment with DFMO, thus a comprehensive approach was developed to analyze serial air conduction audiograms. The generalized estimating equation method estimated the mean difference between treatment arms with regard to change in air conduction pure tone thresholds while accounting for within-subject correlation due to repeated measurements at frequencies. Based on 290 subjects, there was an average difference of 0.50 dB between subjects treated with DFMO plus sulindac compared with those treated with placebo (95% confidence interval, -0.64 to 1.63 dB; P = 0.39), adjusted for baseline values, age, and frequencies. There is a <2 dB difference in mean threshold for patients treated with DFMO plus sulindac compared with those treated with placebo. The results of this study are discussed in greater detail in McLaren *et al.*, 2008, which is incorporated herein by reference in its entirety.

III. NSAIDs

[0053] NSAIDs are anti-inflammatory agents that are not steroids. In addition to anti-inflammatory effects, they are also reported to have analgesic, antipyretic, and platelet-inhibitory effects. They are used, for example, in the treatment of chronic arthritic conditions and certain soft tissue disorders associated with pain and inflammation. They have been reported to act by blocking the synthesis of prostaglandins by inhibiting cyclooxygenase, which converts arachidonic acid to cyclic endoperoxides, precursors of prostaglandins. Inhibition of prostaglandin synthesis accounts for their analgesic, antipyretic, and platelet-inhibitory actions; other mechanisms may contribute to their anti-inflammatory effects. Certain NSAIDs also may inhibit lipoxygenase enzymes or phospholipase C or may modulate T-cell function. See AMA Drug Evaluations Annual, 1814-5, 1994.

[0054] The nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin, ibuprofen, piroxicam (Reddy *et al.*, 1990; Singh *et al.*, 1994), indomethacin (Narisawa, 1981), and sulindac (Piazza *et al.*, 1997; Rao *et al.*, 1995), effectively inhibit colon carcinogenesis in the AOM-treated rat model. NSAIDs also inhibit the development of tumors harboring an activated Ki-ras (Singh and Reddy, 1995). NSAIDs appear to inhibit carcinogenesis via the induction of apoptosis in tumor cells (Bedi *et al.*, 1995; Lupulescu, 1996; Piazza *et al.*, 1995; Piazza *et al.*, 1997b). A number of studies suggest that the chemopreventive properties of the NSAIDs, including the induction of apoptosis, are a function of their ability to inhibit prostaglandin synthesis (reviewed in DuBois *et al.*, 1996; Lupulescu, 1996; Vane and Botting, 1997). Studies, however, indicate that NSAIDs may act through both prostaglandin-dependent

and -independent mechanisms (Alberts *et al.*, 1995; Piazza *et al.*, 1997a; Thompson *et al.*, 1995; Hanif, 1996). Sulindac sulfone, a metabolite of the NSAID sulindac, lacks COX-inhibitory activity yet induces apoptosis in tumor cells (Piazza *et al.*, 1995; Piazza *et al.*, 1997b) and inhibits tumor development in several rodent models of carcinogenesis (Thompson *et al.*, 1995; Piazza *et al.*, 1995, 1997a).

[0055] Several NSAIDs have been examined for their effects in human clinical trials. A phase IIa trial (one month) of ibuprofen was completed and even at the dose of 300 mg/day, a significant decrease in prostoglandin E_2 (PGE_2) levels in flat mucosa was seen. A dose of 300 mg of ibuprofen is very low (therapeutic doses range from 1200-3000 mg/day or more), and toxicity is unlikely to be seen, even over the long-term. However, in animal chemoprevention models, ibuprofen is less effective than other NSAIDs.

A. Aspirin

[0056] Aspirin, also known as acetylsalicylic acid, is a salicylate drug, often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever, and as an anti-inflammatory medication. Aspirin was first isolated by Felix Hoffmann, a chemist with the German company Bayer in 1897. Salicylic acid, the main metabolite of aspirin, is an integral part of human and animal metabolism. While in humans much of it is attributable to diet, a substantial part is synthesized endogenously. Today, aspirin is one of the most widely used medications in the world, with an estimated 40,000 tons of it being consumed each year. In countries where aspirin is a registered trademark owned by Bayer, the generic term is acetylsalicylic acid (ASA).

[0057] Aspirin also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecules together to create a patch over damaged walls of blood vessels. Because the platelet patch can become too large and also block blood flow, locally and downstream, aspirin is also used long-term, at low doses, to help prevent heart attacks, strokes, and blood clot formation in people at high risk of developing blood clots. It has also been established that low doses of aspirin may be given immediately after a heart attack to reduce the risk of another heart attack or of the death of cardiac tissue. Aspirin may be effective at preventing certain types of cancer, particularly colorectal cancer.

[0058] Undesirable side effects of taking aspirin orally include gastrointestinal ulcers, stomach bleeding, and tinnitus, especially in higher doses. In children and adolescents, aspirin is no longer indicated to control flu-like symptoms or the symptoms of chickenpox or other viral illnesses, because of the risk of Reye's syndrome.

[0059] Aspirin is part of a group of medications called nonsteroidal anti-inflammatory drugs (NSAIDs), but differs from most other NSAIDS in the mechanism of action. Though aspirin, and others in its group called the salicylates, have similar effects (antipyretic, anti-inflammatory, analgesic) to the other NSAIDs and inhibit the same enzyme cyclooxygenase, aspirin (but not the other salicylates) does so in an irreversible manner and, unlike others, affects more the COX-1 variant than the COX-2 variant of the enzyme.

B. Sulindac and Its Major Metabolites, Sulidac Sulfone and Sulindac Sulfide

[0060] Sulindac is a nonsteroidal, anti-inflammatory indene derivative with the following chemical designation; (Z)-5-fluoro-2-methyl-1-((4-(methylsulfinyl)phenyl)methylene)-1H-indene-3-acetic acid (Physician's Desk Reference, 1999). Without being bound by theory, the sulfinyl moiety is converted *in vivo* by reversible reduction to a sulfide metabolite and by irreversible oxidation to a sulfone metabolite (exisulind). See U.S. Patent 6,258,845, which is incorporated herein by reference. Sulindac, which also inhibits Ki-ras activation, is metabolized to two different molecules, which differ in their ability to inhibit COX, yet both are able to exert chemopreventive effects via the induction of apoptosis. Sulindac sulfone lacks COX-inhibitory activity, and most likely facilitates the induction of apoptosis in a manner independent of prostaglandin synthesis. Available evidence indicates that the sulfide derivative is at least one of the biologically active compounds. Based on this, sulindac may be considered a prodrug.

[0061] Sulindac (Clinoril®) is available, for example, as 150 mg and 200 mg tablets. The most common dosage for adults is 150 to 200 mg twice a day, with a maximal daily dose of 400 mg. After oral administration, about 90% of the drug is absorbed. Peak plasma levels are achieved in about 2 hours in fasting patients and 3 to 4 hours when administered with food. The mean half-life of sulindac is 7.8 hours: the mean half-life of the sulfide metabolite is 16.4 hours. U.S. Pat. Nos. 3,647,858 and 3,654,349 cover preparations of sulindac; both patents are incorporated by reference herein in their entireties.

[0062] Sulindac is indicated for the acute and long-term relief of signs and symptoms of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, acute gout, and acute painful shoulder. The analgesic and anti-inflammatory effects exerted by sulindac (400 mg per day) are comparable to those achieved by aspirin (4 g per day), ibuprofen (1200 mg per day), indometacin (125 mg per day), and phenylbutazone (400 to 600 mg per day). Side effects of sulindac include mild gastrointestinal effects in nearly 20% of patients, with abdominal pain and nausea being the most frequent complaints. CNS side effects are seen in up to 10% of patients, with drowsiness, headache, and nervousness being those most frequently reported. Skin rash and pruritus occur in 5% of patients. Chronic treatment with sulindac can lead to serious gastrointestinal toxicity such as bleeding, ulceration, and perforation.

[0063] The potential use of sulindac for chemoprevention of cancers, and in particular colorectal polyps, has been well studied. For example, U.S. Patents 5,814,625 and 5,843,929, which are both incorporated herein by reference, report potential chemopreventive uses of sulindac in humans. Sulindac has been shown to produce regression of adenomas in Familial Adenomatous Polyposis (FAP) patients (Muscat *et al.*, 1994), although at least one study in sporadic adenomas has shown no such effect (Ladenheim *et al.*, 1995). Sulindac and its sulfone metabolite exisulind have been tested and continue to be tested clinically for the prevention and treatment of several cancer types.

C. Piroxicam

[0064] Piroxicam is a non-steroidal anti-inflammatory agent that is well established in the treatment of rheumatoid arthritis and osteoarthritis with the following chemical designation: 4-hydroxy-2-methyl-*N*-2-pyridyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide. Its usefulness also has been demonstrated in the treatment of musculoskeletal disorders, dysmenorrhea, and postoperative pain. Its long half-life enables it to be administered once daily. The drug has been shown to be effective if administered rectally. Gastrointestinal complaints are the most frequently reported side effects.

[0065] Piroxicam has been shown to be an effective chemoprevention agent in animal models (Pollard and Luckert, 1989; Reddy *et al.*, 1987), although it demonstrated side effects in a recent IIb trial. A large meta-analysis of the side effects of the NSAIDs also indicates that piroxicam has more side effects than other NSAIDs (Lanza *et al.*, 1995).

[0066] The combination of DFMO and piroxicam has been shown to have a synergistic chemopreventive effect in the AOM-treated rat model of colon carcinogenesis (Reddy *et al.*, 1990), although DFMO exerted a greater suppressive effect than piroxicam on Ki-ras mutation and tumorigenesis when each agent was administered separately (Reddy *et al.*, 1990). In one study, administration of DFMO or piroxicam to AOM-treated rats reduced the number of tumors harboring Ki-ras mutations from 90% to 36% and 25%, respectively (Singh *et al.*, 1994). Both agents also reduced the amount of biochemically active p21 ras in existing tumors.

D. Celecoxib

[0067] Celecoxib is a non-steroidal anti-inflammatory agent that is well established in the treatment of osteoarthritis, rheumatoid arthritis, acute pain, ankylosing spondylitis, and to reduce the number of colon and rectal polyps in patients with FAP with the following chemical designation: 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide. Celecoxib is marketed under the brand names Celebrex, Celebra, and Onsenal by Pfizer. Celecoxib is a selective COX-2 inhibitor. Side effects of celecoxib include a 30% increase in rates of heart and blood vessel disease. Additionally, the risks of gastrointestinal side effects are greater than 80%.

E. Combinations of NSAIDs

[0068] Combinations of various NSAIDs may also be used in some embodiments. By using lower doses of two or more NSAIDs, it is possible, in some embodiments, to reduce the side effects or toxicities associated with higher doses of individual NSAIDs. For example, in some embodiments, sulindac may be used together with celecoxib. Examples of NSAIDs that may be used in combination with one another include, but are not limited to: ibuprofen, naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, indomethacin, sulindac, etodolac, diclofenac, piroxicam, meloxicam, tenoxicam, droxicam, lornoxicam, isoxicam, mefenamic acid, meclofenamic acid, flufenamic acid, tolfenamic acid, celecoxib, rofecoxib, valdecoxib, parecoxib, lumiracoxib, and etoricoxib.

IV. Eflornithine/Sulindac Combination Therapy

[0069] The compositions provided herein may be used, in some embodiments, to reduce the number of, inhibit the growth of, and/or prevent the occurrence of cancer cells in patients. Target cancer cells include cancers of the lung, brain, prostate, kidney, liver, ovary, breast, skin, stomach, esophagus, head and neck, testicles, colon, cervix, lymphatic system and

blood. In some embodiments, the compositions may be used to treat and/or prevent colon cancer, familial adenomatous polyposis (FAP), pancreatic cancer, and/or neuroblastoma.

[0070] In some embodiments, the compositions provided herein may be used to treat patients exhibiting pre-cancerous symptoms and thereby prevent the onset of cancer. Target cells and tissues for such preventative treatments include polyps and other precancerous lesions, premalignancies, preneoplastic, or other aberrant phenotype indicating probable progression to a cancerous state. For example, the compositions provided herein may be used to prevent adenomas with little additional toxicities. The Min (multiple intestinal neoplasia) mouse, which shares a mutated *APC/apc* genotype with FAP, serves as a useful experimental animal model for human FAP patients (Lipkin, 1997). The Min mouse can develop greater than 100 gastrointestinal adenomas/adenocarcinomas throughout the gastrointestinal tract by 120 days of life leading to GI bleeding, obstruction and death. A combination therapy of DFMO and sulindac was shown to be effective in reducing adenomas in these mice. See U.S. Patent 6,258,845, which is incorporated herein by reference in its entirety.

V. Fixed Dose Combinations and Routes of Administration

[0071] In one aspect, the present invention provides compositions comprising a fixed dose combination of a pharmaceutically effective amount of eflornithine and a pharmaceutically effective amount of a nonsteroidal anti-inflammatory drug (NSAID) or a metabolite thereof. In some embodiments, the fixed dose combination is a pharmaceutically effective amount of eflornithine and a pharmaceutically effective amount of sulindac.

[0072] In some embodiments, the eflornithine is eflornithine hydrochloride monohydrate. In some embodiments, the eflornithine is eflornithine hydrochloride monohydrate racemate. In some embodiments, the eflornithine hydrochloride monohydrate is a racemic mixture of its two enantiomers.

[0073] In some embodiments, the eflornithine is present in an amount of about 10 to about 1000 mg. In some embodiments, the eflornithine is present in an amount of about 250 to about 500 mg. In some embodiments, the eflornithine is present in an amount of about 300 to about 450 mg. In some embodiments, the eflornithine is present in an amount of about 350 to about 400 mg. In some embodiments, the eflornithine is present in an amount of about 35 to about 60 weight percent. In some embodiments, the eflornithine is present in an amount of about 40 to about 55 weight percent. In some embodiments, the eflornithine is present in an

amount of about 50 to about 55 weight percent. In some embodiments, the eflornithine is present in an amount of about 52 to about 54 weight percent. In some embodiments, the amount of eflornithine hydrochloride monohydrate racemate is from 52 to 54 weight percent. In some embodiments, the eflornithine is present in an amount of about 375 mg. In some embodiments, the amount of eflornithine hydrochloride monohydrate racemate is 375 mg.

[0074] In some embodiments, the sulindac is present in an amount from about 10 to about 1500 mg. In some embodiments, the sulindac is present in an amount of about 50 to about 100 mg. In some embodiments, the sulindac is present in an amount of about 70 to about 80 mg. In some embodiments, the sulindac is present in an amount of about 75 mg. In some embodiments, the amount of sulindac is 75 mg. In some embodiments, the sulindac is present in an amount of about 5 to about 20 weight percent. In some embodiments, the sulindac is present in an amount of about 8 to about 15 weight percent. In some embodiments, the sulindac is present in an amount of about 10 to about 12 weight percent. In some embodiments, the amount of sulindac is from 10 to 11 weight percent.

[0075] In some embodiments, the eflornithine is present in an amount of about 375 mg and the sulindac is present in an amount of about 75 mg.

[0076] In some embodiments, the formulation further comprises an excipient. In some embodiments, the excipient is starch, colloidal silicon dioxide, or silicified microcrystalline cellulose. In some embodiments, the excipient is colloidal silicon dioxide. In some embodiments, the formulation further comprises a second excipient. In some embodiments, the second excipient is silicified microcrystalline cellulose.

[0077] In some embodiments, the formulation further comprises a lubricant. In some embodiments, the lubricant is magnesium stearate, calcium stearate, sodium stearate, glyceryl monostearate, aluminum stearate, polyethylene glycol, boric acid or sodium benzoate. In some embodiments, the lubricant is magnesium stearate. In some embodiments, magnesium stearate is present in an amount of about 0.25 to about 2 weight percent. In some embodiments, the amount of magnesium stearate is from about 0.75 to about 2 weight percent. In some embodiments, the amount of magnesium stearate is from about 1 to about 1.5 weight percent. In some embodiments, the amount of magnesium stearate is about 1.1 weight percent. In some embodiments, magnesium stearate is present in an amount of about 1.5 weight percent.

[0078] In some embodiments, the compositions are in the form of a capsule, tablet, mini tablets, granules, pellets, solution, gel, cream, foam or patch. In some embodiments, the composition is in the form of a tablet, for example, a monolayer tablet.

[0079] In some embodiments, the weight of the tablet is from about 650 mg to about 1,000 mg. In some embodiments, the weight of the tablet is from about 675 mg to about 725 mg. In some embodiments, the weight of the tablet is about 700 mg.

[0080] In some embodiments, the tablet further comprises a coating. In some embodiments, the coating is a modified release coating or an enteric coating. In some embodiments, the coating is a pH-responsive coating. In some embodiments, the coating comprises cellulose acetate phthalate (CAP), cellulose acetate trimelletate (CAT), poly (vinyl acetate) phthalate (PVAP), hydroxypropylmethylcellulose phthalate (HP), poly(methacrylate ethylacrylate) (1:1) copolymer (MA-EA), poly(methacrylate methylmethacrylate) (1:1) copolymer (MA MMA), poly(methacrylate methylmethacrylate) (1:2) copolymer, or hydroxypropylmethylcellulose acetate succinate (HPMCAS). In some embodiments, the coating masks the taste of eflornithine. In some embodiments, the coating comprises hydroxypropyl methylcellulose, titanium dioxide, polyethylene glycol, and iron oxide yellow.

[0081] In some embodiments, the amount of coating is from about 1 to about 5 weight percent. In some embodiments, the amount of coating is from about 2 to about 4 weight percent. In some embodiments, the amount of coating is about 3 weight percent. In some embodiments, the amount of coating is from about 5 mg to about 30 mg. In some embodiments, the amount of coating is from about 15 mg to about 25 mg. In some embodiments, the amount of coating is about 21 mg.

[0082] In some embodiments, the weight of the tablet comprising a coating is from about 675 mg to about 750 mg. In some embodiments, the weight of the tablet comprising a coating is from about 700 mg to about 725 mg. In some embodiments, the weight of the tablet comprising a coating is about 721 mg.

[0083] In one aspect, the present invention provides compositions comprising a fixed dose combination of a pharmaceutically effective amount of eflornithine and a pharmaceutically effective amount of sulindac. In some embodiments, the compositions are in the form of a capsule, tablet, mini tablets, granules, pellets, solution, gel, cream, foam or patch.

In some embodiments, the compositions are solid and take the form of a tablet, for example, a monolayer tablet. In some embodiments, the tablet is film coated.

[0084] In some aspects, the present disclosure provides oral fixed dose combination formulations of eflornithine and an NSAID. In some embodiments, pharmaceutical compositions are provided that comprise a pharmaceutically effective amount eflornithine and a pharmaceutically effective amount of an NSAID. In some embodiments, the NSAID is sulindac, aspirin, piroxicam or celecoxib. In some preferred embodiments, the NSAID is sulindac.

[0085] In some embodiments, the pharmaceutical compositions and formulations of the present invention are for enteral, such as oral, and also rectal or parenteral, with the compositions comprising the pharmacologically active compounds either alone or together with pharmaceutical auxiliary substances (excipients). Pharmaceutical preparations for enteral or parenteral administration are, for example, in unit dose forms, such as coated tablets, tablets, capsules or suppositories and also ampoules. These are prepared in a manner, which is known per se, for example using conventional mixing, granulation, coating, solubilizing or lyophilizing processes. Thus, pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipients, if desired granulating a mixture which has been obtained, and, if required or necessary, processing the mixture or granulate into tablets or coated tablet cores after having added suitable auxiliary substances. In a preferred embodiment, a mixture of active ingredients and excipients are formulated into a tablet form. Appropriate coatings may be applied to increase palatability or delay absorption. For example, a coating may be applied to a tablet to mask the disagreeable taste of the active compound, such as DFMO, or to sustain and/or to delay the release of the active molecules to a certain area in the gastrointestinal tract.

[0086] The therapeutic compound can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The therapeutic compound and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the therapeutic compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, or wafers.

[0087] In certain embodiments, the tablets and/or capsules provided herein comprise the active ingredients and powdered carriers, such as lactose, starch, cellulose derivatives, magnesium stearate, and stearic acid. Similar diluents can be used to make compressed tablets. In other embodiments, tablets and capsules can be manufactured for immediate or modified release. In some embodiments, the tablet and/or capsule is manufactured as a sustained release product to provide for continuous release of medication over a period of hours. In some embodiments, the compressed tablet is sugar-coated and/or film-coated to mask unpleasant taste and/or protect the tablet from the atmosphere. In some embodiments, the tablet is enteric coated for selective disintegration in the gastrointestinal tract.

[0088] In some embodiments, the tablet or capsule is able to disintegrate or dissolve to liberate multiparticulates comprising particles of different populations of a first component and a second component, *e.g.* modified release coated multiparticles. In some of these embodiments, the tablet or capsule may disintegrate or dissolve in the mouth, stomach, small intestine, terminal ileum, or colon. In some of these embodiments, the tablet or capsule may release the multiparticulates with modified release properties.

[0089] In some embodiments, the present invention provides a pharmaceutical oral fixed dose combination in the form of a multilayer tablet. A multilayer tablet has at least two layers (bilayer tablet) or can have three, four, five or more layers. In some embodiments, each of the layers contains not more than one of the active pharmaceutical ingredients (APIs). For example, in some embodiments, the tablet has two layers, with one of the APIs in each of the two layers. In some embodiments, in addition to these two layers, the tablet contains further layers containing only carrier and which may function, *e.g.*, as separation layer(s) or outer coating layer(s). In some embodiments, if more than two layers are present, the components may be present in more than one layer as long as they are not present together in the same layer. In certain embodiments, a monolayer tablet is preferred but all information detailed below is equally applicable to multilayer tablets.

[0090] In some embodiments, the fixed dose combination may be formulated to provide a mean steady state plasma concentration level of total eflornithine and/or sulindac in the range of about 0.1 μM to about 1000 μM and preferably in the range of about 1 μM to 100 μM and more preferably in the range of about 1 μM to about 50 μM.

A. Pharmaceutically Acceptable Excipients

[0091] In some embodiments, the compositions further comprise a pharmaceutically acceptable excipient. In some of these embodiments, the pharmaceutically acceptable excipient may include a pharmaceutically acceptable diluent, a pharmaceutically acceptable disintegrant, a pharmaceutically acceptable binder, a pharmaceutically acceptable stabilizer, a pharmaceutically acceptable lubricant, a pharmaceutically acceptable pigment, or pharmaceutically acceptable glider. In a fixed dose combination formulation of the present invention, an active ingredient may be mixed at a weight ratio of 1:0.25 to 1:20 with a pharmaceutically acceptable excipient.

[0092] Diluents that can be used in pharmaceutical formulations of the present invention include, but are not limited to, microcrystalline cellulose ("MCC"), silicified MCC (*e.g.* PROSOLV™ HD 90), microfine cellulose, lactose, starch, pregelatinized starch, sugar, mannitol, sorbitol, dextrates, dextrin, maltodextrin, dextrose, calcium carbonate, calcium sulfate, dibasic calcium phosphate dihydrate, tribasic calcium phosphate, magnesium carbonate, magnesium oxide, and any mixtures thereof. Preferably, the diluent is silicified MCC. The diluent may be used in an amount of from about 5 to about 95 weight percent based on the total weight of the formulation, and preferably in an amount of from about 25 to about 40 percent weight, such as in an amount of from about 30 to about 35 percent weight. In certain aspects, the diluent can be a soluble diluent. When the diluent is used, its ratio to the active ingredient in each discrete layer is very important. The term "soluble diluents" refers to a diluent which is dissolved in water, like lactose, Ludipress (BASF, a mixture of lactose, crospovidone and povidone (93: 3.5 : 3.5, w/w(%))), mannitol and sorbitol.

[0093] Disintegrants are used to promote swelling and disintegration of the tablet after exposure to fluids in the oral cavity and/or gastrointestinal tract. Examples of disintegrants useful in the fixed dose combination formulation of the present invention include crospovidone, sodium starch glycolate, croscarmellose sodium, low-substituted hydroxypropylcellulose, starch, alginic acid or sodium salt thereof, and a mixture thereof. Other disintegrants that can be used in pharmaceutical formulations of the present invention include, but are not limited to, methylcelluloses, microcrystalline celluloses, carboxymethyl cellulose calcium, carboxymethyl cellulose sodium (*e.g.* AC-DI-SOL™, PRIMELLOSE™), povidones, guar gum, magnesium aluminum silicate, colloidal silicon dioxide (*e.g.* AEROSIL™, CARBOSIL™), polacrilin potassium, starch, pregelatinized starch, sodium starch glycolate

(*e.g.* EXPLOTAB™), sodium alginate, and any mixtures thereof. Preferably, the disintegrant is colloidal silicon dioxide. The disintegrant may be used in an amount of about 0.1 to about 30 weight percent based on the total weight of the formulation, and preferably in an amount of about 0.2 to about 5 weight percent.

[0094] Compositions of the present invention may comprise lubricants. Sticking can occur when granules attach themselves to the faces of tablet press punches. Lubricants are used to promote flowability of powders, and to reduce friction between the tablet punch faces and the tablet punches and between the tablet surface and the die wall. For example, lubricants include magnesium stearate, calcium stearate, zinc stearate, stearic acid, sodium stearyl fumarate, polyethylene glycol, sodium iauryl sulphate magnesium lauryl sulphate, and sodium benzoate. Preferably, the lubricant is magnesium stearate. In the present invention, lubricants preferably comprise 0.25 weight percent to 2 weight percent of the solid dosage form, and preferably in an amount of about 1.5 weight percent. In an exemplary formulation, the lubricant is magnesium stearate present in an amount of about 1.5 weight percent to prevent sticking.

[0095] Binders can be used in the pharmaceutical compositions of the present invention to help hold tablets together after compression. Examples of binders useful for the present invention are acacia, guar gum, alginic acid, carbomers (*e.g.* Carbopol™ products), dextrin, maltodextrin, methylcelluloses, ethylcelluloses, hydroxyethyl celluloses, hydroxypropyl celluloses (*e.g.* KLUCEL™), hydroxypropyl methylcelluloses (*e.g.* METHOCEL™), carboxymethylcellulose sodiums, liquid glucose, magnesium aluminum silicate, polymethacrylates, polyvinylpyrrolidones (*e.g.*, povidone K-90 D, KOLLIDON™), copovidone (PLASDONE™), gelatin, starches, and any mixtures thereof. Preferably, the binder is starch. In the present invention, binders preferably comprise about 1 to about 15 weight percent of the solid dosage form. In other embodiments, the solid dosage form does not comprise a binder.

[0096] In certain embodiments, the stabilizer usable in the fixed dose combination formulation of the present invention may be an anti-oxidant. The use of an antioxidant enhances stability of the active ingredients against the undesirable reaction with other pharmaceutically acceptable additives and against modification by heat or moisture with time. For example, the anti-oxidant is ascorbic acid and its esters, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), α-tocopherol, cystein, citric acid, propyl gallate, sodium bisulfate, sodium pyrosulfite, ethylene diamine tetracetic acid (EDTA), and any mixtures thereof.

B. Tablet Manufacture Processes

[0097] A further aspect of the present invention is providing processes for the manufacturing tablets disclosed herein, including those comprising eflornithine and sulindac. In some embodiments, active agents are prepared by sifting at least one active agent and one or more excipients through a desired mesh size sieve and then mixing, using a rapid mixer granulator, planetary mixer, mass mixer, ribbon mixer, fluid bed processor, or any other suitable device. The blend can be granulated, such as by adding a solution or suspension with or without a binder, whether alcoholic or hydro-alcoholic or aqueous, in a low or high shear mixer, fluidized bed granulator and the like, or by dry granulation. The granules can be dried using a tray dryer, fluid bed dryer, rotary cone vacuum dryer, and the like. The granules can be sized using an oscillating granulator or comminuting mill or any other conventional equipment equipped with a suitable screen. Alternatively, granules can be prepared by extrusion and spheronization, or roller compaction. Also, the manufacture of granules containing active agents can include mixing with directly compressible excipients or roller compaction.

[0098] In other embodiments of the invention, small tablets (mini-tablets) can be made by compressing granules, using dies and punches of various sizes and shapes, as desired. Optionally, a coating can be applied to the tablets, if desired, by techniques known to one skilled in the art such as spray coating, dip coating, fluidized bed coating and the like. In certain embodiments of the present invention, suitable solvent systems such as alcoholic, hydroalcoholic, aqueous, or organic may be used to facilitate processing.

1. Granulation

[0099] Granulation is a process in which powder particles are made to adhere to each other, resulting in larger, multi-particle entities or granules. In embodiments of the invention, granules obtained by a dry or wet technique can be blended with one or more lubricants and/or anti-adherants and then filled into single capsule or into different capsules of different sizes, such that a smaller capsule can be filled into another larger capsule.

[00100] In certain embodiment, dry granulation by compaction is used for the production of the solid dosage composition. In dry granulation, the powder blend is compacted by applying a force onto the powder, which is general causes a considerable size enlargement. In some aspects, slugging is used in the dry granulation process in which a tablet press is used for the compaction process. In other aspects, a roller compactor is used for dry granulation

including a feeding system, compaction unit and size reduction unit. In this method, the powder is compacted between two rolls by applying a force, which is the most important parameter in the dry granulation process. The applied force is expressed in kN/cm, being the force per cm roll width. Occasionally the press force is also indicated in bar. This, however, merely represents the pressure within the hydraulic system, and is in fact not an appropriate measuring unit for the force applied onto the powder. At a given force, depending on the amount of powder conveyed to the rolls, the powder will be compacted to a predefined ribbon thickness.

[00101] In other embodiments, wet granulation is used for the production of the solid dosage composition. Wet granulation of powders improves flow and compactability of the compression mix. In wet granulation, granules are formed by the addition of a granulation liquid onto a powder bed, which is under the influence of an impeller (in a high-shear granulator), screws (in a twin screw granulator) or air (in a fluidized bed granulator). The agitation resulting in the system along with the wetting of the components within the formulation results in the aggregation of the primary powder particles to produce wet granules. The granulation liquid (fluid) contains a solvent, which must be volatile so that it can be removed by drying, and be non-toxic. Typical liquids include water, ethanol and isopropanol either alone or in combination. The liquid solution can be either aqueous based or solvent-based. Aqueous solutions have the advantage of being safer to deal with than organic solvents.

[00102] Tablets may also be formed by tumbling melt granulation (TMG) essentially as described in Maejima *et al,* 1997; which is incorporated herein by reference. Tumbling melt granulation can be used for preparing the melt granulation. It can be done in a tumbling mixer. The molten low melting point compound is sprayed on the crystalline saccharide and powdered saccharide in the blender and are mixed until granules form. In this case, the low melting ingredient is the binder and the crystalline saccharide is the seed. An alternative method is to combine the unmelted low melting point ingredient, crystalline sugar (*e.g.* sucrose or maltose), and water-soluble ingredient in the powder form (*e.g.*, mannitol or lactose) in the tumbling mixer and mix while heating to the melting point of the low melting point binder or higher. The seed should be crystalline or granular water soluble ingredient (saccharide), *e.g.*, granular mannitol, crystalline maltose, crystalline sucrose, or any other sugar. An example of tumbling mixers is the twin-shell blender (V-blender), or any other shape of tumbling mixers. Heating can be achieved by circulating heated air through the chamber of the granulator and by heating the bottom surface of the chamber. As the seed material and the

powdered tablet constituents circulate in the heated chamber, the low-melting point compound melts and adheres to the seeds. The unmelted, powdered material adheres to the seed-bound, molten low-melting point material. The spherical beads, which are formed by this process are then cooled and screen sifted to remove nonadhered powder.

[00103] Spray congealing or prilling can also be used to form the tablet compositions of the invention. Spray congealing includes atomizing molten droplets of compositions which include a low melting point compound onto a surface or, preferably, other tablet constituents. Equipment that can be used for spray congealing includes spray driers (*e.g.*, Nero spray drier) and a fluid bed coater/granulation with top spray (*e.g.*, Glatt fluid bed coater/granulator). In preferred embodiments, a fast-dissolve granulation is formed wherein, preferably a water soluble excipient, more preferably a saccharide, is suspended in a molten low melting point ingredient and spray congealed. After spray congealing, the resulting composition is allowed to cool and congeal. Following congealing of the mixture, it is screened or sieved and mixed with remaining tablet constituents. Spray congealing processes wherein fast-dissolve granulations comprising any combination of low melting point compound and other tablet constituents are melted and spray congealed onto other tablet constituents are within the scope of the present invention. Spray congealing processes wherein all tablet constituents, including the low-melting point compound, are mixed, the low melting point compound is melted and the mixture is spray congealed onto a surface are also within the scope of the invention.

2. Blending

[00104] In certain embodiments, the mixture is blended after granulation. Blending in solid dose manufacturing is to achieve blend uniformity and to distribute the lubricant. In certain aspects, the blend step(s) are designed to achieve homogeneity of all components prior to the final blend of the lubricant. However, blending powders is a challenge due to particle size, moisture content, structure, bulk density and flow characteristics. The key to a successful formula is the order of addition. Typically the component and pharmaceutically acceptable additives are dispatched to a suitable vessel such as a diffusion blender or diffusion mixer. An example of tumbling mixers is the twin-shell blender (V-blender), or any other shape of tumbling mixers.

3. Compression

[00105] Once tablet compositions are prepared, they may be formed into various shapes. In preferred embodiments, the tablet compositions are pressed into a shape. This process may comprise placing the tablet composition into a form and applying pressure to the composition so as to cause the composition to assume the shape of the surface of the form with which the composition is in contact. Compression into a tablet form can be accomplished by a tablet press. A tablet press includes a lower punch that fits into a die from the bottom and an upper punch having a corresponding shape and dimension which enters the die cavity from the top after the tableting material falls into the die cavity. The tablet is formed by pressure applied on the lower and upper punches. The tablets of the invention generally have a hardness of about 20 kP or less; preferably the tablets have a hardness of about 15 kP or less. Typical compression pressures are about 5 kN to about 40 kN and will vary based on the desired size and hardness of the tablet. In some aspects, the compression pressure is about 25 kN to about 35 kN. In particular aspects, the compression pressure is less than or about 37 kN, such as less than about 30 kN, such as less than about 25 kN. Hydraulic presses such as a Carver Press or rotary tablet presses such as the Stokes Versa Press are suitable means by which to compress the tablet compositions of the invention. Exemplary compression force parameters are shown in Table 3.

[00106] In certain embodiments, the lubricated blend can be compressed using a suitable device, such as a rotary machine to form slugs, which are passed through a mill or fluid energy mill or ball mill or colloid mill or roller mill or hammer mill and the like, equipped with a suitable screen to obtain the milled slugs of actives.

[00107] A pre-compression step can be used such as to prevent capping of the tablet. Capping refers to the split or fracture of the cap or top of a tablet from the body of the tablet. Capping can be caused by non-compressible fine particles that migrate when the air is pushed out during compression. For example, the pre-compression can be at about 5, 10 or 15 percent of the main compression force. In preferred embodiments, the tablet is pre-compressed into the form at a pressure, which will not exceed about 10 kN, preferably less than 5 kN. For example, pressing the tablets at less than 1, 1.5, 2, 2.1, 2.2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, or 10 kN is within the scope of the invention. In particular aspects, the pre-compression force is about 2.5 kN to about 3.5 kN. Exemplary pre-compression force parameters are shown in Table 3.

4. Film Coating

[00108] The composition or solid dosage form according to the invention may also be coated with a film coating, an enteric coating, a modified release coating, a protective coating, or an anti-adhesive coating.

[00109] The composition of the invention may be enteric coated. By enteric coated or coating is meant a pharmaceutically acceptable coating preventing the release of the active agent in the stomach and allowing the release in the upper part of the intestinal tract. In other embodiments, the enteric coating is applied to delay the release of the active agent to the terminal ileum or to the colon. The enteric coating may be added as an overcoat upon the modified release coating. The enteric coating polymers can be used either alone or in combination in the enteric coating formulation. Enteric coatings can be designed as a single layer or as multilayer coating embodiments. The preferred enteric coating for the composition of the invention comprises a film-forming agent selected from cellulose acetate phthalate; cellulose acetate trimellitate; methacrylic acid copolymers, copolymers derived from methylacrylic acid and esters thereof, containing at least 40% methylacrylic acid; hydroxypropyl methylcellulose phthalate; hydroxypropylmethylcellulose acetate succinate or Polyvinylacetatephthalate. Examples of polymers suitable for enteric coating include, for example, cellulose acetate phthalate (CAP), cellulose acetate trimelletate (CAT), poly (vinyl acetate) phthalate (PVAP), hydroxypropylmethylcellulose phthalate (HP), poly(methacrylate ethylacrylate) (1:1) copolymer (MA-EA), poly(methacrylate methylmethacrylate) (1:1) copolymer (MA MMA), poly(methacrylate methylmethacrylate) (1:2) copolymer, EUDRAGIT™ L 30D (MA-EA, 1:1), EUDRAGIT™ 100 55 (MA-EA, 3:1), hydroxypropylmethylcellulose acetate succinate (HPMCAS), SURETERIC (PVAP), AQUATERIC™ (CAP), shellac or AQOAT™ (HPMCAS). Targeted colonic delivery systems which may be used with the present invention are known and employ materials such as hydroxypropylcellulose, microcrystalline cellulose (MCE, AVICEL™ from FMC Corp.), poly(ethylene-vinyl acetate) (60:40) copolymer (EVAC from Aldrich Chemical Co.), 2-hydroxyethylmethacrylate (HEMA), MMA, terpolymers of HEMA:MMA:MA synthesized in the presence of N,N′-bis(methacryloyloxyethyloxycarbonylamino)-azobenzene, azopolymers, enteric coated timed release system (TIME CLOCK® from Pharmaceutical Profiles, Ltd., UK) and calcium pectinate and the osmotic minipump system (ALZA corp.).

[00110] In some embodiments, the film coating comprises a polymer such as hydroxypropylcellulose (HPC), ethylcellulose (EC), hydroxypropylmethylcellulose (HMPC), hydroxyethylcellulose (HEC), sodium carboxymethylcellulose(CMC), poly(vinyl pyrrolidone) (PVP), poly(ethylene glycol) (PEG), dimethylaminoethyl methacrylate-methacrylic acid ester copolymer, or ethylacrylate-methylmethacrylate copolymer (EA-MMA).

[00111] In some embodiments, the composition has a modified release coating. The modified release coating may be a pH-responsive coating which when exposed to a certain pH will deliver the active agent(s) (*e.g.*, to the colorectal tract). In some embodiments, the pH-responsive coating is a pH-responsive polymer that will dissolve when exposed to a pH greater than or equal to about 6; although, the pH-responsive polymer may dissolve at a pH greater than or equal to about 5. The pH-responsive polymer may be, for example, a polymeric compound such as EUDRAGIT™ RS and EUDRAGIT™ RL. The EUDRAGIT™ products form latex dispersions of about 30D by weight. EUDRAGIT™ RS 30D is designed for slow release since it is not very water permeable as a coating and EUDRAGIT™ RS 30D is designed for rapid release since it is relatively water permeable as a coating. These two polymers are generally used in combination. As contemplated herein, the permissible ratios of EUDRAGIT™ RS 30D/EUDRAGIT™ RL 30D is about 10:0 to about 8:2. Ethylcellulose or S100 or other equivalent polymers designed for enteric or colorectal release can also be used in place of the EUDRAGIT™ RS/EUDRAGIT™ RL combination above.

[00112] Optionally, the method comprises the step of film coating the tablet. Film coating can be accomplished using any suitable means. Suitable film coatings are known and commercially available or can be made according to known methods. Typically the film coating material is a polymeric film coating material comprising materials such as polyethylene glycol, talc and colorant. Suitable coating materials are methylcellulose, hydroxypropylmethyl-cellulose, hydroxypropylcellulose, acrylic polymers, ethylcellulose, cellulose acetate phthalate, polyvinyl acetate phthalate, hydroxypropyl methylcellulose phthalate, polyvinylalcohol, sodium carboxymethylcellulose, cellulose acetate, cellulose acetate phthalate, gelatin, methacrylic acid copolymer, polyethylene glycol, shellac, sucrose, titanium dioxide, camauba wax, microcrystalline wax, and zein. In some aspects, the film coating is hydroxypropyl methylcellulose, titanium dioxide, polyethylene glycol, and iron oxide yellow. For example, the film coating is OPADRY® Yellow (Colorcon). Typically, a film coating material is applied in such an amount as to provide a film coating that ranges of from 1% to 6% by weight of the

film-coated tablet, such as from 2% to 4%, such as about 3%. Plasticizers and other ingredients may be added in the coating material. The same or different active substance may also be added in the coating material.

[00113] In some embodiments, the coating of the tablet can improve palatability such as to mask the disagreeable taste of the active ingredient(s) such as DFMO. For example, the tablet coating composition can include a cellulose polymer, a plasticizer, a sweetener, or a powdered flavor composition, the powdered flavor composition including a flavorant associated with a solid carrier.

C. Administration Schedules and Protocols

[00114] In some embodiments, the agent(s) may be administered on a routine schedule. As used herein, a routine schedule refers to a predetermined designated period of time. The routine schedule may encompass periods of time which are identical or which differ in length, as long as the schedule is predetermined. For instance, the routine schedule may involve administration twice a day, every day, every two days, every three days, every four days, every five days, every six days, a weekly basis, a monthly basis or any set number of days or weeks there-between. Alternatively, the predetermined routine schedule may involve administration on a twice daily basis for the first week, followed by a daily basis for several months, *etc.* In other embodiments, the invention provides that the agent(s) may be taken orally and that the timing of which is or is not dependent upon food intake. Thus, for example, the agent can be taken every morning and/or every evening, regardless of when the subject has eaten or will eat.

VI. Diagnosis and Treatment of Patients

[00115] In some embodiments, the treatment methods may be supplemented with diagnostic methods to improve the efficacy and/or minimize the toxicity of the anti-cancer therapies comprising administration of the compositions provided herein. Such methods are described, for example, in U.S. Patents 8,329,636 and 9,121,852, U.S. Patent Publications US2013/0217743 and US2015/0301060, and PCT Patent Publications WO2014/070767 and WO2015/195120, which are all incorporated herein by reference.

[00116] In some embodiments, compositions and formulations of the present disclosure may be administered to a subject with a genotype at position +316 of at least one

allele of the ODC1 gene promoter is G. In some embodiments, the genotype at position +316 of both alleles of the patient's ODC1 gene promoters may be GG. In some embodiments, the genotype at position +316 of both alleles of the patient's ODC1 gene promoters may be GA. A statistically significant interaction was detected for *ODC1* genotype and treatment in a full model for adenoma recurrence, such that the pattern of adenoma recurrence among placebo patients was: GG 50%, GA 35%, AA 29% versus eflornithine/sulindac patients: GG 11%, GA 14%, AA 57%. The adenoma-inhibitory effect of eflornithine and sulindac was greater among those with the major G homozygous *ODC1* genotype, in contrast to prior reports showing decreased risk of recurrent adenoma among CRA patients receiving aspirin carrying at least one A allele (Martinez *et al.*, 2003; Barry *et al.*, 2006; Hubner *et al.*, 2008). These results demonstrate that *ODC1* A allele carriers at position +316 differ in response to prolonged exposure with eflornithine and sulindac compared to GG genotype patients, with A allele carriers experiencing less benefit in terms of adenoma recurrence, and potential for elevated risk of developing ototoxicity, especially among the AA homozygotes.

[00117] In some embodiments, the invention provides methods for the preventative or curative treatment of colorectal carcinoma in a patient comprising: (a) obtaining results from a test that determines the patient's genotype at position +316 of at least one *ODC1* promoter gene allele; and (b) if the results indicate that the patient's genotype at position +316 of at least one allele of the *ODC1* promoter gene is G, then administering to the patient a composition provided herein. In some embodiments, the invention provides methods for the treatment of colorectal carcinoma risk factors in a patient comprising: (a) obtaining results from a test that determines the patient's genotype at position +316 of at least one *ODC1* promoter gene allele; and (b) if the results indicate that the patient's genotype at position +316 of at least one allele of the *ODC1* promoter gene is G, then administering to the patient a composition provided herein, wherein the methods prevent the formation of new aberrant crypt foci, new adenomatous polyps or new adenomas with dysplasia in the patient. See U.S. Patent 8,329,636, which is incorporated herein by reference.

[00118] In some embodiments, the invention provides methods for the preventative or curative treatment of familial adenomatous polyposis (FAP) or neuroblastoma in a patient comprising: (a) obtaining results from a test that determines the patient's genotype at position +316 of at least one *ODC1* promoter gene allele; and (b) if the results indicate that the patient's genotype at position +316 of at least one allele of the *ODC1* promoter gene is G,

then administering to the patient a composition provided herein. In some embodiments, the invention provides methods for the treatment of familial adenomatous polyposis or neuroblastoma risk factors in a patient comprising: (a) obtaining results from a test that determines the patient's genotype at position +316 of at least one *ODC1* promoter gene allele; and (b) if the results indicate that the patient's genotype at position +316 of at least one allele of the *ODC1* promoter gene is G, then administering to the patient a composition provided herein, wherein the methods prevent the formation of new aberrant crypt foci, new adenomatous polyps or new adenomas with dysplasia in the patient. See U.S. Patent 9,121,852, which is incorporated herein by reference.

[00119] In some embodiments, the invention provides methods for treating patients with carcinoma comprising administering to the patients a composition provided herein, wherein the patients have been determined to have a dietary polyamine intake, and/or tissue polyamine level, and/or tissue polyamine flux that is not high. In some of these embodiments, the dietary polyamine intake that is not high is 300 μM polyamine per day or lower. In some of these embodiments, the carcinoma is colorectal cancer. See U.S. Patent Publication US2013/0217743, which is incorporated herein by reference.

[00120] In some embodiments, the invention provides methods for the preventative or curative treatment of cancer in a patient comprising: (a) obtaining results from a test that determines an expression level of a *let-7* non-coding RNA, a HMGA2 protein, and/or a LIN28 protein in a cancer cell from the patient; and (b) if the results indicate that the patient's cancer exhibits a reduced *let-7* non-coding RNA expression level as compared to a reference *let-7* non-coding RNA expression level, an elevated HMGA2 protein expression level as compared a reference HMGA2 protein expression level, and/or an elevated LIN28 protein expression level as compared to a reference LIN28 protein expression level, then administering to the patient a composition provided herein. In some of these embodiments, the reference level is a level observed in a non-diseased subject or a level observed in a non-cancerous cell from the patient. In some of these embodiments, "obtaining" comprises providing a sample of the cancer from the patient and assessing an expression level of a *let-7* non-coding RNA, an HMGA2 protein, or a LIN28 protein in a cancer cell from the sample. In some of these embodiments, "assessing an expression level of a *let*-7 non-coding RNA" comprises quantitative PCR or Northern blotting. In some of these embodiments, "assessing an expression level of a HMGA2 protein or a LIN28 protein" comprises immunohistochemistry

or ELISA. In some of these embodiments, the sample is blood or tissue, such as tumor tissue. In some of these embodiments, the patient is a human. In some of these embodiments, the cancer is colorectal cancer, neuroblastoma, breast cancer, pancreatic cancer, brain cancer, lung cancer, stomach cancer, a blood cancer, skin cancer, testicular cancer, prostate cancer, ovarian cancer, liver cancer, esophageal cancer, cervical cancer, head and neck cancer, non-melanoma skin cancer, or glioblastoma. In some of these embodiments, the methods further comprise (c) obtaining results from a test that determines the expression of a *let-7* non-coding RNA in a second cancer cell from said patient at a second time point following the administration of at least one dose of the ODC inhibitor. In some of these embodiments, the methods further comprise increasing the amount of the ODC inhibitor administered to the patient if no or a small increase in *let-7* non-coding RNA is observed. In some of these embodiments, the methods further comprise obtaining results from a test that determines the expression of a HMGA2 protein or a LIN28 protein in a second cancer cell from said patient at a second time point following the administration of at least one dose of the ODC inhibitor. In some of these embodiments, the methods further comprise increasing the amount of the ODC inhibitor administered to the patient if no or a small decrease in HMGA2 protein or LIN28 protein is observed. In some of these embodiments, the methods further comprise (i) obtaining results from a test that determines the patient's genotype at position +316 of at least one allele of the *ODC1* gene promoter; and (ii) if the results indicate that the patient's genotype at position +316 of at least one allele of the *ODC1* gene promoter is G, then administering to the patient a composition provided herein. In some embodiments, the methods comprise diagnosing a cancer or precancerous condition in a patient comprising obtaining a sample from the patient and (b) determining an expression level of at least two markers selected from the group consisting of a *let-7* non-coding RNA, a LIN28 protein, and a HMGA2 protein in the sample, wherein if the expression level of the *let-7* non-coding RNA is decreased or the LIN28 protein or HMGA2 protein is increased in the sample relative to a reference level, then the patient is diagnosed as having cancer or a precancerous condition. In some embodiments, the fixed dose combination of the present invention is administered to a patient with a low cell or tissue *let-7* level. In other aspects, the present compositions are administered to a patient with a high cell or tissue HMGA2 level. In other aspects, the compositions of the present inventions are administered to a patient with a high cell or tissue LIN28 level. See U.S. Patent Publication US2015/0301060, which is incorporated herein by reference.

[00121] In some embodiments, there are provided methods for the preventative or curative treatment of carcinoma in a patient comprising: (a) obtaining results from a test that determines the patient's genotype at position +263 of at least one *ODC1* allele; and (b) if the results indicate that the patient's genotype at position +263 of at least one allele of the *ODC1* gene is T, then administering to the patient a composition provided herein. In some of these embodiments, the test may determine the nucleotide base at position +263 of one allele of the *ODC1* gene in the patient. In some embodiments, the test may determine the nucleotide bases at position +263 of both alleles of the *ODC1* gene in the patient. In some embodiments, the results may indicate that the patient's genotype at position +263 of both alleles of the *ODC1* gene is TT. In some embodiments, the results may indicate that the patient's genotype at position +263 of both alleles of the *ODC1* gene is TG. In some of these embodiments, the method may further comprise obtaining results from a test that determines the patient's genotype at position +316 of at least one *ODC1* allele and only administering to the patient of the composition provided herein if the results indicate that the patient's genotype at position +316 of at least one allele of the *ODC1* gene is G. In another aspect, there are provided methods for the treatment of colorectal carcinoma risk factors in a patient comprising: (a) obtaining results from a test that determines the patient's genotype at position +263 of at least one *ODC1* allele; and (b) if the results indicate that the patient's genotype at position +263 of at least one allele of the *ODC1* gene is T, then administering to the patient a composition provided herein, wherein the method prevents the formation of new aberrant crypt foci, new adenomatous polyps or new adenomas with dysplasia in the patient. In another aspect, there is provided methods for preventing the development or recurrence of a carcinoma in a patient at risk therefor comprising: (a) obtaining results from a test that determines the patient's genotype at position +263 of at least one *ODC1* allele; and (b) if the results indicate that the patient's genotype at position +263 of at least one allele of the *ODC1* gene is T, then administering to the patient a composition provided herein. See PCT Patent Publication WO2015/195120, which is incorporated herein by reference.

[00122] In variations on any of the above embodiments, the carcinoma may be colorectal cancer, neuroblastoma, breast cancer, pancreatic cancer, brain cancer, lung cancer, stomach cancer, a blood cancer, skin cancer, testicular cancer, prostate cancer, ovarian cancer, liver cancer, esophageal cancer, cervical cancer, head and neck cancer, non-melanoma skin cancer, or glioblastoma. In some embodiments, the carcinoma may be colorectal cancer. In some embodiments, the colorectal cancer may be stage I. In some embodiments, the colorectal

cancer may be stage II. In some embodiments, the colorectal cancer may be stage III. In some embodiments, the colorectal cancer may be stage IV. In variations on any of the above embodiments, the methods may prevent the formation of new advanced colorectal neoplasms within the patient. In some embodiments, the method may prevent the formation of new right-sided advanced colorectal neoplasms. In some embodiments, the method may prevent the formation of new left-sided advanced colorectal neoplasms.

[00123] In variations on any of the above embodiments, the patient may have been identified as having one or more adenomatous polyps in the colon, rectum or appendix. In some embodiments, the patient may have been identified as having one or more advanced colorectal neoplasms. In some embodiments, the patient may have been identified as having one or more left-side advanced colorectal neoplasms. In some embodiments, the patient may have been identified as having one or more right-sided advanced colorectal neoplasms. In some embodiments, the patient may have been diagnosed with familial adenomatous polyposis. In some embodiments, the patient may have been diagnosed with Lynch syndrome. In some embodiments, the patient may have been diagnosed with familial colorectal cancer type X. In some embodiments, the patient may satisfy the Amsterdam Criteria or the Amsterdam Criteria II. In some embodiments, the patient may have a history of resection of one or more colorectal adenomas. In some embodiments, the patient may have an intraepithelial neoplasia or a precancerous lesion associated ODC hyperactivity. In some embodiments, the patient may have an intraepithelial neoplasia or a precancerous lesion and elevated cellular polyamine levels.

[00124] In variations on any of the above embodiments, the patient is human.

VII. Definitions

[00125] As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising," the words "a" or "an" may mean one or more than one.

[00126] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[00127] As used herein, the term "bioavailability" denotes the degree means to which a drug or other substance becomes available to the target tissue after administration. In

the present context, the term "suitable bioavailability" is intended to mean that administration of a composition according to the invention will result in a bioavailability that is improved compared to the bioavailability obtained after administration of the active substance(s) in a plain tablet; or the bioavailability is at least the same or improved compared to the bioavailability obtained after administration of a commercially available product containing the same active substance(s) in the same amounts. In particular, it is desired to obtain quicker and larger and/or more complete uptake of the active compound, and thereby provide for a reduction of the administered dosages or for a reduction in the number of daily administrations.

[00128] The terms "compositions," "pharmaceutical compositions," "formulations," and "preparations" are used synonymously and interchangeably herein.

[00129] The terms "comprise," "have" and "include" are open-ended linking verbs. Any forms or tenses of one or more of these verbs, such as "comprises," "comprising," "has," "having," "includes" and "including," are also open-ended. For example, any method that "comprises," "has" or "includes" one or more steps is not limited to possessing only those one or more steps and also covers other unlisted steps.

[00130] The term "derivative thereof" refers to any chemically modified polysaccharide, wherein at least one of the monomeric saccharide units is modified by substitution of atoms or molecular groups or bonds. In one embodiment, a derivative thereof is a salt thereof. Salts are, for example, salts with suitable mineral acids, such as hydrohalic acids, sulfuric acid or phosphoric acid, for example hydrochlorides, hydrobromides, sulfates, hydrogen sulfates or phosphates, salts with suitable carboxylic acids, such as optionally hydroxylated lower alkanoic acids, for example acetic acid, glycolic acid, propionic acid, lactic acid or pivalic acid, optionally hydroxylated and/or oxo-substituted lower alkanedicarboxylic acids, for example oxalic acid, succinic acid, fumaric acid, maleic acid, tartaric acid, citric acid, pyruvic acid, malic acid, ascorbic acid, and also with aromatic, heteroaromatic or araliphatic carboxylic acids, such as benzoic acid, nicotinic acid or mandelic acid, and salts with suitable aliphatic or aromatic sulfonic acids or N-substituted sulfamic acids, for example methanesulfonates, benzenesulfonates, *p*-toluenesulfonates or *N*-cyclohexylsulfamates (cyclamates).

[00131] The term "disintegration" as used herein refers to a process where the pharmaceutical oral fixed dose combination, typically by means of a fluid, falls apart into

separate particles and is dispersed. Disintegration is achieved when the solid oral dosage form is in a state in which any residue of the solid oral dosage form, except fragments of insoluble coating or capsule shell, if present, remaining on the screen of the test apparatus is a soft mass having no palpably firm core in accordance with USP<701>. The fluid for determining the disintegration property is water, such as tap water or deionized water. The disintegration time is measured by standard methods known to the person skilled in the art, see the harmonized procedure set forth in the pharmacopeias USP <701> and EP 2.9.1 and JP.

[00132] The term "dissolution" as used herein refers to a process by which a solid substance, here the active ingredients, is dispersed in molecular form in a medium. The dissolution rate of the active ingredients of the pharmaceutical oral fixed dose combination of the invention is defined by the amount of drug substance that goes in solution per unit time under standardized conditions of liquid/solid interface, temperature and solvent composition. The dissolution rate is measured by standard methods known to the person skilled in the art, see the harmonized procedure set forth in the pharmacopeias USP <711> and EP 2.9.3 and JP. For the purposes of this invention, the test is for measuring the dissolution of the individual active ingredients is performed following pharmacopoeia USP <711> at the pH as set forth herein for the different embodiments. In particular, the test is performed using a paddle stirring element at 75 rpm (rotations per minute). The dissolution medium is preferably a buffer, typically a phosphate buffer (*e.g.*, at pH 7.2). The molarity of the buffer is preferably 0.1 M.

[00133] An "active ingredient" (AI) (also referred to as an active compound, active substance, active agent, pharmaceutical agent, agent, biologically active molecule, or a therapeutic compound) is the ingredient in a pharmaceutical drug or a pesticide that is biologically active. The similar terms active pharmaceutical ingredient (API) and bulk active are also used in medicine, and the term active substance may be used for pesticide formulations.

[00134] A "pharmaceutical drug" (also referred to as a pharmaceutical, pharmaceutical preparation, pharmaceutical composition, pharmaceutical formulation, pharmaceutical product, medicinal product, medicine, medication, medicament, or simply a drug) is a drug used to diagnose, cure, treat, or prevent disease. An active ingredient (AI) (defined above) is the ingredient in a pharmaceutical drug or a pesticide that is biologically active. The similar terms active pharmaceutical ingredient (API) and bulk active are also used in medicine, and the term active substance may be used for pesticide formulations. Some medications and pesticide products may contain more than one active ingredient. In contrast

with the active ingredients, the inactive ingredients are usually called excipients in pharmaceutical contexts.

[00135] The term "effective," as that term is used in the specification and/or claims, means adequate to accomplish a desired, expected, or intended result. "Effective amount," "therapeutically effective amount" or "pharmaceutically effective amount" when used in the context of treating a patient or subject with a compound means that the amount of the compound which, when administered to a subject or patient for treating or preventing a disease, is an amount sufficient to effect such treatment or prevention of the disease.

[00136] "Prevention" or "preventing" includes: (1) inhibiting the onset of a disease in a subject or patient which may be at risk and/or predisposed to the disease but does not yet experience or display any or all of the pathology or symptomatology of the disease, and/or (2) slowing the onset of the pathology or symptomatology of a disease in a subject or patient which may be at risk and/or predisposed to the disease but does not yet experience or display any or all of the pathology or symptomatology of the disease.

[00137] "Treatment" or "treating" includes (1) inhibiting a disease in a subject or patient experiencing or displaying the pathology or symptomatology of the disease (*e.g.*, arresting further development of the pathology and/or symptomatology), (2) ameliorating a disease in a subject or patient that is experiencing or displaying the pathology or symptomatology of the disease (*e.g.*, reversing the pathology and/or symptomatology), and/or (3) effecting any measurable decrease in a disease in a subject or patient that is experiencing or displaying the pathology or symptomatology of the disease.

[00138] "Prodrug" means a compound that is convertible *in vivo* metabolically into an inhibitor according to the present invention. The prodrug itself may or may not also have activity with respect to a given target protein. For example, a compound comprising a hydroxy group may be administered as an ester that is converted by hydrolysis *in vivo* to the hydroxy compound. Suitable esters that may be converted *in vivo* into hydroxy compounds include acetates, citrates, lactates, phosphates, tartrates, malonates, oxalates, salicylates, propionates, succinates, fumarates, maleates, methylene-bis-β-hydroxynaphthoate, gentisates, isethionates, di-*p*-toluoyltartrates, methanesulfonates, ethanesulfonates, benzenesulfonates, *p*-toluenesulfonates, cyclohexylsulfamates, quinates, esters of amino acids, and the like.

Similarly, a compound comprising an amine group may be administered as an amide that is converted by hydrolysis *in vivo* to the amine compound.

[00139] An "excipient" is a pharmaceutically acceptable substance formulated along with the active ingredient(s) of a medication, pharmaceutical composition, formulation, or drug delivery system. Excipients may be used, for example, to stabilize the composition, to bulk up the composition (thus often referred to as "bulking agents," "fillers," or "diluents" when used for this purpose), or to confer a therapeutic enhancement on the active ingredient in the final dosage form, such as facilitating drug absorption, reducing viscosity, or enhancing solubility. Excipients include pharmaceutically acceptable versions of antiadherents, binders, coatings, colors, disintegrants, flavors, glidants, lubricants, preservatives, sorbents, sweeteners, and vehicles. The main excipient that serves as a medium for conveying the active ingredient is usually called the vehicle. Excipients may also be used in the manufacturing process, for example, to aid in the handling of the active substance, such as by facilitating powder flowability or non-stick properties, in addition to aiding *in vitro* stability such as prevention of denaturation or aggregation over the expected shelf life. The suitability of an excipient will typically vary depending on the route of administration, the dosage form, the active ingredient, as well as other factors.

[00140] The term "hydrate" when used as a modifier to a compound means that the compound has less than one (*e.g.*, hemihydrate), one (*e.g.*, monohydrate), or more than one (*e.g.*, dihydrate) water molecules associated with each compound molecule, such as in solid forms of the compound.

[00141] The term "eflornithine" when used by itself refers to 2,5-diamino-2-(difluoromethyl)pentanoic acid is any of its forms, including non-salt and salt forms (*e.g.*, eflornithine HCl), anhydrous and hydrate forms of non-salt and salt forms (*e.g.*, eflornithine hydrochloride monohydrate), solvates of non-salt and salts forms, its enantiomers (*R* and *S* forms, which may also by identified as *d* and *l* forms), and mixtures of these enantiomers (*e.g.*, racemic mixture, or mixtures enriched in one of the enantiomers relative to the other). Specific forms of eflornithine include eflornithine hydrochloride monohydrate (*i.e.*, CAS ID: 96020-91-6; MW: 236.65), eflornithine hydrochloride (*i.e.*, CAS ID: 68278-23-9; MW: 218.63), and free eflornithine (*i.e.*, CAS ID: 70052-12-9; MW: 182.17). Where necessary, the form of eflornithine has been further specified. In some embodiments, the eflornithine of the present disclosure is eflornithine hydrochloride monohydrate (*i.e.*, CAS ID: 96020-91-6). The terms

"eflornithine" and "DFMO" are used interchangeably herein. Other synonyms of eflornithine and DFMO include: α-difluoromethylornithine, 2-(Difluoromethyl)-DL-ornithine, 2-(Difluoromethyl)ornithine, DL-α-difluoromethylornithine, *N*-Difluoromethylornithine, ornidyl, αδ-Diamino-α-(difluoromethyl)valeric acid, and 2,5-diamino-2(difluro)pentanoic acid.

[00142] As used herein, "essentially free," in terms of a specified component, is used herein to mean that none of the specified component has been purposefully formulated into a composition and/or is present only as a contaminant or in trace amounts. The total amount of the specified component resulting from any unintended contamination of a composition is therefore well below 0.05%, preferably below 0.01%. Most preferred is a composition in which no amount of the specified component can be detected with standard analytical methods.

[00143] The term "fixed dose combination" or "FDC" refers to a combination of defined doses of two drugs or active ingredients presented in a single dosage unit (*e.g.*, a tablet or a capsule) and administered as such; further as used herein, "free dose combination" refers to a combination of two drugs or active ingredients administered simultaneously but as two distinct dosage units.

[00144] "Granulation" refers to the process of agglomerating powder particles into larger granules that contain the active pharmaceutical ingredient. "Dry granulation" refers to any process comprising the steps where there is no addition of a liquid to powdered starting materials, agitation, and drying to yield a solid dosage form. The resulting granulated drug product may be further processed into various final dosage forms, *e.g.*, capsules, tablets, wafers, gels, lozenges, etc.

[00145] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." As used herein "another" may mean at least a second or more.

[00146] As used herein, the term "patient" or "subject" refers to a living mammalian organism, such as a human, monkey, cow, sheep, goat, dog, cat, mouse, rat, guinea pig, or transgenic species thereof. In certain embodiments, the patient or subject is a primate. Non-limiting examples of human patients are adults, juveniles, infants and fetuses.

[00147] As generally used herein "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues, organs, and/or bodily fluids of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

[00148] A "pharmaceutically acceptable carrier," "drug carrier," or simply "carrier" is a pharmaceutically acceptable substance formulated along with the active ingredient medication that is involved in carrying, delivering and/or transporting a chemical agent. Drug carriers may be used to improve the delivery and the effectiveness of drugs, including for example, controlled-release technology to modulate drug bioavailability, decrease drug metabolism, and/or reduce drug toxicity. Some drug carriers may increase the effectiveness of drug delivery to the specific target sites. Examples of carriers include: liposomes, microspheres (*e.g.*, made of poly(lactic-co-glycolic) acid), albumin microspheres, synthetic polymers, nanofibers, nanotubes, protein-DNA complexes, protein conjugates, erythrocytes, virosomes, and dendrimers.

[00149] The term "physically separated" as defined herein refers to a pharmaceutical oral fixed dose combination containing both components a) and b) formulated such that they are not mixed with each other in the same carrier but are separated. This separation helps to minimize the interactions between the two components especially upon release of same. Typically the physical separation means that the two components a) and b) are present in different compartments, such as layers, or are present as different entities, such as particulates or granulates, of the formulation. It is not necessary that the two components a) and b) are further separated by additional layers or coating although this may be appropriate from case to case. This physical separation of the two components a) and b) in one dosage form can be achieved by various means known in the art. In one embodiment, this is achieved by formulating the respective components a) and b) into separate layers, *e.g.*, a multi- or bilayer formulation. Specific examples of such formulation techniques are described herein.

[00150] The term "sticking" refers to the attachment of granules to the faces of tablet press punches including within the letter, logo or design on the punch faces.

[00151] The term "capping" refers to the split or fracture of the cap or top of a tablet from the body of the tablet. Capping can be caused by non-compressible fine particles that migrate when the air is pushed out during compression.

[00152] The term "friability" refers herein to the tendency of a tablet to chip, crumble or break following compression. It can be caused by a number of factors including poor tablet design (too sharp edges), low moisture content, insufficient binder, etc. In some aspects, the friability of a tablet sample is given in terms of % weight loss (*i.e.*, loss in weight expressed as a percentage of the original sample weight). Generally, a maximum weight loss of not more than 1% is considered acceptable for most tablets.

[00153] The term "release" as used herein refers to a process by which the pharmaceutical oral fixed dose combination is brought into contact with a fluid and the fluid transports the drug(s) outside the dosage form into the fluid that surrounds the dosage form. The combination of delivery rate and delivery duration exhibited by a given dosage form in a patient can be described as its *in vivo* release profile. The release profiles of dosage forms may exhibit different rates and durations of release and may be continuous. Continuous release profiles include release profiles in which one or more active ingredients are released continuously, either at a constant or variable rate. When two or more components that have different release profiles are combined in one dosage form, the resulting individual release profiles of the two components may be the same or different compared to a dosage form having only one of the components. Thus, the two components can affect each other's release profile leading to a different release profile for each individual component.

[00154] A two-component dosage form can exhibit release profiles of the two components that are identical or different to each other. The release profile of a two-component dosage form where each component has a different release profile may be described as "asynchronous". Such a release profile encompasses both (1) different continuous releases where preferably component b) is released at a slower rate than component a), and (2) a profile where one of components a) and b), preferably component b), is released continuous and the other of components a) and b), preferably component a), is modified to be released continuous with a time delay. Also a combination of two release profiles for one drug is possible (*e.g.* 50% of the drug in continuous and 50% of the same drug continuous with a time delay).

[00155] Immediate release: For the purposes of the present application, an immediate release formulation is a formulation showing a release of the active substance(s), which is not deliberately modified by a special formulation design or manufacturing method.

[00156] Modified release: For the purposes of the present application, a modified release formulation is a formulation showing a release of the active substance(s), which is deliberately modified by a special formulation design or manufacturing method. This modified release can be typically obtained by delaying the time of release of one or both of the components, preferably component a). Typically for the purposes of the present invention, a modified release refers to a release over 5 h, such as a release over 3 h or even shorter. Modified release as used herein is meant to encompass both a different continuous release over time of the two components or a delayed release where one of the components, preferably component a), is released only after a lag time. Such a modified release form may be produced by applying release-modifying coatings, *e.g.* a diffusion coating, to the drug substance(s) or to a core containing the drug substance(s), or by creating a release-modifying matrix embedding the drug substance(s).

[00157] The term "tablet" refers to a pharmacological composition in the form of a small, essentially solid pellet of any shape. Tablet shapes maybe cylindrical, spherical, rectangular, capsular or irregular. The term "tablet composition" refers to the substances included in a tablet. A "tablet composition constituent" or "tablet constituent" refers to a compound or substance which is included in a tablet composition. These can include, but are not limited to, the active and any excipients in addition to the low melting compound and the water soluble excipient.

[00158] The above definitions supersede any conflicting definition in any of the reference that is incorporated by reference herein. The fact that certain terms are defined, however, should not be considered as indicative that any term that is undefined is indefinite. Rather, all terms used are believed to describe the invention in terms such that one of ordinary skill can appreciate the scope and practice the present invention.

[00159] Unit abbreviations used herein include average result (ar), kilopond (kp), kilonewton (kN), percent weight per weight (%w/w), pounds per square inch (psi), RH (relative humidity), color difference delta E (dE), and revolutions per minute (rpm).

VIII. Examples

[00160] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 – Development of Eflornithine HCl and Sulindac Combination Tables

[00161] In the development process of a fixed dose combination (FDC) tablet comprising eflornithine HCl and sulindac, several formulations were tested (Table 1). The parameters that were tested included tablet disintegration time, tablet hardness, and percentage of tablet friability.

[00162] Formulation I was manufactured into a 900 mg tablet by first mixing 1/3 of the silicified MCC (PROSOLV®) with the eflornithine HCl in a 1 quart v-blender. Next, the sulindac and 1/3 of the silicified MCC (PROSOLV®) was pre-mixed in a polyethylene (PE) bag and added to the blender along with the colloidal silicon dioxide (CARBOSIL®) and the pregelatinized corn starch (STARCH 1500®). The PE bag was rinsed with the remaining 1/3 of silicified MCC (PROSOLV®) and added to the blender. The mix was blended for 10 minutes at about 25 rpm before the addition of hand screened magnesium stearate and then blended for an additional 3 minutes. This formulation was found to have some sticking on the punch surface and resulted in a rough tablet surface. Thus, for Formulation II the magnesium stearate was increased from 0.5% to 1% and silicified MCC was decreased from 38.57% to 38.07%.

[00163] Formulation II was manufactured into a 900 mg tablet by pre-mixing CARBOSIL®, STARCH 1500®, and sulindac in a PE bag. Next, ½ of the PROSOLV® and the eflornithine HCl was added to the 8-quart v-blender along with the pre-mix. The remaining ½ of the PROSOLV® was used to rinse the PE bag and added to the blender. The mix was blended for 10 minutes at about 25 rpm. The mix was then removed from the blender and delumped through a Comill 039R screen before returning to the v-blender for an additional 10 minutes of blending. Next, magnesium stearate hand-screened through a 30 mesh (*i.e.*, 590 μm)

screen was added to the v-blender by manual mixing and the mix was blended for 3 minutes at about 25 rpm. The mix was compressed into a tablet on the Key Model BBTS 10 station. The resulting tablet was determined to have a disintegration time of about 29-32 seconds, a friability of 0.077% at 4 minutes and 0.17392% at 8 minutes, and a hardness of about 28 kp (Table 1). The tablet was then film coated with OPADRY® Yellow (Colorcon) at a percent weight of 2.913 to produce a tablet of 927 mg using an O'Hara Labcoat, 12'' pan. The film coated tablets had a hardness of about 36.0-42.1 kp and disintegration time of 1 minute 27 seconds to 1 minute 53 seconds.

[00164] Formulation III was manufactured into a 650 mg tablet by pre-mixing CARBOSIL®, part 2 of the PROSOLV® and sulindac in a PE bag. Next, ½ of part 1 of the PROSOLV® and eflornithine were added to the 8-quart v-blender with the pre-mix. The remaining ½ of part 1 of the PROSOLV® was used to rinse the PE bag and added to the v-blender. The mix was blended for 10 minutes at about 25 rpm. The mix was then removed from the blender and delumped through a Comill 039R screen before returning to the v-blender for an additional 10 minutes of blending. Next, magnesium stearate hand-screened through a 30 mesh (*i.e.*, 590 μm) screen was added to the v-blender by manual mixing and the mix was blended for 3 minutes at about 25 rpm. The mix was compressed into a tablet on the Key Model BBTS 10 station. The resulting tablet was determined to have a disintegration time of about 51-57 seconds, a friability of 0.2607%-0.3373% at 4 minutes and 0.8988%-1.008% at 8 minutes, and a hardness of about 13 kp. The tablet was then film coated with OPADRY® Yellow (Colorcon) at a percent weight of 2.913 to produce a tablet of 669.5 mg using an O'Hara Labcoat, 12'' pan. The film coated tablets had a hardness of about 36.0-42.1 kp and disintegration time of 1 minute 27 seconds to 1 minute 53 seconds. This formulation had a reduced weight from 900 mg to 650 mg and STARCH 1500® was replaced with PROSOLV® to increase the tablet strength. However, capping was observed during the friability testing as well as during the film coating process.

[00165] Formulation IV was manufactured into a 700 mg tablet using the same process as Formulation III. The resulting tablet was determined to have a disintegration time of 1 minutes 10 seconds to about 1 minutes 34 seconds, a friability of 0.1424%-0.1567% at 4 minutes and 0.3186%-0.5166% at 8 minutes, and a hardness of about 20 kp. The tablet was then film coated with OPADRY® Yellow (Colorcon) at a percent weight of 2.913 to produce a tablet of 721 mg using an O'Hara Labcoat, 12'' pan. The film coated tablets had a

disintegration time of 1 minute 43 seconds to 2 minutes 7 seconds. In this formulation, the amount of PROSOLV® was increased and the table weight increased from 650 mg to 700 mg. Although no capping was observed during friability testing, three tablets did have capping during film coating.

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Table 1: Formulations I-IV of Eflornithine HCL and Sulindac Fixed Dose Combination Tablets.

	Formulation I		Formulation II		Formulation III		Formulation IV	
Components	Unit wt (mg)	% W/W	Unit wt (mg)	% W/W	Unit wt (mg)	% W/W	Unit wt (mg)	% W/W
Eflornithine HCl monohydrate racemate	375	41.67	375	41.67	375	57.69	375	53.571
Sulindac	75	8.33	75	8.33	75	11.54	75	10.714
Silicified MCC (part 1)	347.13	38.57	342.63	38.07	149.5	23.0	199.6	28.514
Silicified MCC (part 2)	0	0	0	0	41.075	6.32	41.075	5.868
Pre Gel Corn Starch	96.12	10.68	96.12	10.68	0	0	0	0
Colloidal silicon dioxide	2.25	0.25	2.25	0.25	1.625	1.625	1.625	0.232
Magnesium stearate	4.5	0.5	9	1	7.8	7.8	7.7	1.1
Uncoated Tablet weight	**900**	**100**	**900**	**100**	**650**	**100**	**700**	**100**
OPADRY® Yellow 03B92557			**27.0**		19.5		21.0	
Coated Tablet Weight			**927.0**		**669.5**		**721.0**	

Tablet Characteristics		
	Formulation II	**Formulation IV**
Compression force	NR	85psi
Hardness (kp)	ar 28	ar 20
Disintegration time	ar30s	ar 1min 30s
Friability (4min) (%)	0.08	0.16
Friability (8min) (%)	0.17	0.52 (one capped tablet)

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Table 2: Exemplary Formulation of Eflornithine HCL and Sulindac Fixed Dose Combination Tablet.

Components	Unit weight (mg)	% (w/w)
Eflornithine HCl monohydrate racemate	375.00	52.011
Sulindac	75.00	10.402
Silicified microcrystalline cellulose	237.87	32.992
Colloidal silicon dioxide	1.63	0.226
Magnesium stearate	10.50	1.456
Core tablet weight	700.00	
OPADRY® Yellow	21.00	2.913
Film coated tablet weight	721.00	100

Table 3: Exemplary Tablet Manufacture Parameters.

Variable	7107/2 R3bis	7107/2 R4	7107/3	7107/5 R2	7107/5 R3
Mixer	Turbula	Turbula	Turbula	Turbula	Turbula
Mixing Time	70 cycles	70 cycles	70 cycles	70 cycles	70 cycles
Mg Stearate	1.50%	1.50%	1.50%	1.50%	1.50%
Press	Korsch	Korsch	Korsch	Ronchi	Ronchi
Tool dimensions Tool Coating Engraving Top Engraving Bottom Scored	17.5x8 chrome/RC02 414C wave logo no	17.5x8 chrome/RC02 414C wave logo no	17x9 R6 chrome neutral neutral cleavable	16.5x7 chrome 4141 logo cleavable	16.5x7 chrome 4141 logo cleavable
Compression Force	37 or 30 kN	37 kN	30 kN	37 kN	25 kN
Pre-Compression Force	2.1 kN	2.5 kN	2.0 kN	3.7 kN	2.5 kN
Test Results					
Cleavage	no	no	no	no	no
Sticking	no	no	no	no	no
Top Engraving Intensity	Pass	Pass	Pass	Pass	Pass
Bottom Engraving Intensity	Pass	Pass	Pass	Pass	Pass
Hardness	NA	12.80 kp	8.14 kp	18.46 kp	16.62 kp
Cleavaging Ability	NA	yes	yes	no	no
Disintegration Time	NA	1min15" to 1min25"	40sec to 45sec	2min15" to 2min32"	1min39" to 1min53"
Friability At 4 Minutes	NA	0.08%	0.17%	0.21%	0.37%
Friability At 30 Minutes	NA	1.15%	1.19%	1.80%	2.85%
Tablets Broken/Cleaved	NA	no	no	no	no

Table 4: Materials used for the formulations described in Example 1.

Material	**Supplier**
Efflornithine HCl monohydrate	Scino Pharm
Sulindac	ZACH
Silicified microcrystalline cellulose (MCC) (PROSOLV®)	NF EP
Starch 1500 (Partially pregelatinized Maize Starch)	Colorcon Limited
Colloidal silicon dioxide (CARBOSIL®)	IMCD France SAS
Magnesium Stearate	Mallinkroot-Tyco
OPADRY® Yellow	Colorcon Limited
Equipment	
PK blend master V-blender (1 quart and 8 quart)	
Quadro Comill model 197S with 0.039″ screen	
Key Model BBTS 10 station tableting press	
O'Hara Labcoat 12″ pan, 0.8 mm nozzle	

Example 2– Development of Formulation IV

[00166] From Example 1, Formulation IV was further tested to determine which parameters can be altered to prevent capping and sticking. The first parameter tested was the compression force and the addition of a pre-compression force at about 5-15% of the compression force (Table 5). To evaluate the compression and pre-pressure forces for the Formulation IV 700 mg tablet to reach a hardness of about 20 kp, several trials were performed. In a first trial, a final blend of the Formulation IV 700 mg tablet was manufactured using Equipment C (Table 9). The manufacturing process involved pre-mixing CARBOSIL®, part 2 of the PROSOLV® and sulindac in a PE bag. Next, ½ of part 1 of the PROSOLV® and eflornithine were added to a 10-quart v-blender with the pre-mix. The remaining ½ of part 1 of the PROSOLV® was used to rinse the PE bag and added to the v-blender. The mix was blended for 35 minutes at about 7 rpm. The mix was then removed from the blender and delumped through a Frewitt TC150 1.0 mm screen before returning to the v-blender for an additional 35 minutes of blending. Next, magnesium stearate was hand-screened through a 500µm screen and added to the v-blender by manual mixing for a final blend of 10 minutes at 7 rpm. The compression step was performed on a Courtoy Modul P tableting press equipped with five 17.5x8 mm engraved and chromium plated punches. The parameters were set in order to obtain a hardness of between 17.0 and 22.5 kp. It was found that without pre-pressure, capping was observed. However, the use of a pre-pressure force increased the hardness and avoided capping (Table 10). In addition, the tablets formed with a pre-pressure force were more resistant against attrition (*i.e.*, lower friability). In addition, the 16.5x8mm punch of the Key BBTS 10 station tableting press used in Example 1 appeared to be more prone to attrition.

Table 5: Compression parameters tested for Formulation IV.

	Initial setting	7107/01 setting#3	7107/01 setting#2
Punch shape	16.5x8mm smooth	17.5x8mm engraved	17.5x8mm engraved
Compression force	85 psi	34 kN	35 kN
Pre-pressure force	No	No	Yes (3 kN)
Hardness (kp)	ar 20	ar 13 (*)	ar 17
Disintegration time	ar 1min30s	ar 1min20sec	ar 2min
Friability (4min) (%)	0.16	0.07	0.03
Friability (8min) (%)	0.52 (1 capped tablet)	NA	NA
Friability (10min) (%)	NA	0.27	0.13
Friability (30min) (%)	NA	1.79 (1 capped tablet)	0.54 (no capped tablet)
Thickness (mm)	ar 6.1	ar 5.5	ar 5.4

NA: not applied

(*) maximum hardness that can be reached without precompression.

[00167] In a second trial, the punch surface was varied to determine its effect on the Formulation IV tablet (Table 11). The final blend of the Formulation IV 700 mg tablet was manufactured using Equipment B in this trial. The manufacturing process involved pre-mixing CARBOSIL®, part 2 of the PROSOLV® and sulindac in a PE bag. Next, ½ of part 1 of the PROSOLV® and eflornithine were added to a 10-quart v-blender with the pre-mix. The remaining ½ of part 1 of the PROSOLV® was used to rinse the PE bag and added to the v-blender. The mix was blended for 8 minutes 30 seconds at about 30 cycles per minute. The mix was then removed from the blender and delumped through a CMA 1.0 mm screen before returning to the v-blender for an additional 8.5 minutes of blending. Next, magnesium stearate hand-screened through a 500µm screen and added to the v-blender by manual mixing for a final blend of 2 minutes 20 seconds at 30 cycles per minute. The compression step was performed on a Korsch XL100 tableting press equipped with two 17.5x8mm engraved and anti-sticking chromium plated punches. The pre-pressure was set at 5-10% of the main compression force which was around 30 kN. Several different punch surfaces were also tested including chromium, carbon, tungsten, and Teflon VS stainless steel. In some embodiments, Teflon may be used to reduce the sticking.

[00168] To avoid sticking, several additional variables were tested and a high constraint was applied at the very beginning of the compression. Neither lubrication with 1.1% magnesium stearate nor increasing the lubrication time from 70 rotations to 140 rotations prevented sticking (Tables 11 and 12). However, increasing the ratio of magnesium stearate to 1.5% did prevent sticking (Table 12) along with a slight decrease in tablet hardness at about 20%, but the friability was still very low at less than 0.1% after 4 minutes. With two types of punches, equipped with different kinds of break line (17×9mm and 16.5×7mm), breakability results were compliant for both punches tested. Thus, increasing the magnesium stearate to 1.5% prevents sticking and pre-compression prevents capping of Formulation IV.

Table 6: Batch weights of Formulation IV in Trial 1 and Trial 2.

Components	Unit wt (mg)	Trial 1 wt (g)	Trial 2 wt (g)
Eflornithine HCl	375	1339.500	1340.000
Sulindac	75	268.100	268.027
Silicified MCC (part 1)	199.6	712.800	712.000
Silicified MCC (part 2)	41.075	146.700	146.648
Colloidal silicon dioxide	1.625	5.796	5.8043
Magnesium stearate	7.7	27.515	27.504
Tablet weight	**700.0**	**2500.411**	**2499.983**

Table 7: Varying Amounts of Magnesium Stearate for Formulation IV.

	1.1% of Magnesium stearate formula		1.3% of Magnesium stearate formula (*)		1.5% of Magnesium stearate formula (*)	
Components	Unit wt (mg)	w/w (%)	Unit wt (mg)	w/w (%)	Unit wt (mg)	w/w (%)
Eflornithine HCl	375.000	53.571	374.227	53.461	373.457	53.351
Sulindac	75.000	10.714	74.851	10.693	74.704	10.672
Silicified MCC (part 1)	199.598	28.514	199.192	28.456	198.793	28.399
Silicified MCC (part 2)	41.075	5.868	40.992	5.856	40.908	5.844
Colloidal silicon dioxide	1.625	0.232	1.624	0.232	1.617	0.231
Magnesium stearate	7.700	1.100	9.100	1.300	10.500	1.500
Tablet weight	**700.0**	**100.00**	**700.0**	**100.00**	**700.0**	**100.00**

(*) Formulae obtained after dilution to increase the percentage of magnesium stearate. APIs concentration consequently slightly below the target.

Table 8: Coating of Formulation IV.

Components	Unit wt (mg)	Batch wt (g)
Uncoated tablets	700.00	600.00
OPADRY® Yellow 03B92557	21.00	53.995
Purified water	154.00	395.99
Coated Tablet weight	**721.00**	**653.995**

Table 9: Equipment used for development of Formulation IV.

Equipment A	**Equipment B**	**Equipment C**
PK blend master V-blender 1 quart and 8 quart	Turbula T10A blender 10L container	Servolift blender 10L container
Quadro Comill 197S 0.039″ screen	CMA T1 conical mill 1.00mm screen	Frewitt TC150 conical mill 1.00mm screen
	0.500mm sieving screen	0.500mm sieving screen
Key BBTS 10 station tableting press	Korsch XL100 tableting press	Courtoy Modul P tableting press
O'Hara Labcoat 12″ pan	Mini Glatt coater	

Table 10: First trial parameters and results for testing effect of pre-compression force on Formulation IV.

Compression Parameters					
		7107/01 setting#3	**7107/01 setting#5**	**7107/01 setting#2**	**7107/01 setting#4**
Speed (tpm)		50	50	50	50
Pre-pressure force (kN) / % of the main pressure		0.11 / 0%	1.43 / 5%	3.25 / 10%	4.68 / 15%
Compression force (kN)		33.58	32.23	34.07	33.03
Punches (quantity)		5	5	5	5
Punch shape		17.5x8 mm engraved	17.5x8 mm engraved	17.5x8 mm engraved	17.5x8 mm engraved
Punch surface treatment		Anti-sticking chromium plating	Anti-sticking chromium plating	Anti-sticking chromium plating	Anti-sticking chromium plating
Results					
Test	**Sampling**	**7107/01 setting#3**	**7107/01 setting#5**	**7107/01 setting#2**	**7107/01 setting#4**
Weight (mg) RSD (%)	20 tablets	702.28 1.18	699.14 1.00	703.5 1.06	701.17 0.73
Hardness (kp)	10 tablets	11.5 to 14.4 (Mean value: 13.2)	15.2 to 17.9 (Mean value: 16.6)	15.8 to 18.4 (Mean value: 17.3)	17.0 to 18.7 (Mean value: 17.9)
Friability (%) 4min 10min 30min	According to Pharmacopeia	 0.07/ No capping 0.27/ No capping 1.79/ ***1 capping***	 0.07/ No capping 0.20/ No capping 0.67/ No capping	 0.03/ No capping 0.13/ No capping 0.54/ No capping	 0.08/ No capping 0.19/ No capping 0.59/ No capping
Disintegration time (min)	3 tablets	1min08sec to 1min40sec	1min39sec to 2min17sec	1min51sec to 2min11sec	1min41sec to 1min 57sec
Thickness (mm)	10 tablets	5.4 to 5.6	5.4 to 5.5	5.4 to 5.5	5.4 to 5.5
Sticking		Some sticking	Some sticking	Some sticking	Some sticking

Table 11: Second trial parameters and results for testing effect of punch surface on Formulation IV.

Final blend						
		7107/02 setting#2	7107/02 setting#5	7107/02 setting#6	7107/02 setting#7	7107/02 setting#8
Ratio of Mg stearate (%)		1.1	1.1	1.1	1.1	1.1
Final blend (rotations)		140	140	140	140	140
Compression parameters						
Speed (tpm)		40	40	40	40	40
Pre-pressure force (kN)		2.5	2.2	2.2	2.1	2.1
Compression force (kN)		30	30	30	30	30
Punches (quantity)		2	2	2	2	2
Punch shape		17.5x8 mm engraved	17.5x8 mm engraved	17.5x8 mm engraved	17.5x8 mm engraved	17.5x8 mm engraved
Punch surface treatment		Anti-sticking chromium RC-02	Anti-sticking with carbon RB-01	Anti-sticking with tungsten RD-03	Anti-sticking with teflon RF-03	Steel (no anti-sticking plating)
Results						
test	**sampling**	7107/02 setting#2	7107/02 setting#5	7107/02 setting#6	7107/02 setting#7	7107/02 setting#8
Weight (mg) / RSD (%)	20 tablets	697.12 / 0.38	NA	NA	NA	NA
Hardness (kp)	5 tablets	15.4 to 16.3	NA	NA	NA	NA
Friability (%) 4min 10min 30min	According to Pharmacopeia	 0.02/ No capping 0.04/ No capping 0.69/ No capping	NA	NA	NA	NA
Disintegration time (min)	3 tablets	0min58sec to 1min00sec	NA	NA	NA	NA
Thickness (mm)	10 tablets	5.5 to 5.5	NA	NA	NA	NA
Sticking	10 tablets	Some sticking	Some sticking	Some sticking	Very slightly sticking	Some sticking

Table 12: Second trial parameters and results for testing effect of final mixing duration and magnesium stearate on Formulation IV.

		7107/02 setting#1	7107/02 setting#2	7107/02 setting#3	7107/02 setting#4	7107/02 setting#10
Final blend						
Ratio of Magnesium stearate (%)		1.1	1.1	***1.5***	1.5	***1.3***
Final mixing duration (rotations)		70	***140***	70	70	***140***
Compression parameters						
Speed (tpm)		40	40	40	40	40
Pre-pressure force (kN)		3.5	2.5	2.1	2.5	2.2
Compression force (kN)		>>30	30	30	37	37
Punches (quantity)		2	2	2	2	2
Punch shape		17.5x8 mm engraved	17.5x8 mm engraved	17.5x8 mm engraved	17.5x8 mm engraved	17.5x8 mm engraved
Punch surface treatment		Anti-sticking chromium plating	Anti-sticking chromium plating	Anti-sticking chromium plating	Anti-sticking chromium plating	Anti-sticking chromium plating
Results						
test	**sampling**					
Weight (mg) / RSD (%)	20 tablets	704.01 (*) / 0.23	697.12 / 0.38	695.19 / 0.38	702.61 / 0.39	703.24 / 0.29
Hardness (kp)	5 tablets	17.3 to 17.9	15.4 to 16.3	12.4 to 13.4	11.9 to 14.1	13.4 to 14.7
Friability (%) 4min 10min 30min	According to Pharmacopeia	 NA NA NA	 0.02/ No capping 0.04/ No capping 0.69/ No capping	 0.03/ No capping 0.15/ No capping 1.01/ No capping	 0.08/ No capping 0.11/ No capping 1.15/ No capping	 0.05/ No capping 0.19/ No capping 1.02/ No capping
Disintegration time (min)	3 tablets	1min15sec to 1min20sec	0min58sec to 1min00sec	1min00sec to 1min 10sec	1min15sec to 1min 25sec	1min30sec to 1min45sec
Thickness (mm)	10 tablets	5.3 to 5.4	5.5 to 5.5	5.5 to 5.5	5.5 to 5.5	5.5 to 5.6

		7107/02 setting#1	7107/02 setting#2	7107/02 setting#3	7107/02 setting#4	7107/02 setting#10
Sticking	10 tablets	Decreasing of the sticking, some lower punches are clean	Decreasing of the sticking	Very slightly sticking on the upper punches. No sticking on the lower one	No sticking but tendency to split during the hardness test	Slightly sticking on the upper punch

(*) on 10 tablets

Table 13: Trial parameters and results for testing effect of compression parameters on Formulation IV.

		7107/03 setting#1	7107/05 setting#1	7107/05 setting#2	7107/05 setting#3
Final blend					
Ratio of Mg stearate (%)		1.5	1.5	1.5	1.5
Compression parameters					
Speed (tpm)		40	40	40	40
Pre-pressure force (kN)		2.0	5.0	3.7	2.5
Compression force (kN)		30	24	37	25
Punches (quantity)		2	2	2	2
Punch shape		17x9R6mm breakable	16.5x7mm breakable	16.5x7mm breakable	16.5x7mm breakable
Punch surface treatment		Anti-sticking chromium	Anti-sticking chromium	Anti-sticking chromium	Anti-sticking chromium
Results					
test	**sampling**	**7107/03 setting#1**	**7107/05 setting#1**	**7107/05 setting#2**	**7107/05 setting#3**
Weight (mg) / RSD (%)	20 tablets	700.20 (*) / 0.49	705.29 / 0.59	709.62 / 0.64	700.08 / 0.54
Breaking test RSD on one half (%)	30 tablets	0.97	NA	3.19	2.91
Hardness (kp)	5 tablets	7.6 to 8.8 (**)	18.6 to 19.7	17.3 to 19.3	16.1 to 16.9
Friability (%) 4min 10min 30min	According to Pharmacopeia	 0.17/ No capping 0.32/ No capping 1.19/ No capping	 0.12/ No capping 0.47/ No capping 1.52/ No capping	 0.21/ No capping 0.51/ No capping 1.80/ No capping	 0.37/ No capping 1.04/ No capping 2.85/ No capping
Disintegration time (min)	3 tablets	0min40sec to 0min45sec	1min38sec to 1min43sec	2min15sec to 2min32sec	1min39sec to 1min53sec

		7107/03 setting#1	7107/05 setting#1	7107/05 setting#2	7107/05 setting#3
Thickness (mm)	10 tablets	5.3 to 5.3	6.6 to 6.7	6.5 to 6.7	6.6 to 6.7
Sticking	10 tablets	No sticking	No sticking	No sticking	No sticking

(*) on 30 tablets (**) on 10 tablets NA: not applied

[00169] The stability of the Formulation IV combination tablet, eflornithine single tablet and sulindac single tablet was tested. Stability analysis of the Formulation IV tablets was performed at 6 months using the Karl Fischer titration method for determination of water content (FIG. 1). In FIG. 1, it is shown that the combination tablet of Formulation IV had a lower uptake of water over six months as compared to the eflornithine single tablet. Water can affect drug potency and drug dissolution; for example, water can increase the rate of drug degradation by hydrolysis (Gerhardt, 2009). Thus, in some embodiments, the combination tablets provided herein are more stable than one or both of the single active agent tablets.

[00170] Finally, the dissolution profile of Formulation IV was also tested. The dissolution study was carried out in 50 mM sodium phosphate buffer medium at a pH of 7.2 using a paddle stirring element at 75 rpm (USP <711> Dissolution Apparatus II (Paddle)) (FIGs. 2A-2B). The method was validated level II for the dissolution of elfonithine and sulindac. No interference of active pharmaceutical ingredients eflornithine and sulindac were observed between themselves, with the dissolution medium, with the phosphate buffer solution, or with the excipients. Surprisingly, the fixed dose combination of Formulation IV was observed to have an overlapping *in vitro* dissolution profile as compared to the single agent tablets.

Example 3 – Drug Excipient and Coating Compatibility

[00171] A non-cGMP drug excipient compatibility study for eflornithine HCl/sulindac combination tablet was conducted. Appearance, HPLC Assay and XRPD properties were evaluated using a series of samples. The excipients that were tested included PVP, HPMC, lactose, EXPLOTAB™, Ac-Di-Sol®, PROSOLV®, STARCH 1500®, and OPADRY® Yellow. Samples prepared for the excipient compatibility were all 1:1 physical mixtures of API(s) with excipient(s), except the eflornithine HCl:sulindac preparation that was 5:1, and the eflornithine HCl:sulindac:H_2O preparation that was about 6:1:0.3. Total mass of most samples was approximately 750mg. Preparation involved weigh off of components into 20cc scintillation vials, closed and vortexed for approximately 30 seconds. The samples were then stored in a 40°C/75% RH stability chamber for four weeks. Lids on the vials were loosely secured and were protected from light while stored in the chamber.

[00172] Appearance observations were conducted by visual examination of the vials prepared for HPLC analysis. Excipient compatibility samples were extracted with 50%

acetonitrile in buffer (50 mM phosphate buffer pH 2.55). Samples containing only Sulindac were prepared by weighing out portion (~150 mg) of the sample and extracted in a pre-determined volume such that the final concentration of eflornithine and sulindac is 9.5 mg/mL and 0.1 mg/mL, respectively. The rest of the compatibility samples were prepared by quantitative transfer using the extraction solvent in a pre-determined volume such that the final concentration of eflornithine and sulindac was approximately the same as above. Excipient compatibility samples were analyzed using a method capable of detecting both actives, eflornithine and sulindac (FIG. 4A). The method employs a gradient reverse phase HPLC with Ultraviolet (UV) detection at 195nm.

[00173] XRPD analysis was conducted on a Bruker AXS D8 Advance system with a Bragg-Brentano configuration using the CuKα radiation. Samples were analyzed at room temperature using the following parameters: 40kV, 40mA, 1° divergence and antiscatter slits, a method measuring in continuous mode from 2 – 40°2Θ with a 0.05° step and 1 second/step time. Between 3 and 25 mg of sample was analyzed using a rotating, top-filled steel sample holder in a nine-position auto-sampler accessory. The system was calibrated using traceable standards. Results are shown in FIGs. 4B-4C.

[00174] Eflornithine HCl with PVP K30 showed moisture in the sample starting in the 2 week sample and becoming a liquid at 4 weeks. Sulindac with PVPK30 showed sticking of the sample at 2 weeks and continuing at 4 weeks. PVPK30 excipient only showed moisture starting in the 2 week sample and becoming a liquid at 4weeks. The same behavior was observed with the Eflornithine HCl samples but not with the Sulindac samples. HPLC Assay results for the majority of the samples tested show no distinctive trend (increasing or decreasing) over the different time points. Although a number of samples had unusually low assay values, the assay levels showed more of an increasing trend or remain relatively constant over the 4 weeks period. The highest variability in assay results was observed for the Sulindac/Eflornithine ProSolv SMCC90 sample. The assay value at the 4-week time point was 10.0% higher than the assay results at initial. This variability may be contributed to the method (nonvalidated) and sample consistency at the different time points. While the acceptable random analytical error of a validated method is 2%, the variability of this method is unknown. Except for some of the samples, the assay values in each of the samples tested over the different time points are within the normally acceptable 2% random error of an analytical method. There is no distinctive trend for the API, eflornithine and sulindac under the stressed conditions tested.

The results of this study suggest that both APIs (eflornithine HCl/sulindac) were compatible with the potential excipients.

[00175] The drug excipient compatibility study was conducted by XRPD analysis to determine the crystallinity of the API(s) with potential formulation excipients for eflornithine HCl/sulindac combination product. The XRPD results showed no interaction between the API(s) and excipient at 40°C/75%RH after four weeks. This indicated that both APIs (eflornithine HCl/sulindac) were compatible with the potential excipients.

[00176] Coating trials were carried out on tablets to determine effect on stability at 1 month and 3 months at a moisture content of 25°C/60% RH or 40°C/75% RH. The coatings included OPADRY® Yellow (Colorcon, 03B92557), OPADRY® White (Colorcon Y-1-7000), OPADRY® II White (Colorcon 85F18422), and OPADRY® Clear (Colorcon YS-3-7413) at a 3 percent or 4 percent weight gain. The color eye measurement was taken to evaluate the total color difference, or DE, between the tablets that were on stability and the initial coated tablets.

[00177] The tablet color was tested using a Datacolor Spectraflash 600 Series Spectrophotometer. The data was analyzed using the Commission Internationale de l'Eclairage (CIE) L* a* b* system. In the L* a* b* system color is represented as a coordinate in a three dimensional space. Lightness and darkness are plotted on the L* axis with L=100 representing pure white and L=0 representing pure black. The a* and b* axes represent the two complementary color pairs of red / green and blue / yellow respectively. By plotting colors geometrically the difference between two colors (total color difference = E*) can be determined by calculating the distance between two points using the following equation.

$$DE^* = [(L^*1 - L^*2)2 + (a^*1 - a^*2)2 + (b^*1 - b^*2)2]1/2$$

[00178] Using the Datacolor, each tablet was analyzed at each weight gain of the various coating formulations. The closer the DE value is to zero, the closer the tested tablet color is to the color standard (the initial samples). Colorcon's standard spec for white coatings (to pass QC testing) would be a DE value of less than 1.5. All stability samples with white film coating exceed that 1.5 DE and therefore would not pass Colorcon's standard QC testing (Table 14). The clear coated tablets were also well above the value of 1.5.

Table 14: DE values for coated tablets on stability.

	3% wg Y-1-7000 (white)	4% wg Y-1-7000 (white)	3% wg 85F18422 (white)	4% wg 85F18422 (white)	3% wg 03B92557 (yellow)	4% wg 03B92557 (yellow)	3% wg YS-3-7413 (clear)
1 mo 25/60	1.81	1.64	2.56	2.8	0.27	0.32	1.15
3 mo 25/60	1.97	1.94	2.96	2.31	0.35	0.22	1.1
1 mo 40/75	1.91	2.47	3.58	2.39	0.3	0.29	4.29
3 mo 40/75	2.71	2.66	2.72	3.31	0.64	0.58	7.6

[00179] The best DE results were seen with the tablets coated with the yellow formulation. The DE values were well below 1.5. A DE value (total color difference) of 1 or below is considered imperceptible to the human eye. Colorcon's typical internal specification for yellow coatings tend to be a DE value of 2.5 - 3. Thus, OPADRY® Yellow was used to coat the combination tablets.

Example 4 – Bioequivalence Study of Fixed Co-Formulated Eflornithine/Sulindac

[00180] A pilot study was performed to compare the pharmacokinetic parameters of eflornithine, sulindac, sulindac sulfide, and sulindac sulfone in plasma following oral administration of the co-formulated tablet containing eflornithine/sulindac compared to individual tablets containing eflornithine or sulindac taken alone or co-administered in normal healthy subjects under fasting conditions. The secondary objective of this study was to determine the safety and tolerability of eflornithine/sulindac co-formulated tablets compared to individual formulations taken alone or co-administered in normal healthy subjects.

[00181] The study comprised twelve subjects, male or female, at least 18 years of age but not older than 60 years. The main inclusion criteria were: light-, non- or ex-smokers; body mass index (BMI) $\geq$18.50 kg/m^2 and <30.00 kg/m^2; no clinically significant abnormality found in the 12-lead ECG performed (subjects had to be in a supine position for 10 minutes prior to ECG, and the ECG was performed prior to all requested blood draws); negative pregnancy test for female subjects; and healthy according to medical history, complete physical examination (including vital signs) and laboratory tests (general biochemistry, hematology and urinalysis).

[00182] The subjects were treated in four treatment groups comprising:

- Treatment 1: a single 750/150 mg dose of co-formulated Eflornithine 375 mg /Sulindac 75 mg tablets (2 x 375/75 mg tablets)

- Treatment 2: a single 750 mg dose of Eflornithine 250 mg tablets (3 x 250 mg tablets)
- Treatment 3: a single 150 mg dose Sulindac 150 mg tablets (1 x 150 mg tablet)
- Treatment 4: a single 150 mg dose of Sulindac 150 mg tablets (1 x 150 mg tablet) and a single 750 mg dose of Eflornithine 250 mg tablets (3 x 250 mg tablets) administered concurrently

[00183] Each subject was assigned to receive the 4 different treatments over a 28-day period. A single oral dose of the assigned treatment was administered under fasting conditions in each study period. The treatment administrations were separated by a wash-out of 7 calendar days. A total of 120 blood samples were collected in 80 occasions for each subject. The first blood sample was collected prior to drug administration while the others were collected 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16, 24, 36 and 48 hours post drug administration. The analytes were measured by HPLC with MS/MS detection. The assay range was 35.0 ng/mL to 35000.0 ng/mL for eflornithine, 30.0 ng/mL to 15000.0 ng/mL for sulindac, and 10.0 ng/mL to 8000.0 ng/mL for sulindac sulfone and sulindac sulfide. Safety was evaluated through assessment of adverse events (AEs), standard laboratory evaluations, vital signs, and ECGs.

[00184] Mathematical Model and Statistical Methods of Pharmacokinetic Parameters: The main absorption and disposition parameters were calculated using a non-compartmental approach with a log-linear terminal phase assumption. The trapezoidal rule was used to estimate area under the curve. The terminal phase estimation was based on maximizing the coefficient of determination. The pharmacokinetic parameters of this trial were C_{max}, T_{max}, AUC_{0-T}, $AUC_{0-\infty}$, $AUC_{0-T/\infty}$, λz and T_{half}. The statistical analysis was based on a parametric ANOVA model of the pharmacokinetic parameters; the two-sided 90% confidence interval of the ratio of geometric means for the C_{max}, AUC_{0-T} and $AUC_{0-\infty}$ was based on ln-transformed data; the T_{max} was rank-transformed. The ANOVA model used fixed factors of sequence, period, and treatment; the random factor was subject nested within sequence.

[00185] The pharmacokinetic parameters included C_{max} (Maximum observed plasma concentration), T_{max} (Time of maximum observed plasma concentration; if it occurs at more than one time point, T_{max} is defined as the first time point with this value), T_{LQC} (Time of last observed quantifiable plasma concentration), AUC_{0-T} (Cumulative area under the plasma concentration time curve calculated from 0 to T_{LQC} using the linear trapezoidal method), $AUC_{0-\infty}$ (Area under the plasma concentration time curve extrapolated to infinity, calculated as $AUC_{0-T} + C_{LQC}/\lambda z$, where C_{LQC} is the estimated concentration at time

T_{LQC}), $AUC_{0-T/\infty}$ (Relative percentage of AUC_{0-T} with respect to $AUC_{0-\infty}$), T_{LIN} (Time point where log-linear elimination phase begins), λz (Apparent elimination rate constant, estimated by linear regression of the terminal linear portion of the log concentration versus time curve), and T_{half} (Terminal elimination half-life, calculated as ln(2)/ λz).

Table 15: Pharmacokinetic Parameters for Eflornithine

PARAMETER	Treatment-1 (n=12)		Treatment-2 (n=12)		Treatment-4 (n=12)	
	MEAN	C.V. (%)	MEAN	C.V. (%)	MEAN	C.V. (%)
C_{max}(ng/mL)	10643.8	(21.6)	10234.6	(19.9)	10012.8	(25.5)
ln (C_{max})	9.2525	(2.2)	9.2134	(2.3)	9.1822	(2.8)
T_{max} (hours)*	3.25	(2.00-6.00)	3.50	(2.00-5.00)	4.50	(2.50-5.00)
AUC_{0-T} (ng·h/mL)	71459.8	(20.4)	68962.3	(20.2)	69914.9	(18.3)
ln (AUC_{0-T})	11.1562	(1.9)	11.1229	(1.8)	11.1407	(1.6)
$AUC_{0-\infty}$ (ng·h/mL)	71839.3	(20.3)	69301.2	(20.0)	70326.0	(18.1)
ln ($AUC_{0-\infty}$)	11.1619	(1.9)	11.1281	(1.8)	11.1468	(1.6)
$AUC_{0-T/\infty}$ (%)	99.44	(0.3)	99.48	(0.2)	99.39	(0.3)
λz ($hours^{-1}$)	0.1453	(25.0)	0.1642	(21.5)	0.1630	(26.3)
T_{half} (hours)	5.07	(27.3)	4.43	(24.9)	4.65	(39.0)

* Median (range)

Table 16: Pharmacokinetic Parameters for Sulindac

PARAMETER	Treatment-1 (n=12)**		Treatment-3 (n=12)**		Treatment-4 (n=12)***	
	MEAN	C.V. (%)	MEAN	C.V. (%)	MEAN	C.V. (%)
C_{max} (ng/mL)	4553.4	(31.6)	5236.1	(39.2)	5188.5	(42.9)
ln (C_{max})	8.3788	(3.7)	8.4946	(4.7)	8.4562	(5.7)
T_{max} (hours)*	1.54	(0.75-5.00)	1.50	(1.00-2.50)	1.50	(0.75-5.00)
AUC_{0-T} (ng·h/mL)	11268.3	(32.2)	11569.7	(31.4)	11340.8	(43.9)
ln (AUC_{0-T})	9.2823	(3.5)	9.3114	(3.4)	9.2621	(4.2)
$AUC_{0-\infty}$ (ng·h/mL)	11579.4	(39.9)	12687.8	(34.9)	12023.7	(49.3)
ln ($AUC_{0-\infty}$)	9.2896	(4.2)	9.3924	(3.9)	9.3019	(4.8)
$AUC_{0-T/\infty}$ (%)	96.73	(4.9)	98.14	(1.2)	97.58	(1.6)
λ_Z ($hours^{-1}$)	0.2810	(48.0)	0.3408	(45.9)	0.2034	(58.0)
T_{half} (hours)	4.97	(142.9)	2.88	(83.5)	4.61	(55.3)

* Median (range)

** n=7 for $AUC_{0-\infty}$, λ_Z and T_{half}

*** n=8 for $AUC_{0-\infty}$, λ_Z and T_{half}

[00186] Criteria for Bioequivalence: Statistical inference of eflornithine was to be based on a bioequivalence approach using the ratio of geometric LSmeans with corresponding 90% confidence interval calculated from the exponential of the difference between Treatment 1 *vs* Treatment 2, Treatment 2 *vs* Treatment 4 and Treatment 1 *vs* Treatment 4 for the ln-transformed parameters C_{max}, AUC_{0-T} and $AUC_{0-\infty}$ were all to be compared to the 80.00 to 125.00% range. Statistical inference of sulindac was to be based on a bioequivalence approach using the ratio of geometric LSmeans with corresponding 90% confidence interval calculated from the exponential of the difference between Treatment 1 *vs* Treatment 3, Treatment 3 *vs* Treatment 4 and Treatment 1 *vs* Treatment 4 for the ln-transformed parameters C_{max}, AUC_{0-T} and $AUC_{0-\infty}$ were all to be compared to the 80.00 to 125.00% range. The same criteria were to be applied for sulindac sulfide and sulindac sulfone and the results were to be presented as supportive evidence of comparable therapeutic outcome.

[00187] Safety results: A total of 12 subjects entered the study, and all subjects received the 4 treatments under study. No serious adverse events (SAE) and no deaths were reported for any of the subjects enrolled in this study. No subject was withdrawn by the investigator for safety reasons. A total of 4 treatment-emergent adverse events (TEAEs) were

reported by 4 (33%) of the 12 subjects who participated in this study. Of these events, 2 occurred after administration Treatment 1, 1 after administration of Treatment 3, and the remaining one after administration of Treatment 4. Subjects dosed with Treatment 2 did not report any TEAEs. Half of the TEAEs experienced during the study were considered related to drug administration.

[00188] The TEAEs in this study were experienced with a low incidence; they were experienced by 1 subject (8%) per treatment group. Dry mouth was reported following administration of Treatment 4, upper respiratory tract infection was reported following administration of Treatment 3, and vessel puncture site bruise and headache were each reported following administration of Treatment 1.

[00189] The incidence of TEAEs was the same for subjects dosed with Treatment 3 and Treatment 4 (8%) and slightly lower than the one reported for subjects dosed with Treatment 1 (17%). Drug-related TEAEs were reported with the same incidence for subjects dosed with Treatment 1 and Treatment 4 (8%), whereas subjects dosed with Treatment-3 did not experience drug-related TEAEs. The TEAEs experienced during the study were deemed mild (3/4, 75%) and moderate (1/4, 25%) in intensity. None of the subjects experienced a severe TEAE during the study.

[00190] All the abnormal clinical laboratory values were marginally higher or lower than their reference ranges and none were considered clinically significant by the investigator. Furthermore, there were no clinically significant abnormalities in the vital signs and ECGs of the subjects in this study. All physical examinations were judged normal. Overall, the drugs tested were generally safe and well tolerated by the subjects included in this study.

[00191] Eflornithine Comparison between Treatment 1 and Treatment 2: The pharmacokinetic results demonstrate that the geometric LSmean ratios and the corresponding 90% confidence intervals of C_{max}, AUC_{0-T}, and $AUC_{0-\infty}$ of eflornithine were all included within the range of 80.00% to 125.00%. The results of this comparison indicate that bioequivalence criteria were met when Treatment 1 and Treatment 2 were administered under fasting conditions and demonstrate that eflornithine bioavailability is comparable between the co-formulated tablet containing eflornithine/sulindac and the tablet containing eflornithine alone.

Table 17: Summary of Statistical Analysis of Eflornithine in Treatment 1 vs. Treatment 2

PARAMETER	INTRA-SUBJECT C.V. (%)	GEOMETRIC LSMEANS *		RATIO (%)	90% CONFIDENCE LIMITS (%)	
		Treatment-1 (n=12)	Treatment-2 (n=12)		LOWER	UPPER
C_{max}	16.8	10430.9	10030.8	103.99	92.42	117.01
AUC_{0-T}	13.5	69998.7	67701.4	103.39	94.03	113.69
$AUC_{0-\infty}$	13.4	70395.4	68056.2	103.44	94.17	113.61

* units are ng/mL for C_{max} and ng·h/mL for AUC_{0-T} and $AUC_{0-\infty}$

[00192] Eflornithine Comparison between Treatment 2 and Treatment 4: The pharmacokinetic results demonstrate that the geometric LSmean ratios and the corresponding 90% confidence intervals of C_{max}, AUC_{0-T}, and $AUC_{0-\infty}$ of eflornithine were all included within the range of 80.00% to 125.00%. The results of this comparison indicate that bioequivalence criteria were met when Treatment 2 and Treatment 4 were administered under fasted conditions and demonstrate that co-administration of sulindac with the individual tablet of eflornithine did not influence the bioavailability of eflornithine when administered alone.

Table 18: Summary of Statistical Analysis of Eflornithine in Treatment 2 vs. Treatment 4

PARAMETER	INTRA-SUBJECT C.V. (%)	GEOMETRIC LSMEANS *		RATIO (%)	90% CONFIDENCE LIMITS (%)	
		Treatment-2 (n=12)	Treatment-4 (n=12)		LOWER	UPPER
C_{max}	16.8	10030.8	9722.7	103.17	91.69	116.09
AUC_{0-T}	13.5	67701.4	68916.4	98.24	89.34	108.02
$AUC_{0-\infty}$	13.4	68056.2	69338.0	98.15	89.36	107.81

* units are ng/mL for C_{max} and ng·h/mL for AUC_{0-T} and $AUC_{0-\infty}$

[00193] Eflornithine Comparison between Treatment 1 and Treatment 4: The pharmacokinetic results demonstrate that the geometric LSmean ratios and the corresponding 90% confidence intervals of C_{max}, AUC_{0-T}, and $AUC_{0-\infty}$ of eflornithine were all included within the range of 80.00% to 125.00%. The results of this comparison indicate that bioequivalence criteria were met when Treatment 1 and Treatment 4 were administered under fasted conditions and demonstrate that the bioavailability of eflornithine for the co-formulated tablet containing

eflornithine/sulindac and the co-administration of individual tablets containing each eflornithine or sulindac is similar.

Table 19: Summary of Statistical Analysis of Eflornithine in Treatment 1 vs. Treatment 4

PARAMETER	INTRA-SUBJECT C.V. (%)	GEOMETRIC LSMEANS *		RATIO (%)	90% CONFIDENCE LIMITS (%)	
		Treatment-1 (n=12)	Treatment-4 (n=12)		LOWER	UPPER
C_{max}	16.8	10030.8	9722.7	107.28	95.35	120.72
AUC_{0-T}	13.5	67701.4	68916.4	101.57	92.37	111.68
$AUC_{0-\infty}$	13.4	68056.2	69338.0	101.53	92.43	111.51

* units are ng/mL for C_{max} and ng·h/mL for AUC_{0-T} and $AUC_{0-\infty}$

[00194] Sulindac Comparison between Treatment 1 between Treatment 3: The pharmacokinetic results demonstrate that the geometric LSmean ratios and the corresponding 90% confidence intervals (90CI) of C_{max}, AUC_{0-T}, and $AUC_{0-\infty}$ of sulindac were not all included within the range of 80.00% to 125.00%. The lower bound of the 90CI of C_{max} was below the 80.00% limit. Since the ratios were within the 80.00% to 125.00% range for all PK parameters, the intra-subject variability could account for the lower bound of C_{max} being outside the BE range. The results obtained for this comparison demonstrate that the sample size used in this pilot study was not sufficient to demonstrate equivalence of sulindac bioavailability from the co-formulated tablet and sulindac alone.

Table 20: Summary of the Statistical Analysis of Sulindac in Treatment 1 vs Treatment 3

PARAMETER	INTRA-SUBJECT C.V. (%)	GEOMETRIC LSMEANS *		RATIO (%)	90% CONFIDENCE LIMITS (%)	
		Treatment-1 (n=12)**	Treatment-3 (n=12)**		LOWER	UPPER
C_{max}	24.6	4353.6	4888.5	89.06	75.04	105.69
AUC_{0-T}	11.9	10746.4	11063.6	97.13	89.34	105.60
$AUC_{0-\infty}$	13.6	12029.4	12743.6	94.40	82.27	108.30

* units are ng/mL for C_{max} and ng·h/mL for AUC_{0-T} and $AUC_{0-\infty}$
** n=7 for $AUC_{0-\infty}$

[00195] Based on the data, the intra-subject variation, that incorporates the variability between all comparisons, is about 24.6% for C_{max} and about 12% for AUC_{0-T}. Statistically, given that the expected Treatment 1 to Treatment 3 ratio of geometric LSmeans felt within 90 and 110%, it is estimated that the number of subjects to meet the 80.00 to 125.00% bioequivalence range with a statistical *a priori* power of at least 80% would be about 54 for a future pivotal study. The inclusion of 60 subjects should be sufficient to account for the possibility of drop-outs and variations around the estimated intra-subject CV.

[00196] Sulindac Comparison between Treatment 3 and Treatment 4: The pharmacokinetic results demonstrate that the geometric LSmean ratios and the corresponding 90% confidence intervals of C_{max}, AUC_{0-T}, and $AUC_{0-\infty}$ of sulindac were all included within the range of 80.00% to 125.00%. The results of this comparison indicate that bioequivalence criteria were met when Treatment 3 and Treatment 4 were administered under fasted conditions and demonstrate that the co-administration of individual tablets containing eflornithine or sulindac did not influence the bioavailability of sulindac when administered alone.

Table 21: Summary of the Statistical Analysis of Sulindac in Treatment 3 vs. Treatment 4

PARAMETER	INTRA-SUBJECT C.V. (%)	GEOMETRIC LSMEANS *		RATIO (%)	90% CONFIDENCE LIMITS (%)	
		Treatment-3 (n=12)**	Treatment-4 (n=12)**		LOWER	UPPER
C_{max}	24.6	4888.5	4704.2	103.92	87.56	123.32
AUC_{0-T}	11.9	11063.6	10530.9	105.06	96.63	114.22
$AUC_{0-\infty}$	13.6	12743.6	11834.3	107.68	93.31	124.27

* units are ng/mL for C_{max} and ng·h/mL for AUC_{0-T} and $AUC_{0-\infty}$
** n=7 for $AUC_{0-\infty}$

[00197] Sulindac Comparison between Treatment 1 and Treatment 4: The pharmacokinetic results demonstrate that the geometric LSmean ratios and the corresponding 90% confidence intervals (90CI) of C_{max}, AUC_{0-T}, and $AUC_{0-\infty}$ of sulindac were not all included within the range of 80.00% to 125.00%. The lower bound of the 90CI of C_{max} was below the 80.00% limit. Since the ratios are within the 80.00% to 125.00% range for all PK parameters, the intra-subject variability could account for the lower bound of C_{max} being outside the BE range. The results obtained for this comparison demonstrate that the sample size used in this pilot study was not sufficient to demonstrate bioequivalence of sulindac bioavailability from the co-formulated tablet and the co-administration of individual tablets containing eflornithine or sulindac.

Table 22: Summary of the Statistical Analysis of Sulindac in Treatment 1 vs. Treatment 4

PARAMETER	INTRA-SUBJECT C.V. (%)	GEOMETRIC LSMEANS *		RATIO (%)	90% CONFIDENCE LIMITS (%)	
		Treatment-1 (n=12)**	Treatment-4 (n=12)**		LOWER	UPPER
C_{max}	24.6	4353.6	4704.2	92.55	77.98	109.83
AUC_{0-T}	11.9	10746.4	10530.9	102.05	93.86	110.94
$AUC_{0-\infty}$	13.6	12029.4	11834.3	101.65	88.09	117.30

* units are ng/mL for C_{max} and ng·h/mL for AUC_{0-T} and $AUC_{0-\infty}$
** n=8 for $AUC_{0-\infty}$

[00198] Based on the data, the intra-subject variation, that incorporates the variability between all comparisons, is about 24.6% for C_{max} and about 12% for AUC_{0-T}. Statistically, given that the expected Treatment 1 to Treatment 4 ratio of geometric LSmeans felt within 92.5and 107.5%, it is estimated that the number of subjects to meet the 80.00 to 125.00% bioequivalence range with a statistical *a priori* power of at least 80% would be about

36 for a future pivotal study. The inclusion of 40 subjects should be sufficient to account for the possibility of drop-outs and variations around the estimated intra-subject CV.

* * *

[00199] All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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U.S. Patent 4,413,141

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U.S. Patent 6,258,845

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What Is Claimed Is:

1. A composition comprising a fixed dose combination of a pharmaceutically effective amount of eflornithine and a pharmaceutically effective amount of a nonsteroidal anti-inflammatory drug (NSAID) or a metabolite thereof.

2. The composition of claim 1, wherein the fixed dose combination is a pharmaceutically effective amount of eflornithine and a pharmaceutically effective amount of sulindac.

3. The composition of claim 2, wherein the eflornithine is eflornithine hydrochloride monohydrate.

4. The composition of claim 3, wherein the eflornithine hydrochloride monohydrate is a racemic mixture of its two enantiomers.

5. The composition of claim 3, wherein the eflornithine hydrochloride monohydrate is a substantially optically pure preparation.

6. The composition of claim 4, wherein the amount of eflornithine hydrochloride monohydrate racemate is from about 10 mg to about 1000 mg.

7. The composition of claim 6, wherein the amount of eflornithine hydrochloride monohydrate racemate is from about 250 mg to about 500 mg.

8. The composition of claim 7, wherein the amount of eflornithine hydrochloride monohydrate racemate is from about 300 mg to about 450 mg.

9. The composition of claim 8, wherein the amount of eflornithine hydrochloride monohydrate racemate is from about 350 mg to about 400 mg.

10. The composition of claim 9, wherein the amount of eflornithine hydrochloride monohydrate racemate is about 375 mg.

11. The composition of claim 10, wherein the amount of eflornithine hydrochloride monohydrate racemate is 375 mg.

12. The composition of claim 4, wherein the amount of eflornithine hydrochloride monohydrate racemate is from about 35 to about 60 weight percent.

13. The composition of claim 12, wherein the amount of eflornithine hydrochloride monohydrate racemate is from about 40 to about 55 weight percent.

14. The composition of claim 13, wherein the amount of eflornithine hydrochloride monohydrate racemate is from about 50 to about 55 weight percent.

15. The composition of claim 14, wherein the amount of eflornithine hydrochloride monohydrate racemate is from about 52 to about 54 weight percent.

16. The composition of claim 15, wherein the amount of eflornithine hydrochloride monohydrate racemate is from 52 to 54 weight percent.

17. The composition according to any one of claims 2-16, wherein the amount of sulindac is from about 10 mg to about 250 mg.

18. The composition of claim 17, wherein the amount of sulindac is from about 50 mg to about 100 mg.

19. The composition of claim 18, wherein the amount of sulindac is from about 70 mg to about 80 mg.

20. The composition of claim 19, wherein the amount of sulindac is about 75 mg.

21. The composition of claim 20, wherein the amount of sulindac is 75 mg.

22. The composition according to any one of claims 2-16, wherein the amount of sulindac is from about 5 to about 20 weight percent.

23. The composition of claim 22, wherein the amount of sulindac is from about 8 to about 15 weight percent.

24. The composition of claim 23, wherein the amount of sulindac is from about 10 to about 12 weight percent.

25. The composition of claim 24, wherein the amount of sulindac is from 10 to 11 weight percent.

26. The composition according to any one of claims 2-25, further comprising an excipient.

27. The composition of claim 26, wherein the excipient is starch, colloidal silicon dioxide, or silicified microcrystalline cellulose.

28. The composition of claim 26, wherein the excipient is colloidal silicon dioxide.

29. The composition of claim 28, wherein the composition further comprises a second excipient.

30. The composition of claim 29, wherein the second excipient is silicified microcrystalline cellulose.

31. The composition according to any one of claims 2-30, further comprising a lubricant.

32. The composition of claim 31, wherein the lubricant is magnesium stearate, calcium stearate, sodium stearate, glyceryl monostearate, aluminum stearate, polyethylene glycol, boric acid, or sodium benzoate.

33. The composition of claim 32, wherein the lubricant is magnesium stearate.

34. The composition of claim 33, wherein the amount of magnesium stearate is from about 0.25 to about 2 weight percent.

35. The composition of claim 34, wherein the amount of magnesium stearate is from about 0.75 to about 2 weight percent.

36. The composition of claim 35, wherein the amount of magnesium stearate is from about 1 to about 1.5 weight percent.

37. The composition of claim 36, wherein the amount of magnesium stearate is about 1.1 weight percent.

38. The composition of claim 36, wherein the amount of magnesium stearate is about 1.5 weight percent.

39. The composition according to any one of claims 2-38, wherein the composition is in the form of a capsule, tablet, mini-tablet, granule, pellet, solution, gel, cream, foam, or patch.

40. The composition of claim 39, wherein the composition is in the form of a tablet.

41. The composition of claim 40, wherein the weight of the tablet is from about 650 mg to about 1,000 mg.

42. The composition of claim 41, wherein the weight of the tablet is from about 675 mg to about 725 mg.

43. The composition of claim 42, wherein the weight of the tablet is about 700 mg.

44. The composition of claim 40, wherein the tablet further comprises a coating.

45. The composition of claim 44, wherein the coating is a modified release coating or an enteric coating.

46. The composition of claim 45, wherein the coating is further defined as a pH-responsive coating.

47. The composition of claim 45, wherein the coating comprises cellulose acetate phthalate (CAP), cellulose acetate trimelletate (CAT), poly (vinyl acetate) phthalate (PVAP), hydroxypropylmethylcellulose phthalate (HP), poly(methacrylate ethylacrylate) (1:1) copolymer (MA-EA), poly(methacrylate methylmethacrylate) (1:1) copolymer (MA MMA), poly(methacrylate methylmethacrylate) (1:2) copolymer, or hydroxypropylmethylcellulose acetate succinate (HPMCAS).

48. The composition of claim 44, wherein the coating masks the taste of eflornithine.

49. The composition of claim 49, wherein the coating comprises hydroxypropyl methylcellulose, titanium dioxide, polyethylene glycol, and iron oxide yellow.

50. The composition according to any one of claims 44-49, wherein the amount of coating is from about 1 to about 5 weight percent.

51. The composition of claim 50, wherein the amount of coating is from about 2 to about 4 weight percent.

52. The composition of claim 51, wherein the amount of coating is about 3 weight percent.

53. The composition according to any one of claims 44-49, wherein the amount of coating is from about 5 mg to about 30 mg.

54. The composition of claim 53, wherein the amount of coating is from about 15 mg to about 25 mg.

55. The composition of claim 54, wherein the amount of coating is about 21 mg.

56. The composition of claim 44, wherein the weight of the tablet is from about 675 mg to about 750 mg.

57. The composition of claim 56, wherein the weight of the tablet is from about 700 mg to about 725 mg.

58. The composition of claim 57, wherein the weight of the tablet is about 721 mg.

59. A method of preventing and/or treating a disease or condition in a patient in need thereof, comprising administering to the patient the composition of any one of claims 2-58.

60. The method of claim 59, further comprising administering to the patient a second composition of any one of claims 2-58, wherein the first and the second compositions comprise the same fixed dose combinations.

61. The method of claim 60, wherein the first and the second administration occurs simultaneously.

62. The method of claim 61, wherein the second administration follows the first administration by an interval of 1 second to 1 hour.

63. The method according to any one of claims 60-62, wherein the first and the second compositions are both formulated as tablets and contain the same amounts of eflornithine and sulindac.

64. The method according to any one of claims 59-63, wherein the disease is cancer.

65. The method of claim 64, wherein the cancer is colon cancer, breast cancer, pancreatic cancer, brain cancer, lung cancers, stomach cancer, a blood cancer, skin cancer, testicular cancer, prostate cancer, ovarian cancer, liver cancer, or esophageal cancer.

66. The method of claim 65, wherein the colon cancer is familial adenomatous polyposis.

67. The method of claim 64, wherein the cancer is neuroblastoma.

68. The method of claim 59, wherein the condition is a skin condition.

69. The method of claim 68, wherein the skin condition is facial hirsutism.

70. The method of claim 59, wherein the composition is administered orally, intraarterially, intravenously, or topically.

71. The method of claim 59, wherein the composition is administered orally.

72. The method of claim 59, wherein the composition is administered every 12 hours.

73. The method of claim 59, wherein the composition is administered every 24 hours.

74. The method of claim 59, wherein the composition is administered at least a second time.

75. A method of producing the tablet according to any one of claims 40-58 comprising:

(a) pre-mixing sulindac and an excipient to form a first mixture;

(b) mixing the first mixture with a second mixture comprising eflornithine and an excipient to form a blend;

(c) screening the blend to form a granulated blend;

(d) adding a lubricant to the granulated blend to obtain a final blend; and

(e) applying a compression force to the final blend to form the tablet.

76. The method of claim 75, further comprising mixing the granulated blend prior to step (d) and mixing the final blend prior to step (e).

77. The method of claim 75, wherein there are two excipients in the first mixture, wherein the first excipient is colloidal silicon dioxide, and the second excipient is silicified microcrystalline cellulose.

78. The method of either claim 75 or 77, wherein the excipient of the second mixture is silicified microcrystalline cellulose.

79. The method of claim 75, wherein the pre-mixing is performed in a polyethylene-coated container.

80. The method of claim 75 or 76, wherein the mixing is performed in a diffusion blender.

81. The method according to any one of claims 75-77, wherein the lubricant is magnesium stearate.

82. The method of claim 81, wherein the magnesium stearate is sieved through a screen prior to step (d).

83. The method of claim 82, wherein the screen is a 500 μm screen.

84. The method of claim 75, wherein screening comprises applying the blend to a rotative calibrator.

85. The method of claim 84, wherein the rotative calibrator comprises a 1.0 mm screen.

86. The method of claim 75, further comprising a pre-compression step after step (d) and prior to step (e), wherein the blend is compressed with a force lower than the force of step (e) to form a pre-compressed blend, further wherein the compression force of step (e) then acts on the pre-compressed blend to form the tablet.

87. The method of claim 86, wherein the pre-compression step prevents tablet capping.

88. The method of claim 81, wherein a compression force of the pre-compression step is applied at about 5 to about 15 percent of the compression force applied in step (e).

89. The method according to any one of claims 86-88, wherein the compression force of the pre-compression step is from 2.5 to 3.5 kN.

90. The method of claim 89, wherein the compression force of the pre-compression step is about 3 kN.

91. The method of claim 75, wherein the compression force of step (e) is from 20 to 35 kN.

92. The method of claim 91, wherein the compression force of step (e) is about 25 kN.

93. The method of claim 75, further comprising coating the tablet.

94. The method of claim 93, wherein the coating comprises hydroxypropyl methylcellulose, titanium dioxide, polyethylene glycol, and iron oxide yellow.

Product	Conditions	Packaging	T6 M	T0
Eflornithine/ Sulindac 375mg/75mg	25°C/75% HR	Closed bottles	6,0	6,1
	30°C/65% HR	Closed bottles	6,0	
	40°C/75% HR	Opened bottles	6,4	
	40°C/75% HR	Closed bottles	6,2	
Eflornithine 250mg	25°C/75% HR	Closed bottles	6,9	6,5
	30°C/65% HR	Closed bottles	7,1	
	40°C/75% HR	Opened bottles	7,5	
	40°C/75% HR	Closed bottles	7,3	
Sulindac 150mg	25°C/75% HR	Closed bottles	2,9	2,9
	30°C/65% HR	Closed bottles	3,2	
	40°C/75% HR	Opened bottles	3,5	
	40°C/75% HR	Closed bottles	3,2	

FIG. 1

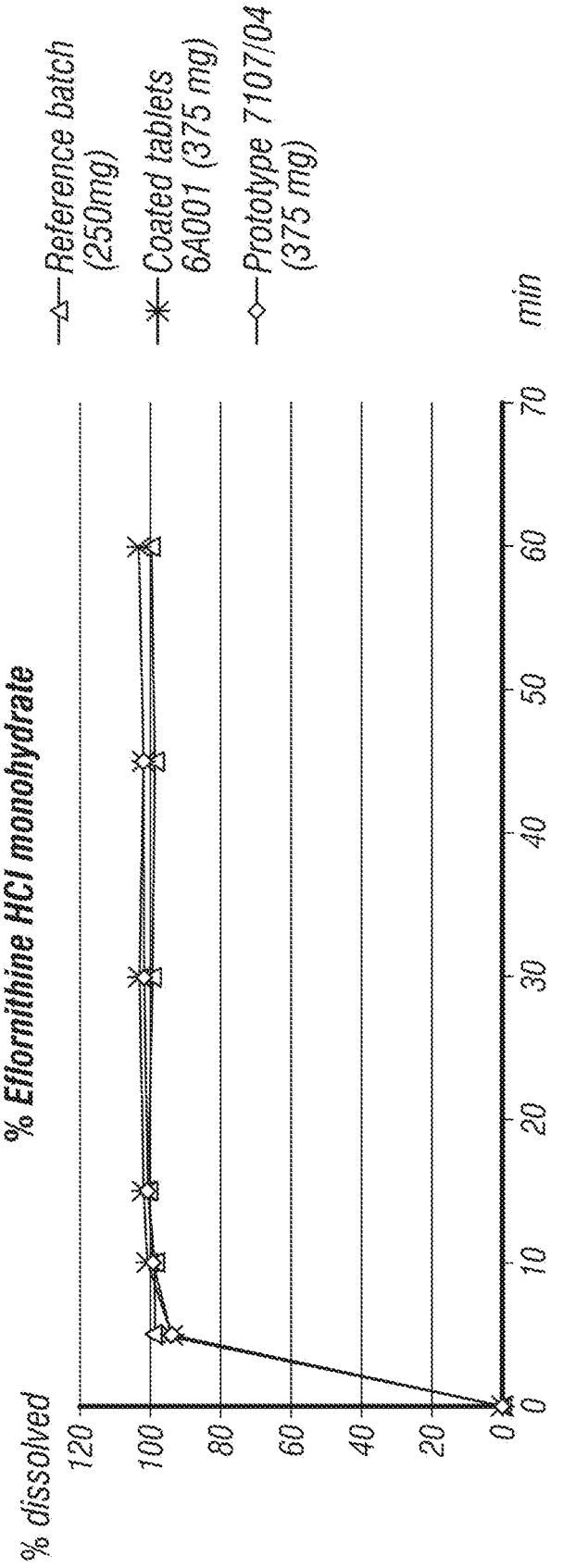
% Eflornithine HCl monohydrate
% dissolved
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
min
Reference batch (250mg)
Coated tablets 6A001 (375 mg)
Prototype 7107/04 (375 mg)

FIG. 2A

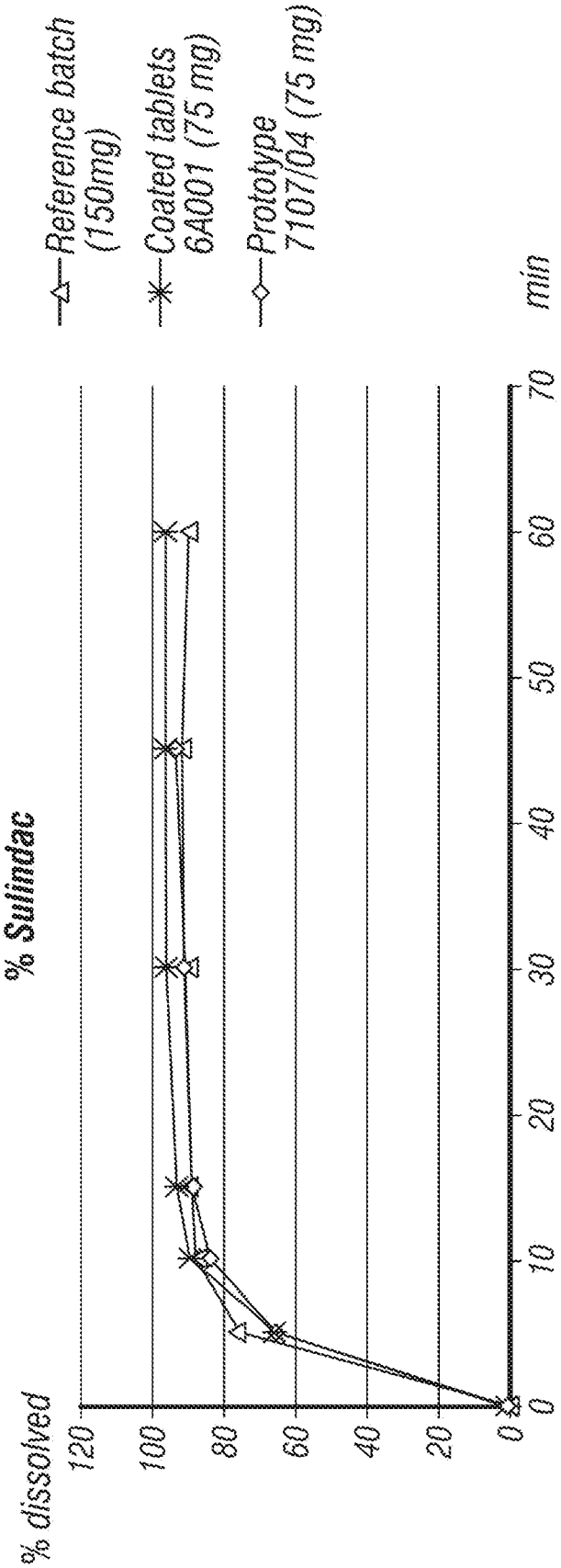
% Sulindac
% dissolved
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
min
Reference batch (150mg)
Coated tablets 6A001 (75 mg)
Prototype 7107/04 (75 mg)

FIG. 2B

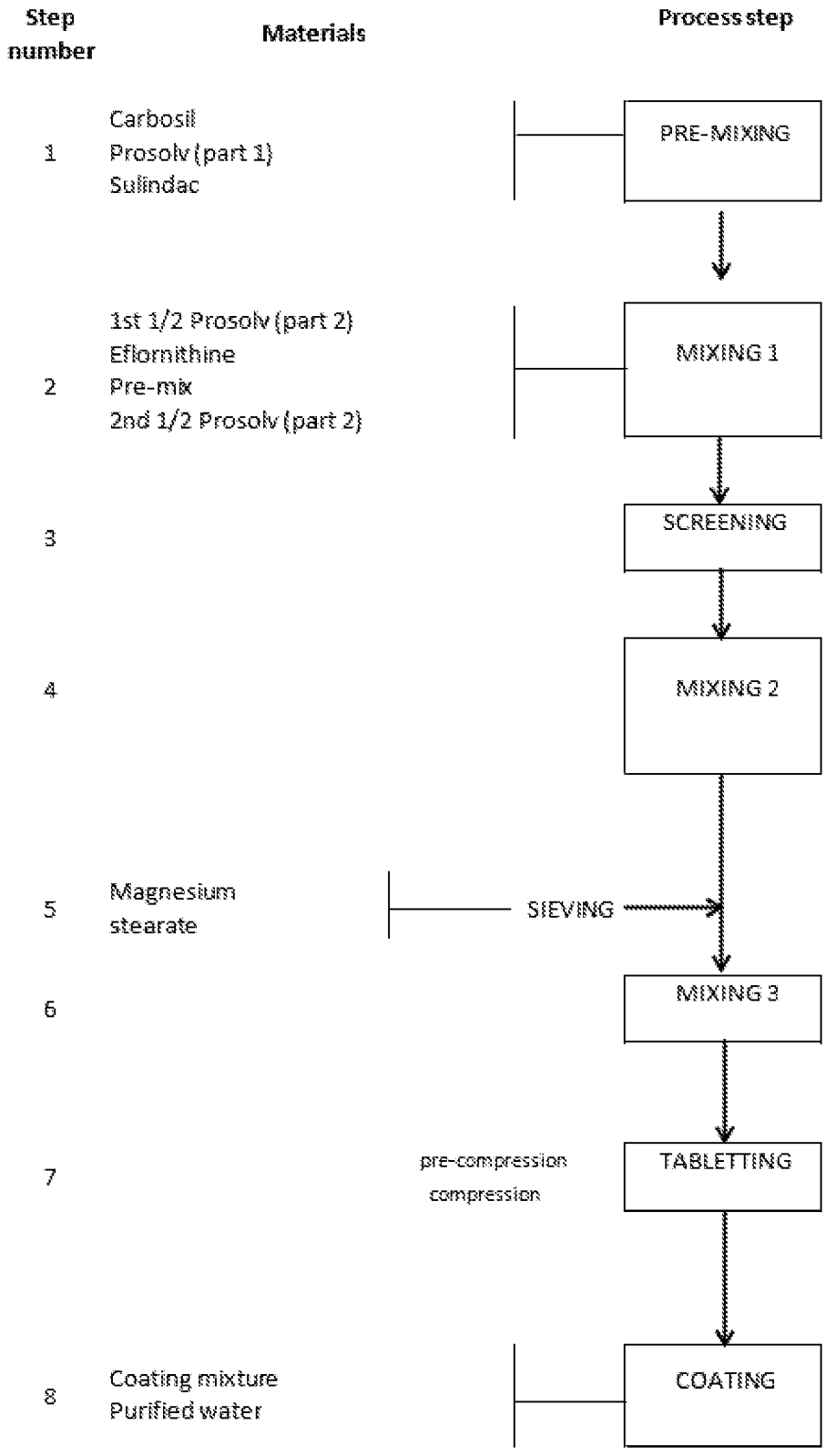
Step number
Materials
Process step
1
Carbosil
Prosolv (part 1)
Sulindac
PRE-MIXING
2
1st 1/2 Prosolv (part 2)
Eflornithine
Pre-mix
2nd 1/2 Prosolv (part 2)
MIXING 1
3
SCREENING
4
MIXING 2
5
Magnesium stearate
SIEVING
6
MIXING 3
7
pre-compression
compression
TABLETTING
8
Coating mixture
Purified water
COATING

FIG. 3

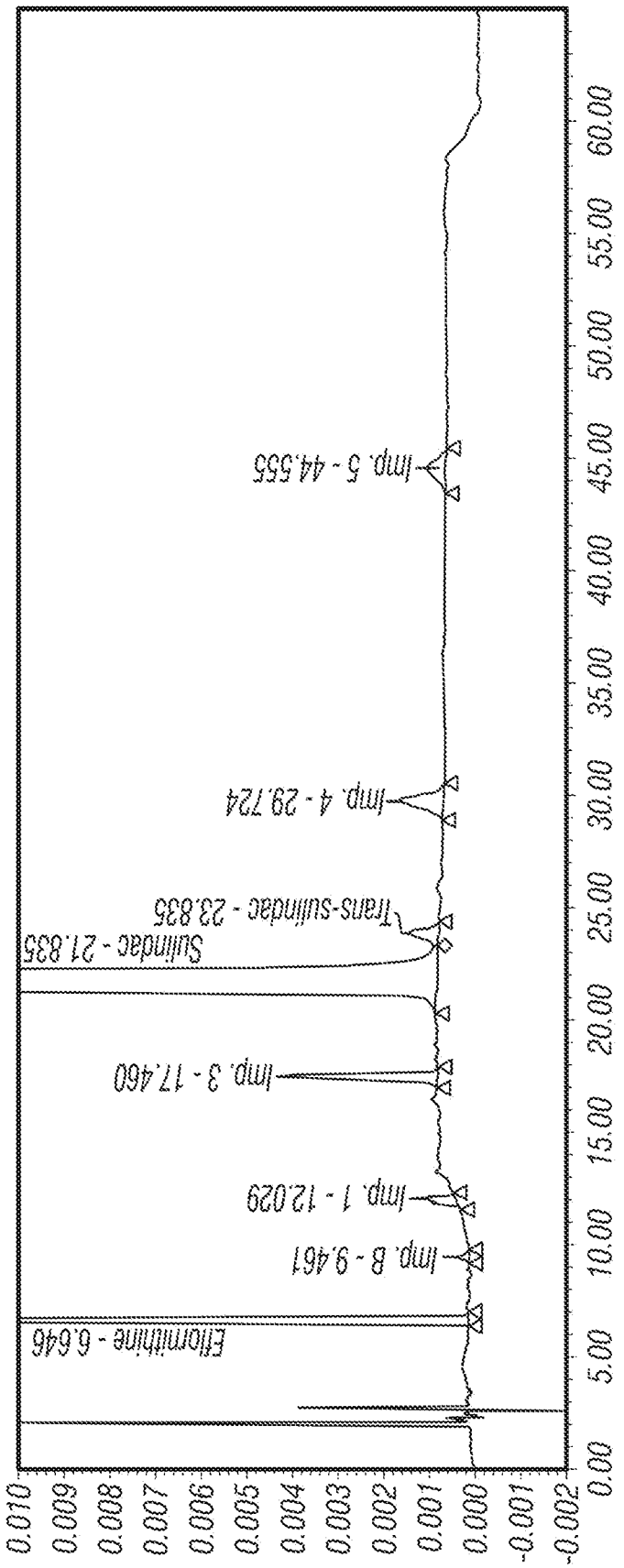
Imp. 5 - 44.555
Imp. 4 - 29.724
Trans-sulindac - 23.835
Sulindac - 21.835
Imp. 3 - 17.460
Imp. 1 - 12.029
Imp. B - 9.461
Eflornithine - 6.646
0.010
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
-0.001
-0.002
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00

FIG. 4A

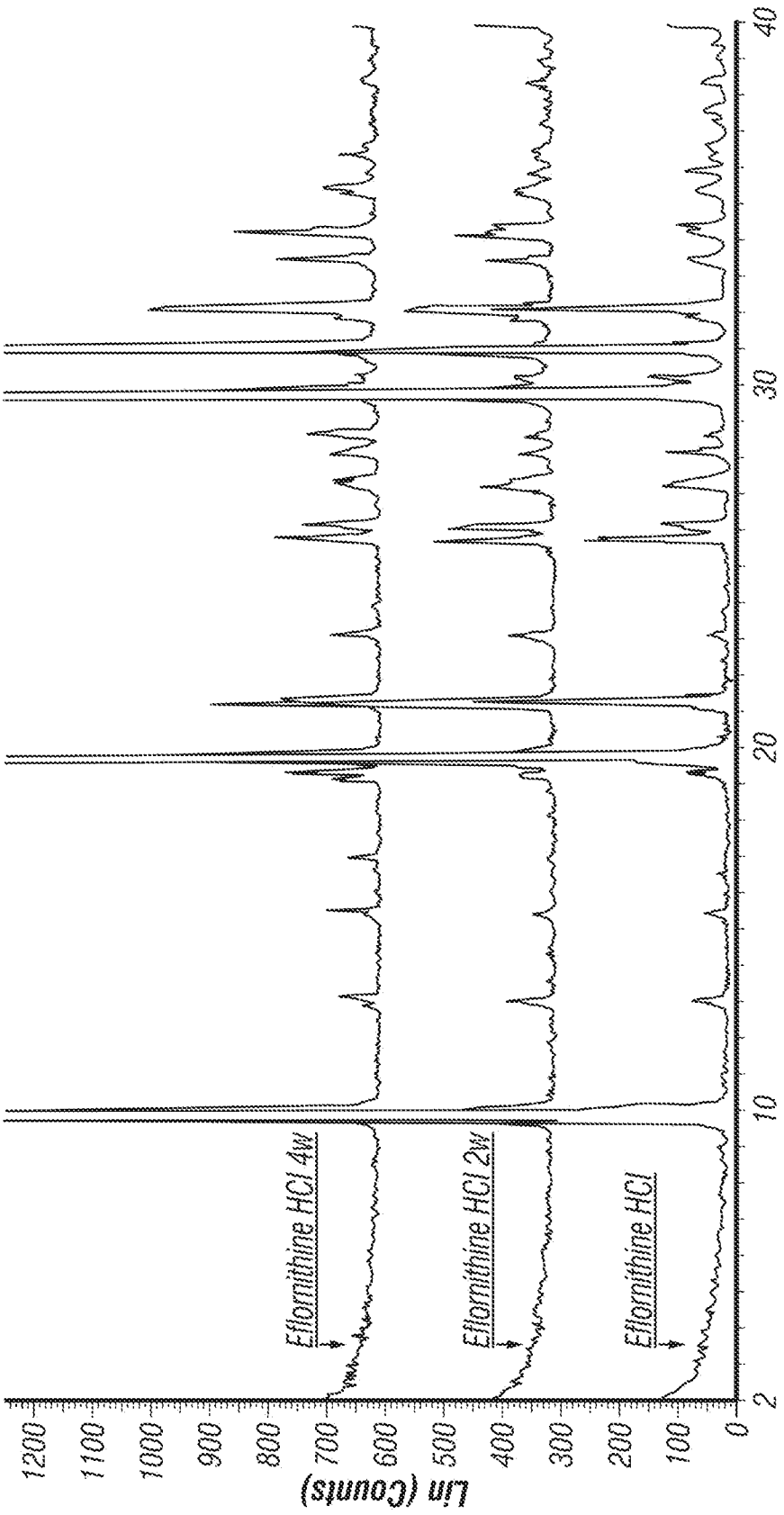
Eflornithine HCl 4w
Eflornithine HCl 2w
Eflornithine HCl
Lin (Counts)
2-Theta - Scale
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
2
10
20
30
40

FIG. 4B

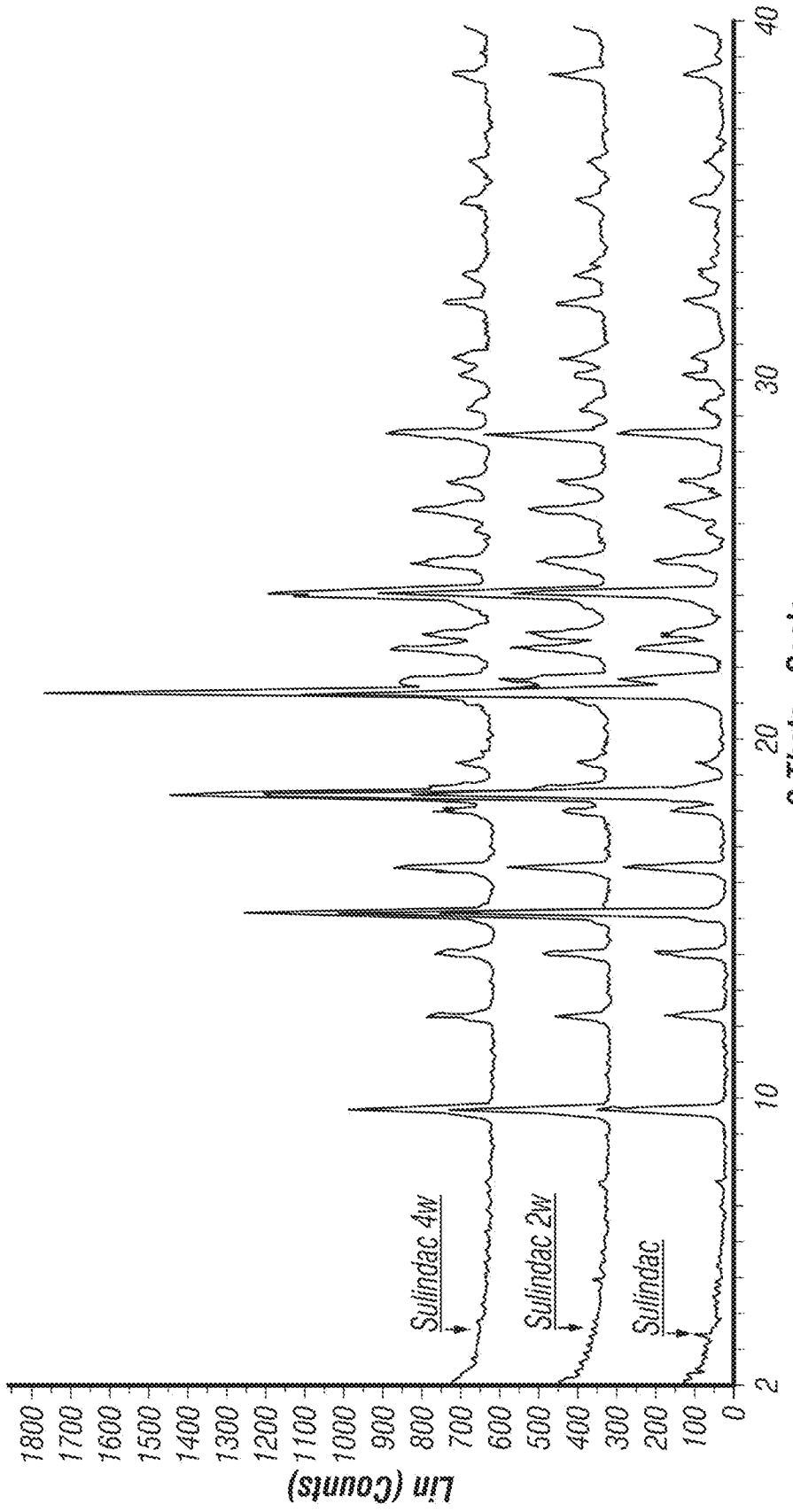
Lin (Counts)
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
2
10
20
30
40
2-Theta - Scale
Sulindac 4w
Sulindac 2w
Sulindac

FIG. 4C

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/059689

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/192 A61K31/198 A61K9/20 A61K9/28 A61K45/06
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)
A61K

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, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/49859 A1 (UNIV ARIZONA [US]; UNIV CALIFORNIA [US]; GERNER EUGENE W [US]; MEYSKEN) 7 October 1999 (1999-10-07)	1-27, 31-43, 59-66, 68,70-74
Y	page 5, lines 3-26 page 20, line 26 - page 21, line 25 page 36, lines 22-23 claims	1-94

X	Anonymous: "NCT01483144 on 2015_07_28: ClinicalTrials.gov Archive", , 28 July 2015 (2015-07-28), XP055337328, Retrieved from the Internet: URL:https://clinicaltrials.gov/archive/NCT01483144/2015_07_28 [retrieved on 2017-01-20]	1-26, 39-43, 59-66, 70-74
Y	the whole document	1-94

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"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
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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	Gradassi, Giulia

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Anonymous: "NCT01245816 on 2015_04_23: ClinicalTrials.gov Archive", , 23 April 2015 (2015-04-23), XP055337339, Retrieved from the Internet: URL:https://clinicaltrials.gov/archive/NCT01245816/2015_04_23 [retrieved on 2017-01-20]	1-26, 39-43, 59-66, 70-74
Y	the whole document	1-94
X	F. L. MEYSKENS ET AL: "Difluoromethylornithine Plus Sulindac for the Prevention of Sporadic Colorectal Adenomas: A Randomized Placebo-Controlled, Double-Blind Trial", CANCER PREVENTION RESEARCH, vol. 1, no. 1, 14 April 2008 (2008-04-14), pages 32-38, XP055085335, ISSN: 1940-6207, DOI: 10.1158/1940-6207.CAPR-08-0042	1-26, 39-43, 59-66, 70-74
Y	abstract page 33, left-hand column, paragraph 5 table 2	1-94
X	WO 2010/056919 A2 (KYPHIA PHARMACEUTICALS INC [US]; XU FENG [US]) 20 May 2010 (2010-05-20)	1
Y	paragraphs [0012], [0036], [0037], [0078] - [0081], [0085] paragraphs [00109], [00208]	1-94
Y	US 2002/110590 A1 (SHAKED ZE EV [US] ET AL) 15 August 2002 (2002-08-15) examples 11,18	1-94
Y	US 4 309 442 A (BEY PHILIPPE ET AL) 5 January 1982 (1982-01-05) examples 11,13	1-94
Y	US 2005/059690 A1 (NEWMAN KENNETH [US] ET AL) 17 March 2005 (2005-03-17) paragraphs [0015], [0050] - [0056]; example 5	1-94
Y	EP 2 438 919 A1 (HORIZON PHARMA USA INC [US]) 11 April 2012 (2012-04-11) paragraphs [0102], [0108], [0133], [0199] - [0211], [0194] - [0136], [0144]	1-94

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/059689

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62/358,698 2016.07.06 US

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(71)申请人 癌症预防制药股份有限公司
地址 美国亚利桑那州

(72)发明人 派脆克·雪南
罗伯特欧·卡罗斯·布拉弗欧 甘萨莱斯
吉恩·杜卡萨屋

(74)专利代理机构 北京世峰知识产权代理有限公司 11713
代理人 康健 王思琪

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A61K 9/20(2006.01)
A61K 9/28(2006.01)
A61K 45/06(2006.01)
A61P 35/00(2006.01)

权利要求书4页 说明书45页 附图7页

(54)发明名称

依氟鸟氨酸和舒林酸,固定剂量的组合制剂

(57)摘要

本文提供了药学上有效量的依氟鸟氨酸与药学上有效量的舒林酸的固定剂量组合制剂。还提供了这些制剂的使用方法和制造方法。

CN 108366982 A

1.包含药学上有效量的依氟鸟氨酸和药学上有效量的非甾体抗炎药(NSAID)或其代谢物的固定剂量组合的组合物。

2.如权利要求1所述的组合物,其中所述固定剂量组合是药学上有效量的依氟鸟氨酸和药学上有效量的舒林酸。

3.如权利要求2所述的组合物,其中所述依氟鸟氨酸是依氟鸟氨酸盐酸盐一水合物。

4.如权利要求3所述的组合物,其中所述依氟鸟氨酸盐酸盐一水合物是其两种对映异构体的外消旋混合物。

5.如权利要求3所述的组合物,其中所述依氟鸟氨酸盐酸盐一水合物是基本上光学纯的制剂。

6.如权利要求4所述的组合物,其中依氟鸟氨酸盐酸盐一水合物外消旋物的量为约10mg至约1000mg。

7.如权利要求6所述的组合物,其中依氟鸟氨酸盐酸盐一水合物外消旋物的量为约250mg至约500mg。

8.如权利要求7所述的组合物,其中依氟鸟氨酸盐酸盐一水合物外消旋物的量为约300mg至约450mg。

9.如权利要求8所述的组合物,其中依氟鸟氨酸盐酸盐一水合物外消旋物的量为约350mg至约400mg。

10.如权利要求9所述的组合物,其中依氟鸟氨酸盐酸盐一水合物外消旋物的量为约375mg。

11.如权利要求10所述的组合物,其中依氟鸟氨酸盐酸盐一水合物外消旋物的量为375mg。

12.如权利要求4所述的组合物,其中依氟鸟氨酸盐酸盐一水合物外消旋物的量为约35重量%至约60重量%。

13.如权利要求12所述的组合物,其中依氟鸟氨酸盐酸盐一水合物外消旋物的量为约40重量%至约55重量%。

14.如权利要求13所述的组合物,其中依氟鸟氨酸盐酸盐一水合物外消旋物的量为约50重量%至约55重量%。

15.如权利要求14所述的组合物,其中依氟鸟氨酸盐酸盐一水合物外消旋物的量为约52重量%至约54重量%。

16.如权利要求15所述的组合物,其中依氟鸟氨酸盐酸盐一水合物外消旋物的量为52-54重量%。

17.如权利要求2-16中任一项所述的组合物,其中舒林酸的量为约10mg至约250mg。

18.如权利要求17所述的组合物,其中舒林酸的量为约50mg至约100mg。

19.如权利要求18所述的组合物,其中舒林酸的量为约70mg至约80mg。

20.如权利要求19所述的组合物,其中舒林酸的量为约75mg。

21.如权利要求20所述的组合物,其中舒林酸的量为75mg。

22.如权利要求2-16中任一项所述的组合物,其中舒林酸的量为约5重量%至约20重量%。

23.如权利要求22所述的组合物,其中舒林酸的量为约8重量%至约15重量%。

24.如权利要求23所述的组合物，其中舒林酸的量为约10重量％至约12重量％。

25.如权利要求24所述的组合物，其中舒林酸的量为10-11重量％。

26.如权利要求2-25中任一项所述的组合物，进一步包含赋形剂。

27.如权利要求26所述的组合物，其中所述赋形剂为淀粉、胶体二氧化硅或硅化微晶纤维素。

28.如权利要求26所述的组合物，其中所述赋形剂为胶体二氧化硅。

29.如权利要求28所述的组合物，其中所述组合物进一步包含第二赋形剂。

30.如权利要求29所述的组合物，其中所述第二赋形剂为硅化微晶纤维素。

31.如权利要求2-30中任一项所述的组合物，进一步包含润滑剂。

32.如权利要求31所述的组合物，其中所述润滑剂为硬脂酸镁、硬脂酸钙、硬脂酸钠、单硬脂酸甘油酯、硬脂酸铝、聚乙二醇、硼酸或苯甲酸钠。

33.如权利要求32所述的组合物，其中所述润滑剂为硬脂酸镁。

34.如权利要求33所述的组合物，其中硬脂酸镁的量为约0.25重量％至约2重量％。

35.如权利要求34所述的组合物，其中硬脂酸镁的量为约0.75重量％至约2重量％。

36.如权利要求35所述的组合物，其中硬脂酸镁的量为约1重量％至约1.5重量％。

37.如权利要求36所述的组合物，其中硬脂酸镁的量为约1.1重量％。

38.如权利要求36所述的组合物，其中硬脂酸镁的量为约1.5重量％。

39.如权利要求2-38中任一项所述的组合物，其中所述组合物呈胶囊、片剂、微型片剂、颗粒、丸剂、溶液、凝胶、乳膏、泡沫或贴剂的形式。

40.如权利要求39所述的组合物，其中所述组合物呈片剂的形式。

41.如权利要求40所述的组合物，其中所述片剂的重量为约650mg至约1,000mg。

42.如权利要求41所述的组合物，其中所述片剂的重量为约675mg至约725mg。

43.如权利要求42所述的组合物，其中所述片剂的重量为约700mg。

44.如权利要求40所述的组合物，其中所述片剂进一步包含包衣。

45.如权利要求44所述的组合物，其中所述包衣是改性释放包衣或肠溶衣。

46.如权利要求45所述的组合物，其中所述包衣被进一步限定为pH响应性包衣。

47.如权利要求45所述的组合物，其中所述包衣包含乙酸邻苯二甲酸纤维素(CAP)、乙酸偏苯三酸纤维素(CAT)、聚(乙酸乙烯酯)邻苯二甲酸酯(PVAP)、羟丙基甲基纤维素邻苯二甲酸酯(HP)、聚(甲基丙烯酸酯乙基丙烯酸酯)(1:1)共聚物(MA-EA)、聚(甲基丙烯酸酯甲基丙烯酸甲酯)(1:1)共聚物(MA MMA)、聚(甲基丙烯酸酯甲基丙烯酸甲酯)(1:2)共聚物或乙酸羟丙基甲基纤维素琥珀酸酯(HPMCAS)。

48.如权利要求44所述的组合物，其中所述包衣掩盖依氟鸟氨酸的味道。

49.如权利要求49所述的组合物，其中所述包衣包含羟丙基甲基纤维素、二氧化钛、聚乙二醇以及氧化铁黄。

50.如权利要求44-49中任一项所述的组合物，其中包衣的量为约1重量％至约5重量％。

51.如权利要求50所述的组合物，其中包衣的量为约2重量％至约4重量％。

52.如权利要求51所述的组合物，其中包衣的量为约3重量％。

53.如权利要求44-49中任一项所述的组合物，其中包衣的量为约5mg至约30mg。

54.如权利要求53所述的组合物，其中包衣的量为约15mg至约25mg。

55.如权利要求54所述的组合物，其中包衣的量为约21mg。

56.如权利要求44所述的组合物，其中所述片剂的重量为约675mg至约750mg。

57.如权利要求56所述的组合物，其中所述片剂的重量为约700mg至约725mg。

58.如权利要求57所述的组合物，其中所述片剂的重量为约721mg。

59.预防和/或治疗有需要的患者的疾病或病况的方法，包括向所述患者施用权利要求2-58中任一项所述的组合物。

60.如权利要求59所述的方法，进一步包括向所述患者施用权利要求2-58中任一项所述的第二组合物，其中第一组合物和第二组合物包含相同的固定剂量组合。

61.如权利要求60所述的方法，其中第一次施用和第二次施用同时发生。

62.如权利要求61所述的方法，其中所述第二次施用以1秒至1小时的间隔在第一次施用之后进行。

63.如权利要求60-62中任一项所述的方法，其中所述第一组合物和所述第二组合物都被配制为片剂，并且含有相同量的依氟鸟氨酸和舒林酸。

64.如权利要求59-63中任一项所述的方法，其中所述疾病是癌症。

65.如权利要求64所述的方法，其中所述癌症是结肠癌、乳腺癌、胰腺癌、脑癌、肺癌、胃癌、血癌、皮肤癌、睾丸癌、前列腺癌、卵巢癌、肝癌或食管癌。

66.如权利要求65所述的方法，其中所述结肠癌是家族性腺瘤性息肉病。

67.如权利要求64所述的方法，其中所述癌症是神经母细胞瘤。

68.如权利要求59所述的方法，其中所述病况是皮肤病况。

69.如权利要求68所述的方法，其中所述皮肤病况是面部多毛症。

70.如权利要求59所述的方法，其中口服、动脉内、静脉内或局部施用所述组合物。

71.如权利要求59所述的方法，其中口服施用所述组合物。

72.如权利要求59所述的方法，其中每12小时施用所述组合物。

73.如权利要求59所述的方法，其中每24小时施用所述组合物。

74.如权利要求59所述的方法，其中至少第二次施用所述组合物。

75.生产如权利要求40-58中任一项所述的片剂的方法，包括：

(a)预混合舒林酸和赋形剂以形成第一混合物；

(b)将所述第一混合物与包含依氟鸟氨酸和赋形剂的第二混合物混合以形成共混物；

(c)筛选所述共混物以形成粒状共混物；

(d)将润滑剂加入到所述粒状共混物中以获得最终共混物；以及

(e)对所述最终共混物施加压缩力以形成所述片剂。

76.如权利要求75所述的方法，进一步包括在步骤(d)之前混合所述粒状共混物，以及在步骤(e)之前混合所述最终共混物。

77.如权利要求75所述的方法，其中在所述第一混合物中存在两种赋形剂，其中第一赋形剂是胶体二氧化硅，并且第二赋形剂是硅化微晶纤维素。

78.如权利要求75或77所述的方法，其中所述第二混合物的赋形剂是硅化微晶纤维素。

79.如权利要求75所述的方法，其中在聚乙烯涂覆的容器中进行所述预混合。

80.如权利要求75或76所述的方法，其中在扩散搅拌机中进行所述混合。

81.如权利要求75-77中任一项所述的方法，其中所述润滑剂是硬脂酸镁。

82.如权利要求81所述的方法，其中在步骤(d)之前用筛网筛选所述硬脂酸镁。

83.如权利要求82所述的方法，其中所述筛网是500μm筛网。

84.如权利要求75所述的方法，其中筛选包括将所述共混物施加到旋转校准器。

85.如权利要求84所述的方法，其中所述旋转校准器包括1.0mm的筛网。

86.如权利要求75所述的方法，进一步包括在步骤(d)之后且在步骤(e)之前的预压缩步骤，其中用低于步骤(e)的力的力压缩所述共混物以形成预压缩共混物，进一步其中步骤(e)的压缩力然后作用于所述预压缩共混物以形成片剂。

87.如权利要求86所述的方法，其中所述预压缩步骤防止片剂加帽。

88.如权利要求81所述的方法，其中以步骤(e)中施加的压缩力的约5％至约15％施加所述预压缩步骤的压缩力。

89.如权利要求86-88中任一项所述的方法，其中所述预压缩步骤的压缩力为2.5-3.5kN。

90.如权利要求89所述的方法，其中所述预压缩步骤的压缩力为约3kN。

91.如权利要求75所述的方法，其中步骤(e)的压缩力为20-35kN。

92.如权利要求91所述的方法，其中步骤(e)的压缩力为约25kN。

93.如权利要求75所述的方法，进一步包括对所述片剂进行包衣。

94.如权利要求93所述的方法，其中所述包衣包含羟丙基甲基纤维素、二氧化钛、聚乙二醇和氧化铁黄。

依氟鸟氨酸和舒林酸,固定剂量的组合制剂

[0001] 本申请要求以下申请的优先权权益:2015年10月30日提交的美国临时申请第62/248,810号、2016年7月6日提交的美国临时申请第62/358,698号、2016年10月28日提交的欧洲申请第16306429.8号,以及2016年10月28日提交的欧洲申请第16306430.6号,通过引用将上述申请中的每一个整体专门并入本文。

背景技术

1.技术领域

[0002] 本发明大体上涉及癌症生物学和医学领域。具体而言,涉及用于预防和治疗癌的组合物。

[0003] 2.相关技术的描述

[0004] 癌细胞能共同选择多种途径以满足其对特定代谢物增加的需求(Vander Heiden,2011)。非甾体抗炎药(NSAID),包括阿司匹林、布洛芬、吡罗昔康(Reddy et al.,1990;Singh et al.,1994)、吲哚美辛(Narisawa,1981)和舒林酸(Piazza et al.,1997;Rao et al.,1995),在AOM处理的大鼠模型中有效抑制结肠癌变。舒林酸砜是NASAID舒林酸代谢物,尽管缺乏COX抑制活性,但仍然诱导肿瘤细胞凋亡(Piazza et al.,1995;Piazza et al.,1997b),并在几种啮齿动物癌变模型中抑制肿瘤发展(Thompson et al.,1995;Piazza et al.,1995,1997a)。

[0005] α-二氟甲基鸟氨酸(DFMO)是鸟氨酸脱羧酶(ODC)的酶激活的不可逆抑制剂,并导致腐胺及其衍生物亚精胺的细胞内浓度耗尽(Pegg,1988)。在实验动物模型中,DFMO是癌变的有效抑制剂,其在预防许多器官的致癌物诱导的上皮癌(包括结肠的那些)中尤其有效(Weeks et al.,1982;Thompson et al.,1985;Nowels et al.,1986;Nigro et al.,1987)。

[0006] 将癌症化学预防研究转化为临床实践的主要障碍是边际药剂效力和超出效益的毒性(Psaty and Potter,2006;Lippman,2006)。例如,已经在结肠直肠腺瘤(CRA)患者中证实了长期每日口服D,L-α二氟甲基鸟氨酸(DFMO,依氟鸟氨酸)和舒林酸的多胺抑制组合的显著疗效(Meyskens et al.,2008),然而,治疗与轻度的亚临床耳毒性相关(McLaren et al.,2008),以及在具有高基线心血管风险患者中与更多的心血管事件有关(Zell et al.,2009)。

[0007] 与定期施用许多单独剂量的两种或更多种药物相反,共同施用单位剂型中的两种或更多种活性药物成分的便利性已经在制药领域中得到认可,并且描述于美国专利第6,428,809号和第6,702,683号中。对患者和临床医生的潜在优势包括(1)最小化或消除局部和/或全身副作用;(2)更有效地治疗并存的疾病状况;(3)改善多重用药;(4)患者对整体疾病管理的依从性更好,这反过来可由于减少拜访医生的次数、减少住院和提高患者舒适度而导致成本降低。将两种或更多种制剂组合或共同配制成单一剂型的固定剂量组合产品可用于需要改善临床效果、增强患者依从性和简化剂量给药的多药治疗方案。然而,即使对于

单一活性药物成分(API)制剂而言,固体口服剂型的药物产品的开发在研究和开发水平以及商业制造水平上都是复杂的。对于多种API,预计会有其他复杂因素,包括(1)药物药物相互作用,(2)药物-赋形剂相互作用,(3)同时释放曲线,(4)差异释放曲线和(5)每种药物成分的混合均匀性。鉴于这些障碍,开发具有与单一实体药物产品相同或相似释放曲线的固定剂量组合特别代表着重大挑战。克服一些或全部这些挑战的依氟鸟氨酸和舒林酸的固定剂量组合,对于有效治疗和/或预防多种疾病或病症包括家族性腺瘤息肉病(FAP)具有显著的潜在影响。

发明内容

[0008] 一方面,本发明提供了包含药学上有效量的依氟鸟氨酸和药学上有效量的非甾体抗炎药(NSAID)或其代谢物的固定剂量组合的组合物。在一些实施方案中,固定剂量组合是药学上有效量的依氟鸟氨酸和药学上有效量的舒林酸。

[0009] 在一些实施方案中,依氟鸟氨酸是依氟鸟氨酸盐酸盐一水合物。在一些实施方案中,依氟鸟氨酸是依氟鸟氨酸盐酸盐一水合物外消旋物。在一些实施方案中,依氟鸟氨酸盐酸盐一水合物是其两种对映异构体的外消旋混合物。在一些实施方案中,依氟鸟氨酸盐酸盐一水合物实质上是光学纯的制剂。在一些实施方案中,依氟鸟氨酸盐酸盐一水合物是L-依氟鸟氨酸盐酸盐一水合物或D-依氟鸟氨酸盐酸盐一水合物。在一些实施方案中,依氟鸟氨酸是无水游离碱依氟鸟氨酸。

[0010] 在一些实施方案中,依氟鸟氨酸以约10mg至约1000mg的量存在。在一些实施方案中,依氟鸟氨酸以约250mg至约500mg的量存在。在一些实施方案中,依氟鸟氨酸以约300mg至约450mg的量存在。在一些实施方案中,依氟鸟氨酸以约350mg至约400mg的量存在。在一些实施方案中,依氟鸟氨酸以约35重量%至约60重量%的量存在。在一些实施方案中,依氟鸟氨酸以约40重量%至约55重量%的量存在。在一些实施方案中,依氟鸟氨酸以约50重量%至约55重量%的量存在。在一些实施方案中,依氟鸟氨酸以约52重量%至约54重量%的量存在。在一些实施方案中,依氟鸟氨酸盐酸盐一水合物外消旋物的量为52-54重量%。在一些实施方案中,依氟鸟氨酸以约375mg的量存在。在一些实施方案中,依氟鸟氨酸盐酸盐一水合物外消旋体的量为375mg。

[0011] 在一些实施方案中,舒林酸以约10mg至约1500mg的量存在。在一些实施方案中,舒林酸以约50mg至约100mg的量存在。在一些实施方案中,舒林酸以约70mg至约80mg的量存在。在一些实施方案中,舒林酸以约75mg的量存在。在一些实施方案中,舒林酸的量是75mg。在一些实施方案中,舒林酸以约5重量%至约20重量%的量存在。在一些实施方案中,舒林酸以约8重量%至约15重量%的量存在。在一些实施方案中,舒林酸以约10重量%至约12重量%的量存在。在一些实施方案中,舒林酸的量为10重量%至11重量%。

[0012] 在一些实施方案中,依氟鸟氨酸以约375mg的量存在,并且舒林酸以约75mg的量存在。

[0013] 在一些实施方案中,制剂进一步包含赋形剂。在一些实施方案中,赋形剂是淀粉、胶体二氧化硅或硅化微晶纤维素。在一些实施方案中,赋形剂是胶体二氧化硅。在一些实施方案中,制剂还包含第二赋形剂。在一些实施方案中,第二赋形剂是硅化微晶纤维素。

[0014] 在一些实施方案中,制剂进一步包含润滑剂。在一些实施方案中,润滑剂是硬脂酸

镁、硬脂酸钙、硬脂酸钠、单硬脂酸甘油酯、硬脂酸铝、聚乙二醇、硼酸或苯甲酸钠。在一些实施方案中，润滑剂是硬脂酸镁。在一些实施方案中，硬脂酸镁以约0.25重量％至约2重量％的量存在。在一些实施方案中，硬脂酸镁的量为约0.75重量％至约2重量％。在一些实施方案中，硬脂酸镁的量为约1重量％至约1.5重量％。在一些实施方案中，硬脂酸镁的量为约1.1重量％。在一些实施方案中，硬脂酸镁以约1.5重量％的量存在。

[0015] 在一些实施方案中，组合物采用胶囊、片剂、微型片剂(mini tablet)、颗粒、丸剂(pellet)、溶液、凝胶、乳膏、泡沫或贴剂的形式。在一些实施方案中，组合物采用片剂形式，例如单层片剂。

[0016] 在一些实施方案中，片剂的重量为约10mg至约2,500mg。在一些实施方案中，片剂的重量为约250mg至约1,500mg。在一些实施方案中，片剂的重量为约650mg至约1,000mg。在一些实施方案中，片剂的重量为约675mg至约725mg。在一些实施方案中，片剂的重量为约700mg。

[0017] 在一些实施方案中，胶囊、微型片剂、颗粒或丸剂的重量为约10mg至约2,500mg。在一些实施方案中，胶囊、微型片剂、颗粒或丸剂的重量为约250mg至约1,500mg。在一些实施方案中，胶囊、微型片剂、颗粒或丸剂的重量为约650mg至约1,000mg。在一些实施方案中，胶囊、微型片剂、颗粒或丸剂的重量为约675mg至约725mg。在一些实施方案中，胶囊、微型片剂、颗粒或丸剂的重量为约700mg。

[0018] 在一些实施方案中，片剂进一步包含包衣。在一些实施方案中，包衣是改性释放包衣或肠溶衣。在一些实施方案中，包衣是pH响应性包衣。在一些实施方案中，包衣包含乙酸邻苯二甲酸纤维素(CAP)、乙酸偏苯三酸纤维素(cellulose acetate trimelletate,CAT)，聚(乙酸乙烯酯)邻苯二甲酸酯(PVAP)、羟丙基甲基纤维素邻苯二甲酸酯(HP)、聚(甲基丙烯酸酯乙基丙烯酸酯)(1:1)共聚物(MA-EA)、聚(甲基丙烯酸酯甲基丙烯酸甲酯)(1:1)共聚物(MA MMA)、聚(甲基丙烯酸酯-甲基丙烯酸甲酯)(1:2)共聚物或乙酸羟丙基甲基纤维素琥珀酸酯(HPMCAS)。在一些实施方案中，包衣掩盖依氟鸟氨酸的味道。在一些实施方案中，包衣包含羟丙基甲基纤维素、二氧化钛、聚乙二醇和氧化铁黄。

[0019] 在一些实施方案中，包衣的量为约1重量％至约5重量％。在一些实施方案中，包衣的量为约2重量％至约4重量％。在一些实施方案中，包衣的量为约3重量％。在一些实施方案中，包衣的量为约5mg至约30mg。在一些实施方案中，包衣的量为约15mg至约25mg。在一些实施方案中，包衣的量为约21mg。

[0020] 在一些实施方案中，包含包衣的片剂的重量为约675mg至约750mg。在一些实施方案中，包含包衣的片剂的重量为约700mg至约725mg。在一些实施方案中，包含包衣的片剂的重量为约721mg。

[0021] 一方面，提供了用于预防和/或治疗有需要的患者的疾病或病况的方法，包括向患者施用本文提供的药学上有效量的依氟鸟氨酸和药学上有效量的舒林酸的固定剂量组合。

[0022] 在一些实施方案中，所述方法进一步包括向患者施用包含本文提供的药学上有效量的依氟鸟氨酸和药学上有效量的舒林酸的固定剂量组合的第二组合物。在一些实施方案中，第一组合物和第二组合物包含相同的固定剂量组合。在一些实施方案中，第一施用和第二施用同时发生。在一些实施方案中，第二施用在第一施用之后进行，间隔1秒至1小时。在一些实施方案中，第一组合物和第二组合物都配制成片剂并含有相同量的依氟鸟氨酸和舒

林酸。

[0023] 在一些实施方案中，疾病是癌症。在一些实施方案中，癌症是结肠癌、乳腺癌、胰腺癌、脑癌、肺癌、胃癌、血癌、皮肤癌、睾丸癌、前列腺癌、卵巢癌、肝癌或食管癌。在一些实施方案中，结肠癌是家族性腺瘤性息肉病。在一些实施方案中，癌症是神经内分泌瘤。在一些实施方案中，神经内分泌瘤是神经母细胞瘤。

[0024] 在一些实施方案中，病况是皮肤病况。在一些实施方案中，皮肤病况是面部多毛症。

[0025] 在一些实施方案中，口服、动脉内、静脉内或局部施用组合物。在一些实施方案中，口服施用组合物。

[0026] 在一些实施方案中，口服施用组合物。在一些实施方案中，每12小时施用组合物。在一些实施方案中，每24小时施用组合物。在一些实施方案中，至少第二次施用组合物。

[0027] 另一方面，提供了生产包含约375g依氟鸟氨酸盐酸盐和约75g舒林酸的片剂的方法，包括：(a)预混合舒林酸和赋形剂以形成第一混合物；(b)将所述第一混合物与包含依氟鸟氨酸和赋形剂的第二混合物混合以形成共混物；(c)筛选所述共混物以形成粒状共混物；(d)将润滑剂加入所述粒状共混物中以获得最终共混物；和(e)对最终共混物施加压缩力以形成片剂。在一些实施方案中，所述方法进一步包括在步骤(d)之前混合所述粒状共混物和在步骤(e)之前混合所述最终共混物。

[0028] 在一些实施方案中，第一混合物中存在两种赋形剂，其中第一赋形剂是胶体二氧化硅，第二赋形剂是硅化微晶纤维素。在一些实施方案中，第二混合物的赋形剂是硅化微晶纤维素。

[0029] 在一些实施方案中，预混合在聚乙烯涂覆的容器中进行。在一些实施方案中，混合在扩散搅拌机中进行。

[0030] 在一些实施方案中，润滑剂是硬脂酸镁。在一些实施方案中，在步骤(d)之前通过筛网(screen)筛选硬脂酸镁。在一些实施方案中，筛网是500μm的筛网。

[0031] 在一些实施方案中，筛选包括将共混物施加到旋转校准器。在一些实施方案中，旋转校准器包括1.0mm的筛网。

[0032] 在一些实施方案中，所述方法进一步包括在步骤(d)之后且在步骤(e)之前的预压缩步骤，其中用低于步骤(e)的力的力压缩所述共混物，以形成预压缩共混物，进一步其中步骤(e)的压缩力然后作用于所述预压缩共混物以形成片剂。在一些实施方案中，所述预压缩步骤防止片剂加帽(capping)。在一些实施方案中，以步骤(e)中施加的压缩力的约5％至约15％，施加所述预压缩步骤的压缩力。在一些实施方案中，所述预压缩步骤的压缩力为2.5-3.5kN。在一些实施方案中，所述预压缩步骤的压缩力为约3kN。在一些实施方案中，步骤(e)的压缩力为20-35kN。在一些实施方案中，步骤(e)的压缩力为约25kN。

[0033] 在一些实施方案中，所述方法进一步包括对片剂进行包衣。在一些实施方案中，包衣包含羟丙基甲基纤维素、二氧化钛、聚乙二醇和氧化铁黄。

[0034] 根据以下详细描述，本发明的其他目的、特征和优点会变得显而易见。然而，应当该理解的是，尽管指出了本发明的优选实施方案，但是详细描述和具体实例仅仅是以说明的方式给出，因为根据所述详细描述，在本发明的精神和范围内的各种改变和修改对于本领域技术人员而言会是显而易见的。

附图说明

[0035] 以下附图形成本说明书的一部分并且被包括在内以进一步说明本发明的某些方面。通过参考一个或多个这些附图并结合本文给出的具体实施方案的详细描述，可以更好地理解本发明。

[0036] 图1：依氟鸟氨酸HCl一水合物(375mg)和舒林酸(75mg)的700mg片剂的7107/04批原型的稳定性分析。片剂具有3％w/w的包衣。在零时(T0)和6个月(T6)时使用经验证的卡尔费休滴定法(Karl Fischer titration method)分析样品，用来测定含水量。样品在经过验证的稳定室中储存在有盖和没有盖的HDPE瓶中保存。数值表示在特定条件下每个片剂中水的百分比。

[0037] 图2A-2B：7107/04和6A001批包衣片剂的溶解分析结果。包括250mg依氟鸟氨酸HCl一水合物和市售150mg舒林酸的参照片剂，用于比较。共同配制的片剂含有375mg依氟鸟氨酸HCl一水合物和75mg舒林酸以及3％w/w的包衣。

[0038] 图3：简化方案，其描述了含有依氟鸟氨酸HCl一水合物和舒林酸的片剂的制造工艺。

[0039] 图4A-4C：(A)依氟鸟氨酸HCl一水合物和舒林酸共配制的片剂的典型HPLC色谱证明了测量所选杂质的能力。(B-C)与片剂赋形剂混合的依氟鸟氨酸HCl一水合物和舒林酸活性成分在0时、2周、4周的X射线粉末衍射(XRPD)图。变化的缺乏支持了赋形剂兼容性和晶型稳定性。

[0040] 说明性实施方案的描述

[0041] 在几个方面，提供了依氟鸟氨酸和舒林酸的固定剂量组合(FDC)的组合物。还提供了用于制造本发明的固定剂量组合的方法，其克服了与现有方法相关的问题。已经设计了制造方法来解决包括药物-药物相互作用、药物-赋形剂相互作用以及每种药物成分的混合均匀性在内的问题。因此，本发明的固定剂量组合可以用于使局部和/或全身副作用最小化，提供更有效的治疗，改善多重用药，并提供更好的患者依从性。

[0042] I.家族性腺瘤性息肉病

[0043] 长期以来，过量多胺的形成与上皮癌变，特别是结直肠癌变有关。多胺是参与各种过程的小型遍在分子，包括例如转录、RNA稳定化和离子通道门控(Wallace，2000)。鸟氨酸脱羧酶(ODC)是多胺合成中的第一种酶，对哺乳动物的正常发育和组织修复至关重要，但在大多数成人组织中下调(Gerner and Meyskens，2004)。多胺代谢和转运控制中的多种异常导致多胺水平升高，这可以促进几种组织中的肿瘤发生(Thomas and Thomas，2003)。

[0044] 家族性腺瘤性息肉病(FAP)是与结肠癌和其他癌症的高风险相关的综合征。FAP是由腺瘤性结肠息肉病(APC)肿瘤抑制基因中的突变引起的，并且已经证明APC信号传导在人类细胞(Fultz and Gerner，2002)和在小鼠FAP模型(Erdman et al.，1999)中调节ODC表达。在患有FAP的人的肠上皮组织中多胺代谢上调(Giardiello et al.，1997)。

[0045] 野生型APC表达导致ODC表达减少，而突变型APC导致ODC表达增加。ODC的APC依赖性调节机制涉及E-盒转录因子，包括转录激活剂c-MYC和转录阻遏物MAD1(Fultz and Gerner，2002；Martinez et al.，2003)。其他人证明，c-MYC调节ODC转录(Bellofemandez et al.，1993)。参与多胺代谢的几个基因是在大多数生物体中用于最佳生长的重要基因，

并且在非增殖细胞和/或成体细胞和组织中下调(Gerner and Meyskens,2004)。正如其他文献所做的综述(Childs et al.,2003),多胺影响特定细胞表型,部分是通过影响基因表达的模式。

[0046] 家族性腺瘤性息肉病(FAP),即遗传性息肉综合征,是腺瘤性结肠息肉病(APC)肿瘤抑制基因的生殖系突变的结果(Su et al.,1992)。这种具有可变表达的常染色体显性病况与数百个结肠腺瘤的发展相关,这些腺瘤在40岁时一律发展成腺癌,比散发性结肠癌的平均年龄诊断早20年(Bussey,1990)。在对患有FAP的症状前个体的先前研究中,与正常家族成员对照相比,在看似正常的结肠直肠活检中检测到多胺亚精胺和精胺及其二胺前体腐胺的水平增加(Giardiello et al.,1997)。鸟氨酸脱羧酶(ODC)——哺乳动物多胺合成中的第一种酶和限速酶——活性在FAP患者看似正常的结肠粘膜活检中也有所增加(Giardiello et al.,1997;Luk and Baylin,1984)。这些研究结果令人感兴趣,因为多胺是最佳细胞增殖必需的(Pegg,1986)。此外,使用酶激活的不可逆抑制剂DFMO抑制ODC活性,抑制致癌物处理的啮齿动物的结肠癌变(Kingsnorth et al.,1983;Tempero et al.,1989)。

[0047] 与FAP共享突变的APC/apc基因型的Min(多发性肠瘤(multiple intestinal neoplasia))小鼠用作人类FAP患者的有用实验动物模型(Lipkin,1997)。Min小鼠在120天的生命中在整个胃肠道内能够发展超过100个胃肠腺瘤/腺癌,从而导致GI出血、梗阻和死亡。已经证明,DFMO和舒林酸的联合疗法有效减少这些小鼠中的腺瘤。参见美国专利第6,258,845号和Gerner and Meyskens,2004,两者通过引用并入本文。

[0048] II.依氟鸟氨酸

[0049] 当单独使用并且无上下文时,术语“依氟鸟氨酸”是指任何形式的2,5-二氨基-2-(二氟甲基)戊酸,包括非盐形式和盐形式(例如,依氟鸟氨酸HCl),非盐和盐形式的无水形式和水合形式(例如依氟鸟氨酸盐酸盐一水合物),非盐形式和盐形式的溶剂合物,其对映异构体(R和S形式,其也可以称为d和l形式)和这些对映异构体的混合物(例如外消旋混合物)。“实质上光学纯的制剂”是指含有约5重量%或更少的相反对映异构体的第一对映异构体的制剂。依氟鸟氨酸的具体形式包括依氟鸟氨酸盐酸盐一水合物(即CAS ID:96020-91-6;MW:236.65)、依氟鸟氨酸盐酸盐(即CAS ID:68278-23-9;MW:218.63)和无水游离碱依氟鸟氨酸(即,CAS ID:70052-12-9;MW:182.17)。必要时,已进一步指定了依氟鸟氨酸的具体形式。在一些实施方案中,本公开的依氟鸟氨酸为依氟鸟氨酸盐酸盐一水合物(即,CAS ID:96020-91-6)。术语“依氟鸟氨酸”和“DFMO”在本文中可互换使用。DFMO是二氟甲基鸟氨酸的缩写。依氟鸟氨酸和DFMO的其他同义词包括:α-二氟甲基鸟氨酸、2-(二氟甲基)-DL-鸟氨酸、2(二氟甲基)-dl-鸟氨酸、2-(二氟甲基)鸟氨酸、DL-α-二氟甲基鸟氨酸、M二氟甲基鸟氨酸、αδ-二氨基-α-(二氟甲基)戊酸和2,5-二氨基-2-(二氟甲基)戊酸。

[0050] 依氟鸟氨酸是鸟氨酸脱羧酶(ODC)的酶激活的不可逆抑制剂,ODC是多胺生物合成途径的限速酶。由于这种多胺合成的抑制作用,该化合物有效预防许多器官系统中的癌症形成,抑制癌生长并减小肿瘤大小。它还与其他抗肿瘤药物协同作用。

[0051] 已经证明,依氟鸟氨酸降低小鼠的APC依赖性肠肿瘤发生(Erdman et al.,1999)。每日口服施用于人的依氟鸟氨酸抑制许多上皮组织中的ODC酶活性和多胺含量(Love et al.,1993;Gerner et al.,1994;Meyskens et al.,1994;Meyskens et al.,1998;

Simoneau et al.,2001;Simoneau et al.,2008)。据报道,与非甾体抗炎药(NSAID)舒林酸组合的依氟鸟氨酸,与安慰剂相比,在随机临床试验中显著降低患有结肠腺瘤的个体的腺瘤复发率(Meyskens et al.,2008)。

[0052] 依氟鸟氨酸最初由斯特拉斯堡的Center de Recherche Merrell合成。目前美国食品和药品管理局(FDA)的批准包括:

[0053] • 非洲昏睡病。高剂量全身IV剂型-未上市(Sanofi/WHO)

[0054] • 多毛症(Hirsutis,雄激素诱导的过量毛发生长)局部剂型

[0055] 尽管依氟鸟氨酸的口服制剂尚未获得FDA批准,但局部和注射形式已获得批准。Vaniqa®是一种乳膏,其在用于局部给药的乳膏中含有15%w/w依氟鸟氨酸盐酸盐一水合物,分别相当于11.5%w/w无水依氟鸟氨酸(EU),13.9%w/w无水依氟鸟氨酸盐酸盐(美国)。Ornidyl®是适于注射或输注的依氟鸟氨酸HCl溶液。它以每毫升200mg依氟鸟氨酸盐酸盐一水合物(20g/100mL)的浓度提供。

[0056] 依氟鸟氨酸及其在治疗良性前列腺肥大中的用途描述于美国专利第4,413,141号和第4,330,559号中。'141专利描述了依氟鸟氨酸在体外和体内都是ODC的强效抑制剂。据报道,依氟鸟氨酸的施用导致细胞中腐胺和亚精胺的浓度降低,在这些细胞中通常活跃产生这些多胺。此外,已经证明,当在标准肿瘤模型中测试时,依氟鸟氨酸能够减缓肿瘤细胞增殖。'559专利描述了依氟鸟氨酸和依氟鸟氨酸衍生物用于治疗良性前列腺肥大的用途。良性前列腺肥大,如许多以快速细胞增殖为特征的疾病状态一样,伴随着多胺浓度的异常升高。

[0057] 可潜在地持续给予依氟鸟氨酸,带来显著的抗肿瘤效果。这种药物在0.4g/m^2/天低剂量时对人类是相对无毒的,同时对肿瘤中腐胺的合成产生抑制。在大鼠肿瘤模型中的研究证明,依氟鸟氨酸输注可以在不抑制外周血小板计数的情况下使肿瘤腐胺水平降低90%。

[0058] 用依氟鸟氨酸观察到的副作用包括在4g/m^2/天的高剂量时对听力产生的影响,所述影响在依氟鸟氨酸中断时获得解除。在以0.4g/m^2/天的低剂量施用达1年时,并未观察到对听力的这些影响(Meyskens et al.,1994)。另外,观察到少数头晕/眩晕情况,但在药物停止时,所述情况解除。据报道,主要在使用高"治疗"剂量依氟鸟氨酸(>1.0g/m^2/天)的研究中,以及主要在先前接受过化疗的癌症患者或骨髓受损的患者中,报道了血小板减少症。尽管与依氟鸟氨酸治疗相关的毒性通常并不像其他类型的化疗一样严重,但在有限的临床试验中,已发现其促进剂量相关性血小板减少症。此外,大鼠研究表明,与对照相比,依氟鸟氨酸持续输注12天明显降低血小板计数。其他研究也进行了类似的观察,其中血小板减少症是连续静脉内依氟鸟氨酸疗法的主要毒性。这些发现表明,依氟鸟氨酸可能显著抑制巨核细胞骨髓前体的ODC活性。依氟鸟氨酸可抑制增殖性修复过程,如上皮伤口愈合。

[0059] III期临床试验评估了用DFMO加舒林酸或匹配的安慰剂治疗36个月后腺瘤性息肉的复发。临时性听力损失是已知用DFMO进行治疗的毒性,因此开发了分析连续气导听力图的综合方法。广义估计方程方法估计治疗组之间相对于气导纯音阈值变化的平均差异,同时考虑由于在频率处重复测量引起的对象内相关性。基于290名受试者,与用安慰剂治疗的受试者相比,用DFMO加舒林酸治疗的受试者的平均差异为0.50分贝(95%置信区间,-0.64至1.63分贝;P=0.39),针对基线值、年龄和频率被调整。与用安慰剂治疗的患者相比,用

DFMO加舒林酸治疗的患者的平均阈值差异＜2dB。该研究的结果在McLaren et al.,2008中有更详细的讨论,该文献通过引用整体并入本文。

[0060] III.NSAID

[0061] NSAID是非甾体抗炎剂。除抗炎作用外,还报道它们具有镇痛、解热和抑制血小板效果。例如,它们用于治疗与疼痛和炎症有关的慢性关节炎病况和某些软组织病症。据报道,它们通过抑制环氧合酶来阻断前列腺素的合成而发挥作用,环氧合酶将花生四烯酸转化为环内过氧化物即前列腺素前体。抑制前列腺素合成是是它们镇痛、解热和血小板抑制作用的原因;其他机制可归因于其抗炎作用。某些NSAID也可以抑制脂氧合酶或磷脂酶C或可以调节T细胞功能。参见AMA Drug Evaluations Annual,1814-5,1994。

[0062] 包括阿司匹林、布洛芬、吡罗昔康(Reddy et al.,1990;Singh et al.,1994)、吲哚美辛(Narisawa,1981)和舒林酸(Piazza et al.,1997;Rao et al.,1995)在内的非甾体抗炎药(NSAID)在AOM处理的大鼠模型中有效抑制结肠癌变。NSAID还抑制携带激活的Ki-ras的肿瘤的发展(Singh and Reddy,1995)。NSAID似乎通过诱导肿瘤细胞凋亡来抑制癌变(Bedi et al.,1995;Lupulescu,1996;Piazza et al.,1995;Piazza et al.,1997b)。许多研究表明,NSAID的化学预防特性,包括诱导细胞凋亡,是它们能抑制前列腺素合成的功能(DuBois et al.,1996的中的综述;Lupulescu,1996;Vane and Botting,1997)。然而,研究表明,NSAID可能通过前列腺素依赖性和非依赖性机制发挥作用(Alberts et al.,1995;Piazza et al.,1997a;Thompson et al.,1995;Hanif,1996)。舒林酸砜是NSAID舒林酸的代谢物,其缺乏COX抑制活性,但仍然诱导肿瘤细胞的细胞凋亡(Piazza et al.,1995;Piazza et al.,1997b),并在几种啮齿动物癌变模型中抑制肿瘤发展(Thompson et al.,1995;Piazza et al.,1995,1997a)。

[0063] 已经检查了几种NSAID在人临床试验中的作用。完成了布洛芬的IIa期试验(一个月),甚至在300mg/天的剂量下,观察到平粘膜中的前列腺素E_2(PGE_2)水平显著降低。300mg布洛芬的剂量非常低(治疗剂量的范围从1200-3000mg/天或更多),并且即使在长期内也不大可能观察到毒性。然而,在动物化学预防模型中,布洛芬不如其他NSAID有效。

[0064] A.阿司匹林

[0065] 阿司匹林,也称为乙酰水杨酸,是水杨酸盐药物,通常用作镇痛剂来缓解轻微疼痛,用作为解热剂来减轻发烧,并用作抗炎药物。阿司匹林首先由德国拜耳公司的化学家菲利克斯霍夫曼(Felix Hoffmann)在1897年分离。水杨酸是阿司匹林的主要代谢产物,是人类和动物新陈代谢的组成部分。虽然在人类中许多是由饮食引起的,但大部分是内源性合成的。今天,阿司匹林是世界上最广泛使用的药物之一,估计每年消费40000吨。在阿司匹林被注册为拜耳公司所拥有商标的国家,通用术语是乙酰水杨酸(ASA)。

[0066] 阿司匹林还通过抑制血栓素的产生而具有抗血小板作用,所述血栓素在正常情况下将血小板分子结合在一起以在损伤的血管壁上形成补丁(patch)。由于血小板补丁可变得太大并且还阻断局部和下游的血流,阿司匹林也长期低剂量使用,以帮助预防发展血块高风险人的心脏病发作、中风和血块形成。也已经确定,可以在心脏病发作后立即给予低剂量阿司匹林,以降低再次心脏病发作或心脏组织死亡风险。阿司匹林可以有效预防某些类型的癌症,特别是结肠直肠癌。

[0067] 口服阿司匹林的不良副作用包括胃肠溃疡、胃出血和耳鸣,尤其是在较高剂量时。

在儿童和青少年中，由于瑞氏综合征(Reye's syndrome)的风险，阿司匹林不再用于控制流感样症状或水痘或其他病毒性疾病的症状。

[0068] 阿司匹林是一组称为非甾体抗炎药(NSAID)的一部分，但其作用机制与大多数其他NSAID不同。尽管阿司匹林和该组中其他称为水杨酸盐的成员，具有与其他NSAID相似的作用(解热、抗炎、镇痛)并抑制相同的酶环氧合酶，但阿司匹林(非其他水杨酸盐)以不可逆的方式进行，且与其他酶不同，与所述酶的COX-2变体相比，其更多地影响COX-1变体。

[0069] B.舒林酸及其主要代谢物舒林酸砜和舒林酸硫化物

[0070] 舒林酸是具有以下化学名称的非甾体抗炎茚衍生物：(Z)-5-氟-2-甲基-1-((4-)甲基亚磺酰基)苯基)亚甲基)-1H-茚-3-乙酸(Physician's Desk Reference，1999)。在不受理论束缚的情况下，亚磺酰基部分通过可逆还原转化成硫化物代谢物，并通过不可逆氧化在体内转化成砜代谢物(依昔舒林(exisulind))。参见美国专利第6,258,845号，该专利通过引用并入本文。舒林酸也抑制Ki-ras激活，被代谢成两种不同的分子，这两种不同的分子抑制COX的能力不同，但都能够通过诱导细胞凋亡发挥化学预防作用。舒林酸砜缺乏COX抑制活性，并且最有可能以独立于前列腺素合成的方式诱导细胞凋亡。现有证据表明，硫化物衍生物是至少一种生物活性化合物。基于此，舒林酸可以视为前药。

[0071] 舒林酸(Clinoril®)可以例如以150mg和200mg的片剂获得。对成人而言，最常见的剂量是150-200mg，每天两次，最大日剂量为400mg。口服给药后，约90％的药物被吸收。在空腹患者中在约2小时内达到峰值血浆水平，与食物一起施用时在3-4小时内达到峰值血浆水平。舒林酸的平均半衰期为7.8小时；硫化物代谢物的平均半衰期为16.4小时。美国专利第3,647,858号和第3,654,349号涵盖了舒林酸制剂；通过引用将这两个专利的全部内容并入本文。

[0072] 舒林酸用于短期期缓和长期缓解骨关节炎、类风湿性关节炎、强直性脊柱炎、急性痛风和急性肩痛的体征和症状。舒林酸(每天400mg)的镇痛和抗炎作用与阿司匹林(每天4g)、布洛芬(每天1200mg)、吲哚美辛(每天125mg)和保泰松(每天400-600mg)相当。舒林酸的副作用包括近20％的患者中出现轻微的胃肠道反应，腹痛和恶心是最常见的不适。在高达10％的患者中观察到CNS副作用，其中最常报道的是嗜睡、头痛和紧张。5％的患者出现皮疹和瘙痒。用舒林酸进行慢性治疗可导致严重的胃肠毒性，如出血、溃疡和穿孔。

[0073] 舒林酸用于化学预防癌症，特别是结肠直肠息肉的潜在用途已有充分的研究。例如，美国专利第5,814,625号和第5,843,929号报道了舒林酸在人类中的潜在化学预防用途，这两篇专利均通过引用并入本文。已证实舒林酸可导致家族性腺瘤性息肉病(FAP)患者的腺瘤退化(Muscat et al.，1994)，虽然在至少一项关于散发性腺瘤的研究中表明没有这种效果(Ladenheim et al.，1995)，。已经测试了舒林酸及其砜代谢产物依昔舒林，并在临床上继续测试，用于预防和治疗几种癌症类型。

[0074] C.吡罗昔康

[0075] 吡罗昔康是一种非甾体抗炎药，在治疗类风湿性关节炎和骨关节炎中已得到很好的确认，其化学名称如下：4-羟基-2-甲基-N-2-吡啶基-2H-1，2-苯并噻嗪-3-甲酰胺1，1-二氧化物。已证实其在治疗肌肉骨骼病症、痛经和术后疼痛方面也有用。它的长半衰期能使其每天给药一次。如果直肠给药，已证明该药物是有效的。胃肠不适是最常报告的副作用。

[0076] 已证明吡罗昔康在动物模型中是有效的化学预防剂(Pollard and Luckert，

1989;Reddy et al.,1987),但吡罗昔康在最近的IIb试验中显示了副作用。NSAID副作用的大型荟萃分析也表明,吡罗昔康比其他NSAID具有更多的副作用(Lanza et al.,1995)。

[0077] 已证明,DFMO和吡罗昔康的组合在AOM处理的大鼠结肠癌变模型中具有协同化学预防作用(Reddy et al.,1990),尽管在DFMO和吡罗昔康单独施用时,DFMO比吡罗昔康对Ki-ras突变和肿瘤发生产生更大的抑制作用(Reddy et al.,1990)。在一项研究中,向AOM处理的大鼠施用DFMO或吡罗昔康分别将携带Ki-ras突变的肿瘤数量从90%减少到36%和25%(Singh et al.,1994)。这两种药剂还减少了现有肿瘤中生化活性p21ras的量。

[0078] D.塞来昔布(Celecoxib)

[0079] 塞来昔布是非载体抗炎剂,在骨关节炎、类风湿性关节炎、急性疼痛、强直性脊柱炎的治疗中完全确立,并完全确立以减少患有FAP的患者中结肠息肉和直肠息肉的数量,其具有以下化学名称:4-[5-(4-甲基苯基)-3-(三氟甲基)吡唑-1-基]苯磺酰胺。塞来昔布以辉瑞(Pfizer)的商标名称Celebrex、Celebra和Onsenal销售。塞来昔布是选择性COX-2抑制剂。塞来昔布的副作用包括心脏和血管疾病率增加30%。此外,胃肠副作用的风险大于80%。

[0080] E.NSAID的组合

[0081] 各种NSAID的组合也可用于一些实施方案中。在一些实施方案中,通过使用较低剂量的两种或更多种NSAID,有可能减少与更高剂量各NSAID相关的副作用或毒性。例如,在一些实施方案中,舒林酸可以与塞来昔布一起使用。可以彼此组合使用的NSAID的实例包括但不限于:布洛芬、萘普生、非诺洛芬、酮洛芬、氟比洛芬、奥沙普秦、吲哚美辛、舒林酸、依托度酸、双氯芬酸、吡罗昔康、美洛昔康、替诺昔康、屈昔康、氯诺昔康、伊索昔康(isoxicam)、甲芬那酸、甲氯芬那酸、氟芬那酸、托芬那酸、塞来昔布、罗非昔布、伐地昔布、帕瑞昔布、罗美昔布和依托昔布。

[0082] IV.依氟鸟氨酸/舒林酸组合疗法

[0083] 在一些实施方案中,本文提供的组合物可用于减少患者癌细胞的数量、抑制其生长和/或预防其发生。靶癌细胞包括肺癌、脑癌、前列腺癌、肾癌、肝癌、卵巢癌、乳腺癌、皮肤癌、胃癌、食管癌、头颈癌、睾丸癌、结肠癌、宫颈癌、淋巴系统癌和血癌。在一些实施方案中,组合物可用于治疗和/或预防结肠癌、家族性腺瘤性息肉病(FAP)、胰腺癌和/或神经母细胞瘤。

[0084] 在一些实施方案中,本文提供的组合物可用于治疗表现癌前症状的患者并由此预防癌症发作。用于这种预防性治疗的靶细胞和组织包括息肉和其他癌前病损(precancerous lesions)、癌前病变(premalignancies)、肿瘤前的(preneoplastic)或表明可能向癌性状态发展的其他异常表型。例如,本文提供的组合物可用于预防腺瘤,伴有很小的附加毒性。与FAP共有突变的APC/apc基因型的Min(多发性肠瘤)小鼠用作人FAP患者的有用实验动物模型(Lipkin,1997)。在120天的生命期中,Min小鼠可在整个胃肠道内发展超过100个胃肠腺瘤/腺癌,从而导致GI出血、梗阻和死亡。已经证明,DFMO和舒林酸的联合疗法有效减少这些小鼠中的腺瘤。参见美国专利第6,258,845号,其全部内容通过引用并入本文。

[0085] V.固定剂量组合和给药途径

[0086] 一方面,本发明提供包含药学上有效量的依氟鸟氨酸和药学上有效量的非甾体抗

炎药(NSAID)或其代谢物的固定剂量组合的组合物。在一些实施方案中,固定剂量组合是药学上有效量的依氟鸟氨酸和药学上有效量的舒林酸。

[0087] 在一些实施方案中,依氟鸟氨酸是依氟鸟氨酸盐酸盐一水合物。在一些实施方案中,依氟鸟氨酸是依氟鸟氨酸盐酸盐一水合物外消旋物。在一些实施方案中,依氟鸟氨酸盐酸盐一水合物是其两种对映异构体的外消旋混合物。

[0088] 在一些实施方案中,依氟鸟氨酸以约10mg至约1000mg的量存在。在一些实施方案中,依氟鸟氨酸以约250mg至约500mg的量存在。在一些实施方案中,依氟鸟氨酸以约300mg至约450mg的量存在。在一些实施方案中,依氟鸟氨酸以约350mg至约400mg的量存在。在一些实施方案中,依氟鸟氨酸以约35重量%至约60重量%的量存在。在一些实施方案中,依氟鸟氨酸以约40重量%至约55重量%的量存在。在一些实施方案中,依氟鸟氨酸以约50重量%至约55重量%的量存在。在一些实施方案中,依氟鸟氨酸约52重量%至约54重量%的量存在。在一些实施方案中,依氟鸟氨酸盐酸盐一水合物外消旋物的量为52重量%至54重量%。在一些实施方案中,依氟鸟氨酸以约375mg的量存在。在一些实施方案中,依氟鸟氨酸盐酸盐一水合物外消旋物的量为375mg。

[0089] 在一些实施方案中,舒林酸以约10mg至约1500mg的量存在。在一些实施方案中,舒林酸以约50mg至约100mg的量存在。在一些实施方案中,舒林酸以约70mg至约80mg的量存在。在一些实施方案中,舒林酸以约75mg的量存在。在一些实施方案中,舒林酸的量是75mg。在一些实施方案中,舒林酸以约5重量%至约20重量%的量存在。在一些实施方案中,舒林酸以约8重量%至约15重量%的量存在。在一些实施方案中,舒林酸以约10重量%至约12重量%的量存在。在一些实施方案中,舒林酸的量为10-11重量%。

[0090] 在一些实施方案中,依氟鸟氨酸以约375mg的量存在,并且舒林酸以约75mg的量存在。

[0091] 在一些实施方案中,该制剂进一步包含赋形剂。在一些实施方案中,赋形剂是淀粉、胶体二氧化硅或硅化微晶纤维素。在一些实施方案中,赋形剂是胶体二氧化硅。在一些实施方案中,该制剂进一步包含第二赋形剂。在一些实施方案中,第二赋形剂是硅化微晶纤维素。

[0092] 在一些实施方案中,该制剂进一步包含润滑剂。在一些实施方案中,润滑剂是硬脂酸镁、硬脂酸钙、硬脂酸钠、单硬脂酸甘油酯、硬脂酸铝、聚乙二醇、硼酸或苯甲酸钠。在一些实施方案中,润滑剂是硬脂酸镁。在一些实施方案中,硬脂酸镁以约0.25重量%至约2重量%的量存在。在一些实施方案中,硬脂酸镁的量为约0.75重量%至约2重量%。在一些实施方案中,硬脂酸镁的量为约1重量%至约1.5重量%。在一些实施方案中,硬脂酸镁的量为约1.1重量%。在一些实施方案中,硬脂酸镁以约1.5重量%的量存在。

[0093] 在一些实施方案中,组合物采用胶囊、片剂、微型片剂、颗粒、丸剂、溶液、凝胶、乳膏、泡沫或贴剂的形式。在一些实施方案中,组合物采用片剂形式,例如单层片剂。

[0094] 在一些实施方案中,片剂的重量为约650mg至约1,000mg。在一些实施方案中,片剂的重量为约675mg至约725mg。在一些实施方案中,片剂的重量为约700mg。

[0095] 在一些实施方案中,片剂进一步包含包衣。在一些实施方案中,该包衣是改性释放包衣或肠溶衣。在一些实施方案中,包衣是pH响应性包衣。在一些实施方案中,包衣包含乙酸邻苯二甲酸纤维素(CAP)、乙酸偏苯三酸纤维素(CAT)、聚(乙酸乙烯酯)邻苯二甲酸酯

(PVAP)、羟丙基甲基纤维素邻苯二甲酸酯(HP)、聚(甲基丙烯酸酯乙基丙烯酸酯)(1:1)共聚物(MA-EA)、聚(甲基丙烯酸酯甲基丙烯酸甲酯)(1:1)共聚物(MA MMA)、聚(甲基丙烯酸酯甲基丙烯酸甲酯)(1:2)共聚物或乙酸羟丙基甲基纤维素琥珀酸酯(HPMCAS)。在一些实施方案中,包衣掩盖依氟鸟氨酸的味道。在一些实施方案中,包衣包含羟丙基甲基纤维素、二氧化钛、聚乙二醇和氧化铁黄。

[0096] 在一些实施方案中,包衣的量为约1重量%至约5重量%。在一些实施方案中,包衣的量为约2重量%至约4重量%。在一些实施方案中,包衣的量为约3重量%。在一些实施方案中,包衣的量为约5mg至约30mg。在一些实施方案中,包衣的量为约15mg至约25mg。在一些实施方案中,包衣的量为约21mg。

[0097] 在一些实施方案中,包含包衣的片剂的重量为约675mg至约750mg。在一些实施方案中,包含包衣的片剂的重量为约700mg至约725mg。在一些实施方案中,包含包衣的片剂的重量为约721mg。

[0098] 一方面,本发明提供了包含药学上有效量的依氟鸟氨酸和药学上有效量的舒林酸的固定剂量组合的组合物。在一些实施方案中,组合物采用胶囊、片剂、微型片剂、颗粒、丸剂、溶液、凝胶、乳膏、泡沫或贴剂的形式。在一些实施方案中,组合物是固体并且采用片剂形式,例如单层片剂。在一些实施方案中,片剂是薄膜包衣的。

[0099] 在一些方面,本公开提供了依氟鸟氨酸和NSAID的口服固定剂量组合制剂。在一些实施方案中,提供了包含药学上有效量的依氟鸟氨酸和药学上有效量的NSAID的药物组合物。在一些实施方案中,NSAID是舒林酸、阿司匹林、吡罗昔康或塞来昔布。在一些优选的实施方案中,NSAID是舒林酸。

[0100] 在一些实施方案中,本发明的药物组合物和制剂用于肠内,例如口服,以及直肠或肠胃外,其中所述组合物包含单独的药物活性化合物,或包含药物活性化合物与药物助剂(赋形剂)。用于肠内或肠胃外给药的药物制剂为例如单位剂量形式,例如包衣片剂、片剂、胶囊或栓剂以及安瓿。它们以本身已知的方式制备,例如使用常规混合、制粒、包衣、增溶或冻干方法。因此,口服使用的药物制剂可通过将活性化合物与固体赋形剂混合,如果需要将已获得的混合物制粒,并且如果需要或必要的话,在加入合适的助剂后将混合物或颗粒加工成片剂或包衣片剂芯。在优选的实施方案中,将活性成分和赋形剂的混合物配制成片剂形式。可以使用适当的包衣来增加适口性或延迟吸收。例如,可以将包衣施用于片剂以掩盖活性化合物如DFMO的不愉快味道,或维持和/或延缓活性分子释放至胃肠道中的某个区域。

[0101] 治疗性化合物可以口服施用,例如,用惰性稀释剂或可吸收的可食载体。也可以将治疗性化合物和其他成分封装在硬壳或软壳明胶胶囊中,压制成片剂或直接掺入受试者的饮食中。对于口服治疗给药,可以将治疗性化合物与赋形剂混合,并以可摄取的片剂、口含片剂、锭剂、胶囊、酏剂、混悬剂、糖浆剂或薄片剂(wafer)的形式使用。

[0102] 在某些实施方案中,本文提供的片剂和/或胶囊包含活性成分和粉状载体,例如乳糖、淀粉、纤维素衍生物、硬脂酸镁和硬脂酸。类似的稀释剂可用于制备压缩片剂。在其他实施方案中,可制造用于立即释放或改性释放的片剂和胶囊。在一些实施方案中,将片剂和/或胶囊制成在数小时内提供药物的连续释放的缓释产品。在一些实施方案中,压缩片剂是糖包衣的和/或膜包衣的,以掩盖令人不愉快的味道和/或保护片剂免受空气影响。在一些实施方案中,片剂是肠溶包衣的,以在胃肠道中选择性崩解。

[0103] 在一些实施方案中，片剂或胶囊能够崩解或溶解以释放包含第一组分和第二组分的不同颗粒群体的多颗粒，例如，改性释放包衣多颗粒。在这些实施方案中的一些中，片剂或胶囊可以在口腔、胃、小肠、末端回肠或结肠中崩解或溶解。在这些实施方案中的一些中，片剂或胶囊剂可以以改性的释放特性释放多颗粒。

[0104] 在一些实施方案中，本发明提供了多层片剂形式的药物口服固定剂量组合。多层片剂具有至少两层(双层片剂)或可具有三层、四层、五层或更多层。在一些实施方案中，每个层都含有不超过一种活性药物成分(API)。例如，在一些实施方案中，片剂具有两层，其中两层中的每一层都具有一种API。在一些实施方案中，除了这两层以外，片剂进一步含有仅含载体并且可以用作例如分离层或外部包衣层的其他层。在一些实施方案中，如果存在多于两个层，则组分可以存在于多于一个层中，只要它们不在同一层中即可。在某些实施方案中，单层片剂是优选的，但以下详述的所有信息同样适用于多层片剂。

[0105] 在一些实施方案中，可配制固定剂量组合以提供约0.1μM至约1000μM范围的总依氟鸟氨酸和/或舒林酸的平均稳态血浆浓度水平，并且优选约1μM到100μM的范围，更优选约1μM到大约50μM的范围。

[0106] A.药学上可接受的赋形剂

[0107] 在一些实施方案中，组合物还包含药学上可接受的赋形剂。在这些实施方案的一些中，药学上可接受的赋形剂可以包括药学上可接受的稀释剂、药学上可接受的崩解剂、药学上可接受的粘合剂、药学上可接受的稳定剂、药学上可接受的润滑剂、药学上可接受的颜料或药学上可接受的滑行剂(glider)。在本发明的固定剂量组合制剂中，活性成分可以以1:0.25至1:20的重量比与药学上可接受的赋形剂混合。

[0108] 可用于本发明药物制剂的稀释剂包括但不限于微晶纤维素(“MCC”)、硅化MCC(例如PROSOLV™ HD 90)、微细纤维素、乳糖、淀粉、预胶化淀粉、糖、甘露醇、山梨醇、葡聚糖(dextrate)、糊精、麦芽糖糊精、右旋糖、碳酸钙、硫酸钙、磷酸氢钙二水合物、磷酸三钙、碳酸镁、氧化镁及其任何混合物。优选地，稀释剂是硅化MCC。基于制剂的总重量，稀释剂的用量可以为约5重量％至约95重量％，优选约25重量％至约40重量％，例如约30重量％到约35％的重量。在某些方面，稀释剂可以是可溶性稀释剂。当使用稀释剂时，其与每个离散层中活性成分的比例非常重要。术语“可溶性稀释剂”是指溶解于水中的稀释剂，例如乳糖、Ludipress(BASF，乳糖、交聚维酮和聚维酮(93:3.5:3.5，w/w(％)的混合物)、甘露醇和山梨醇。

[0109] 片剂在暴露于口腔和/或胃肠道中的流体之后，使用崩解剂来促进片剂的溶胀和崩解。可用于本发明的固定剂量组合制剂中的崩解剂的实例包括交聚维酮、羟基乙酸淀粉钠、交联羧甲基纤维素钠、低取代羟丙基纤维素、淀粉、海藻酸或其钠盐及其混合物。可用于本发明的药物制剂中的其他崩解剂包括但不限于甲基纤维素、微晶纤维素、羧甲基纤维素钙、羧甲基纤维素钠(例如AC-DI-SOL™，PRIMELLOSE™)、聚维酮、瓜尔胶、硅酸镁铝、胶体二氧化硅(例如AEROSIL™，CARBOSIL™)、波拉克林钾、淀粉、预胶化淀粉、羟基乙酸淀粉钠(例如EXPLOTAB™)、海藻酸钠及其任何混合物。优选地，崩解剂是胶体二氧化硅。基于制剂的总重量，崩解剂的用量可以为约0.1重量％至约30重量％，并且优选为约0.2重量％至约5重量％。

[0110] 本发明的组合物可以包含润滑剂。当颗粒将自身附着在压片冲头(tablet press

punch)的面时，会发生粘连。润滑剂用于促进粉末的流动性，并降低片剂冲面与片剂冲头之间以及片剂表面与模具壁(die wall)之间的摩擦。例如，润滑剂包括硬脂酸镁、硬脂酸钙、硬脂酸锌、硬脂酸、硬脂酰富马酸钠、聚乙二醇、月桂基硫酸钠、月桂基硫酸镁和苯甲酸钠。优选地，润滑剂是硬脂酸镁。在本发明中，润滑剂优选占固体剂型的0.25重量％至2重量％，并且优选约1.5重量％的量。在示例性制剂中，润滑剂是硬脂酸镁，其存在量为约1.5重量％以防止粘连。

[0111] 粘合剂可以用于本发明的药物组合物中以帮助在压缩后将片剂保持在一起。可用于本发明的粘合剂的例子是阿拉伯胶、瓜尔胶、海藻酸、卡波姆(例如Carbopol™产品)、糊精、麦芽糖糊精、甲基纤维素、乙基纤维素、羟乙基纤维素、羟丙基纤维素(例如KLUCEL™)、羟丙基甲基纤维(例如METHOCEL™)、羧甲基纤维素钠、液体葡萄糖、硅酸镁铝、聚甲基丙烯酸酯、聚乙烯吡咯烷酮(例如聚维酮K-90D，KOLLIDON™)、共聚维酮(PLASDONE™)、明胶、淀粉及其任何混合物。优选地，粘合剂是淀粉。在本发明中，粘合剂优选占固体剂型的约1重量％至约15重量％。在其他实施方案中，固体剂型不包含粘合剂。

[0112] 在某些实施方案中，可用于本发明的固定剂量组合制剂中的稳定剂可以是抗氧化剂。抗氧化剂的使用增强了活性成分的稳定性，防止与其他药学上可接受的添加剂的不希望的反应和随时间因热或湿度造成的改性。例如，抗氧化剂是抗坏血酸及其酯、丁基化苯甲醇(BHT)、丁羟茴醚(BHA)、α-生育酚、半胱氨酸(cystein)、柠檬酸、没食子酸丙酯、硫酸氢钠、焦亚硫酸钠、乙二胺四乙酸(EDTA)及其任何混合物。

[0113] B.片剂制造方法

[0114] 本发明的另一方面是提供了用于制造本文公开的片剂的方法，所述片剂包括那些包含依氟鸟氨酸和舒林酸的片剂。在一些实施方案中，活性剂通过以下方式制备：用期望筛目的筛子筛选至少一种活性剂和一种或多种赋形剂，然后使用快速混合制粒机、行星式混合机、大容量混合机、螺带式混合机、流化床处理机或任何其他合适的装置混合。例如通过在低剪切或高剪切混合机、流化床制粒机等中，通过添加具有或不具有粘合剂的溶液或悬浮液(无论是醇或水醇或水性溶液或悬浮液)来对共混物进行制粒，或通过干法制粒。可以使用盘式干燥器、流化床干燥器、旋转锥形真空干燥器等干燥颗粒。可以使用振动式制粒机或粉碎机或任何其他配有合适的筛网的常规设备来定制颗粒大小。或者，可以通过挤出和滚圆或辊压制备颗粒。含有活性剂的颗粒的制造还可以包括用可直接压缩的赋形剂或辊压混合。

[0115] 在本发明的其他实施方案中，小片剂(微型片剂)可以通过使用各种尺寸和形状的模具和冲头按需要压制颗粒来制备。任选地，如果需要，可以通过本领域技术人员已知的技术例如喷涂、浸涂、流化床涂布等将包衣施加于片剂。在本发明的某些实施方案中，合适的溶剂体系如醇、水醇、水性或有机溶剂体系可用于促进加工。

[0116] 1.制粒

[0117] 制粒是使粉末状颗粒彼此粘附从而产生更大的多颗粒实体或粒的过程。在本发明的实施方案中，通过干法技术或湿法技术获得的颗粒可以与一种或多种润滑剂和/或防粘剂混合，然后填充到单个胶囊中或不同大小的不同胶囊中，使得可以将更小的胶囊填充到另一个更大的胶囊。

[0118] 在某些实施方案中，通过压实进行干法制粒用于生产固体剂型组合物。在干法制

粒中，通过在粉末上施加力来压实粉末状共混物，这通常导致相当大的尺寸增大。在一些方面，在使用压片机进行压实过程的干法制粒工艺中使用压片(slugging)。在其他方面，碾压机用于干法制粒，包括进料系统、压实单元和尺寸减小单元。在这种方法中，通过施加力，将粉末在两个辊之间压实，这是干法制粒工艺中最重要的参数。施加的力以kN/cm表示，即每厘米辊宽度的力。有时候，压力也以巴(bar)显示。然而，这只是表示液压系统内的压力，实际上并不是施加在粉末上的力的适当测量单位。在给定的力下，根据运送到辊上的粉末量，会将粉末压实到预定的带厚度。

[0119] 在其他实施方案中，湿法制粒用于生产固体剂型组合物。对粉末进行湿法制粒提高压缩混合物的流动性和压实性。在湿法制粒中，通过将制粒液体添加到受推进器(在高剪切制粒机中)、螺杆(在双螺杆制粒机中)或空气(在流化床制粒机中)影响的粉末床上来形成颗粒。导致该体系的搅拌以及对制剂内组分的润湿导致初级粉末颗粒聚集以产生湿颗粒。制粒液体(流体)含有溶剂，所述溶剂必须是挥发性的，以便可以通过干燥除去，并且必须是无毒的。典型的液体包括水、乙醇和异丙醇，单独或组合。液体溶液可以是水基或溶剂基的。与有机溶剂相比，水溶液具有处理更安全的优点。

[0120] 基本上如Maejima et al，1997所述，其通过引用并入本文，还可以通过滚动熔融制粒(tumbling melt granulation，TMG)形成片剂。滚动熔融制粒可以用于制备熔融制粒。它可以在滚动混合机中进行。将熔融的低熔点化合物喷洒在在搅拌机中的结晶糖和粉末状糖中，并混合直至形成颗粒。在这种情况下，低熔融成分是粘合剂，结晶糖是种子。可替代方法是将未熔融的低熔点成分、结晶糖(例如蔗糖或麦芽糖)和粉末形式的水溶性成分(例如甘露糖醇或乳糖)在滚动混合机中合并，并混合，同时加热至低熔点粘合剂的熔点或以上。种子应该是晶体或粒状水溶性成分(糖)，例如粒状甘露糖醇、结晶麦芽糖、结晶蔗糖或任何其他糖。滚动混合机的实例是双壳搅拌机(V型搅拌机)或任何其他形状的滚动混合机。加热可以通过使加热的空气循环通过制粒机的腔室并通过加热腔室的底部表面来实现。当种子材料和粉状片剂组分在加热的腔室中循环时，低熔点化合物熔融并粘附到种子上。未熔融的粉末状材料粘附在结合了种子的熔融的低熔点材料上。然后将通过该方法形成的球形珠冷却并用筛网筛选以除去未粘附的粉末。

[0121] 喷雾凝结或造粒(prilling)也可用于形成本发明的片剂组合物。喷雾凝结包括将包括低熔点化合物的组合物的熔融微滴雾化到表面上，或优选其他片剂组分上。可用于喷雾凝结的设备包括喷雾干燥器(例如Nero喷雾干燥器)和具有顶喷的的流化床包衣机/制粒机(例如Glatt流化床包衣机/制粒机)。在优选的实施方案中，形成快速溶解颗粒，其中优选将水溶性赋形剂，更优选糖类悬浮在熔融的低熔点成分中并喷雾凝结。在喷雾凝结之后，使所得的组合物冷却并凝结。在混合物凝结之后，将其筛分或筛选并与剩余的片剂组分混合。喷雾凝结过程在本发明的范围内，其中，将包含低熔点化合物和其他片剂组分的任何组合的快速溶解制粒熔化并喷雾凝结到其他片剂组分上。这样的喷雾凝结过程也在本发明的范围内，其中混合包括低熔点化合物在内的所有片剂组分，将低熔点化合物熔化，并将混合物喷雾凝结到表面上。

[0122] 2.混合

[0123] 在某些实施方案中，在制粒后混合混合物。在固体剂型制造中，混合是为了实现混合均匀性和分配润滑剂。在某些方面，将混合步骤设计为在最终混合润滑剂之前实现所有

组分的均匀性。然而，由于粒径、含水量、结构、体积密度和流动特性，混合粉末是一项挑战。成功的配方的关键是添加顺序。通常将组分和药学上可接受的添加剂分配到合适的容器例如扩散搅拌机或扩散混合机中。滚动混合机的实例是双壳搅拌机(V-搅拌机)或任何其他形状的滚动混合机。

[0124] 3.压缩

[0125] 一旦制备了片剂组合物，它们可以形成各种形状。在优选的实施方案中，将片剂组合物压成一定形状。该工艺可以包括将片剂组合物置于一种形状中，并向该组合物施加压力以使该组合物呈现组合物接触的形状表面的形状。压缩成片剂形状可以通过压片机完成。压片机包括从底部适配模具的下冲头和具有相应的形状和尺寸的上冲头，所述上冲头在压片材料落入所述模具腔内之后从顶部进入所述模具腔。片剂通过施加在下冲头和上冲头上的压力形成。本发明的片剂的硬度通常为约20kP或更小；优选片剂的硬度为约15kP或更小。典型的压缩压力为约5kN至约40kN，并且会基于片剂的期望尺寸和硬度而变化。在一些方面，压缩压力为约25kN至约35kN。在具体方面，压缩压力小于37kN或约为37kN，例如小于约30kN，例如小于约25kN。诸如Carver压片机的液压机或诸如Stokes Versa压片机的旋转式压片机是压缩本发明的片剂组合物的合适手段。表3示出了示例性压缩力参数。

[0126] 在某些实施方案中，被润滑地共混物可以使用合适的装置例如旋转机器压缩以形成锭(slug)，所述锭通过装备有合适筛网的研磨机或流体能量磨机或球磨机或胶体研磨机或辊研磨机或锤研磨机等，以获得活性成分的研磨锭。

[0127] 可以使用预压缩步骤以例如防止片剂加帽。加帽指的是片剂的帽或顶部从片剂本体上分裂或断裂。压缩过程中空气被挤出时迁移的不可压缩的细小颗粒能引起加帽。例如，预压缩可以为主压缩力的约5％、10％或15％。在优选的实施方案中，在不超过约10kN，优选不超过5kN的压力下，将片剂预压缩所成形状。例如，在小于1、1.5、2、2.1、2.2、2.5、3、3.5、4、4.5、5、6、7、8、9或10kN压片在本发明的范围内。在具体方面中，预压缩力为约2.5kN至约3.5kN。表3示出了示例性预压缩力参数。

[0128] 4.薄膜包衣

[0129] 本发明的组合物或固体剂型也可以用薄膜包衣、肠溶衣、改性的释放包衣、保护性包衣或抗粘合包衣进行包衣。

[0130] 本发明的组合物可以是肠溶包衣的。肠溶包衣的或肠溶衣是指防止活性剂在胃中释放并允许在肠道上部释放的药学上可接受的包衣。在其他实施方案中，施加肠溶衣以延迟活性剂释放至回肠末端或结肠。可以添加肠溶衣，作为改性的释放包衣上的外层。在肠溶衣制剂中，可以单独或组合使用肠溶衣聚合物。可以将肠溶衣设计为单层包衣或多层包衣实施方案。用于本发明组合物的优选肠溶衣包含选自以下的成膜剂：乙酸邻苯二甲酸纤维素；乙酸偏苯三酸纤维素；甲基丙烯酸共聚物，衍生自甲基丙烯酸及其酯的共聚物，其含有至少40％的甲基丙烯酸；羟丙基甲基纤维素邻苯二甲酸酯；乙酸羟丙基甲基纤维素琥珀酸酯或聚乙酸乙烯邻苯二甲酸酯(Polyvinylacetatephthalate)。适用于肠溶衣的聚合物的实例包括，例如乙酸邻苯二甲酸纤维素(CAP)、乙酸偏苯三酸纤维素(CAT)、聚(乙酸乙烯酯)邻苯二甲酸酯(PVAP)、羟丙基甲基纤维素邻苯二甲酸酯(HP)、聚(甲基丙烯酸酯乙基丙烯酸酯)(1∶1)共聚物(MA-EA)、聚(甲基丙烯酸酯甲基丙烯酸甲酯)(1∶1)共聚物(MA MMA)、聚(甲基丙烯酸酯甲基丙烯酸甲酯)(1∶2)共聚物、EUDRAGIT™ L 30D(MA-EA，1∶1)、EUDRAGIT™

100 55(MA-EA,3:1)、乙酸羟丙基甲基纤维素琥珀酸酯(HPMCAS)、SURETERIC(PVAP)、AQUATERIC™(CAP)、紫胶或AQOAT™(HPMCAS)。可以与本发明一起使用的靶向结肠递送系统是已知的,并且其所使用的材料为诸如羟丙基纤维素、微晶纤维素(FMC公司的MCE、AVICEL™)、聚(乙烯-乙酸乙烯酯)(60:40)共聚物(Aldrich Chemical公司的EVAC)、甲基丙烯酸2-羟乙酯(HEMA)、MMA、在N,N′-双(甲基丙烯酰氧乙氧基羰基氨基)-偶氮苯存在的情况下合成的HEMA:MMA:MA的三元共聚物、偶氮聚合物、肠溶包衣的延时释放体系(TIME CLOCK®,来自Pharmaceutical Profiles有限公司,UK),以及果胶钙和渗透性微型泵体系(ALZA公司)。

[0131] 在一些实施方案中,薄膜包衣包含聚合物例如羟丙基纤维素(HPC)、乙基纤维素(EC)、羟丙基甲基纤维素(HMPC)、羟乙基纤维素(HEC)、羧甲基纤维素钠(CMC)、聚乙烯吡咯烷酮(PVP)、聚乙二醇(PEG)、甲基丙烯酸二甲氨基乙酯-甲基丙烯酸酯共聚物或丙烯酸乙酯-甲基丙烯酸甲酯共聚物(EA-MMA)。

[0132] 在一些实施方案中,组合物具有改性释放包衣。改性释放包衣可以是pH-响应性包衣,当暴露于某pH时,其会递送活性剂(例如,至结肠直肠道)。在一些实施方案中,尽管pH响应性聚合物可以在大于或等于约5的pH下溶解;但pH响应性包衣是当暴露于大于或等于约6的pH时会溶解的pH响应性聚合物。pH响应性聚合物可以是,例如,聚合化合物,例如EUDRAGIT™ RS和EUDRAGIT™ RL。EUDRAGIT™产品形成基于重量约30D的乳胶分散体。EUDRAGIT™ RS 30D被设计用于缓慢释放,因为其作为包衣不是特别水渗透性的,且EUDRAGIT™ RS 30D被设计为快速释放,因为其作为包衣是相对而言水可渗透的。通常组合使用这两种聚合物。正如本文所考虑的,EUDRAGIT™ RS 30D/EUDRAGIT™ RL 30D的可允许比例为约10:0至约8:2。也可以使用乙基纤维素或S100或设计用于肠释放或结肠直肠释放的其他等同聚合物,代替上文的EUDRAGIT™ RS/EUDRAGIT™ RL组合。

[0133] 任选地,该方法包括对片剂进行薄膜包衣的步骤。可以使用任何合适的方式来实现薄膜包衣。合适的薄膜包衣是已知的并且可商购获得,或者可以根据已知方法制备。通常,薄膜包衣材料是包含诸如聚乙二醇、滑石和着色剂的材料的聚合薄膜包衣材料。合适的包衣材料是甲基纤维素、羟丙基甲基纤维素、羟丙基纤维素、丙烯酸聚合物、乙基纤维素、乙酸邻苯二甲酸纤维素、聚乙酸乙烯邻苯二甲酸酯、羟丙基甲基纤维素邻苯二甲酸酯、聚乙烯醇、羧甲基纤维素钠、乙酸纤维素、乙酸邻苯二甲酸纤维素、明胶、甲基丙烯酸共聚物、聚乙二醇、紫胶、蔗糖、二氧化钛、巴西棕榈蜡、微晶蜡和玉米醇溶蛋白。在一些方面,薄膜包衣是羟丙基甲基纤维素、二氧化钛、聚乙二醇和氧化铁黄。例如,薄膜包衣是OPADRY®黄(Colorcon)。通常,以这样的量施加薄膜包衣材料,以提供基于重量占薄膜包衣片剂1%至6%范围的薄膜包衣,例如2%至4%,如约3%。可以将增塑剂和其他成分加入到包衣材料中。也可以在包衣材料中加入相同或不同的活性物质。

[0134] 在一些实施方案中,片剂的包衣可以善适口性,例如掩盖活性成分例如DFMO的不愉快味道。例如,片剂包衣组合物可包含纤维素聚合物、增塑剂、增甜剂或粉末状芳香组合物,所述粉末状芳香组合物包括与固体载体结合的调味剂(flavorant)。

[0135] C.给药时间表和方案

[0136] 在一些实施方案中,可以按照常规时间表施用所述药剂。如本文所用的,常规时间表是指预定的指定时间段。常规时间表可以涵盖长度相同或者不同的时间段,只要时间表

是预定的。例如，常规时间表可涉及一天给药两次，每天给药，每两天给药，每三天给药，每四天给药，每五天给药，每六天给药，每周给药，每月给药或之间任何设定的天数或周数。或者，预定的常规时间表可以包括第一周每天两次给药，然后几个月每天给药等。在其他实施方案中，本发明公开了可以口服药剂，并且口服时机依赖于或不依赖于进食。因此，例如，可以在每天早上和/或每天晚上服用药剂，而不管受试者何时已进食或将进食。

[0137] VI.患者的诊断和治疗

[0138] 在一些实施方案中，可用诊断方法增补治疗方法，以改善抗癌疗法的疗效和/或使其毒性最小化，所述抗癌疗法包括施用本文提供的组合物。这样的方法描述于例如美国专利第8,329,636号和第9,121,852号，美国专利公开US2013/0217743和US2015/0301060，以及PCT专利公开WO2014/070767和WO2015/19512，在此通过引用并入所有这些专利。

[0139] 在一些实施方案中，可将本公开的组合物和制剂施用于在ODC1基因启动子的至少一个等位基因的位置+316的基因型为G的受试者。在一些实施方案中，在患者ODC1基因启动子的两个等位基因的位置+316处的基因表型可以是GG。在一些实施方案中，在患者ODC1基因启动子的两个等位基因的位置+316处的基因型可以是GA。在腺瘤复发的全模型中检测到ODC1基因型和治疗的统计学上显著的相互作用，使得安慰剂患者中腺瘤复发的模式为：GG50％，GA35％，AA29％，相比于依氟鸟氨酸/舒林酸患者:GG 11％，GA 14％，AA 57％。依氟鸟氨酸和舒林酸的腺瘤抑制效果在具有主要G纯合ODC1基因型的患者中较大，这与先前表明接受阿司匹林、携带至少一个A等位基因的CRA患者中复发性腺瘤风险降低的报道(Martinez et al.,2003;Barry et al.,2006;Hubner et al.,2008)相反。这些结果表明，与GG基因型患者相比，在位置+316处ODC1 A等位基因携带者对长时间暴露于依氟鸟氨酸和舒林酸的反应不同，A等位基因携带者在腺瘤复发方面经历的益处较小，发生耳毒性风险的可能性增加，尤其是在AA纯合子中。

[0140] 在一些实施方案中，本发明提供了预防性治疗或治愈性治疗患者的结肠直肠癌的方法，包括：(a)由确定患者在至少一个ODC1启动子基因等位基因的位置+316处的基因型的测试获得结果；和(b)如果所述结果表明患者在ODC1启动子基因的至少一个等位基因的位置+316处的基因型为G，则向患者施用本文提供的组合物。在一些实施方案中，本发明提供了用于治疗患者的结肠直肠癌风险因素的方法，包括：(a)由确定患者在至少一个ODC1启动子基因等位基因的位置+316处的基因型的测试获得结果；和(b)如果所述结果表明患者在ODC1启动子基因的至少一个等位基因的位置+316处的基因型为G，则向患者施用本文提供的组合物，其中所述方法预防患者形成新的异常隐窝病灶，新的腺瘤性息肉或伴有发育不良的新腺瘤。参见美国专利第8,329,636号，其通过引用并入本文。

[0141] 在一些实施方案中，本发明提供了用于预防性治疗或治愈性治疗患者的家族性腺瘤性息肉病(FAP)或神经母细胞瘤的方法，包括：(a)由确定患者在至少一个ODC1启动子基因等位基因的位置+316处的基因型的测试获得结果；和(b)如果所述结果表明患者在ODC1启动子基因的至少一个等位基因的位置+316处的基因型为G，则向患者施用本文提供的组合物。在一些实施方案中，本发明提供了治疗患者的家族性腺瘤性息肉病或神经母细胞瘤风险因素的方法，包括：(a)由确定患者在至少一个ODC1启动子基因等位基因的位置+316处的基因型的测试获得结果；和(b)如果所述结果表明患者在ODC1启动子基因的至少一个等位基因的位置+316处的基因型为G，则向患者施用本文提供的组合物，其中所述方法预防患

者形成新的异常隐窝病灶，新的腺瘤性息肉病或伴有发育不良的新腺瘤。参见美国专利第9,121,852号，其通过引用并入本文。

[0142] 在一些实施方案中，本发明提供了用于治疗患有癌症的患者的方法，其包括向患者施用本文提供的组合物，其中患者已经确定为具有饮食性多胺摄入和/或组织多胺水平，和/或不高的组织多胺流量。在这些实施方案的一些中，不高的饮食性多胺摄入是每天300μM多胺或以下。在这些实施方案中的一些中，癌症是结肠直肠癌。参见美国专利公开第US2013/0217743号，其通过引用并入本文。

[0143] 在一些实施方案中，本发明提供用于预防或治愈性治疗患者癌症的方法，包括：(a)由测定患者癌细胞中let-7非编码RNA、HMGA2蛋白和/或LIN28蛋白的表达水平的测试获得结果；和(b)如果所述结果表明，患者的癌症表现出：与参照let-7非编码RNA表达水平相比降低的let-7非编码RNA表达水平，与参照HMGA2蛋白表达水平相比升高的HMGA2蛋白表达水平，和/或与参照LIN28蛋白质表达水平相比升高的LIN28蛋白质表达水平，则向患者施用本文提供的组合物。在这些实施方案中的一些中，参照水平是在未患病受试者中观察到的水平，或在患者的非癌性细胞中观察到的水平。在这些实施方案的一些中，“获得”包括提供来自患者的癌症样品，并评估来自所述样品的癌细胞中let-7非编码RNA、HMGA2蛋白或LIN28蛋白的表达水平。在这些实施方案中的一些中，“评估let-7非编码RNA的表达水平”包括定量PCR或Northern印迹。在这些实施方案中的一些中，“评估HMGA2蛋白或LIN28蛋白的表达水平”包括免疫组织化学或ELISA。在这些实施方案的一些中，样品是血液或组织，例如肿瘤组织。在这些实施方案中的一些中，患者是人。在这些实施方案的一些中，癌症是结直肠癌、神经母细胞瘤、乳腺癌、胰腺癌、脑癌、肺癌、胃癌、血癌、皮肤癌、睾丸癌、前列腺癌、卵巢癌、肝癌、食管癌、宫颈癌、头颈癌、非黑素瘤皮肤癌或成胶质细胞瘤。在这些实施方案中的一些中，所述方法还包括(c)获得测试的结果，所述测试在施用至少一种剂量的ODC抑制剂后，在第二时间点确定来自所述患者的第二癌细胞中let-7非编码RNA的表达。在这些实施方案中的一些中，所述方法还包括如果没有观察到let-7非编码RNA增加或观察到小幅增加，则增加向患者施用的ODC抑制剂的量。在这些实施方案中的一些中，所述方法还包括获得测试的结果，所述测试在施用至少一剂量的ODC抑制剂后，在第二时间点确定来自所述患者的第二癌细胞中HMGA2蛋白或LIN28蛋白的表达。在这些实施方案中的一些中，所述方法还包括如果没有观察到HMGA2蛋白或LIN28蛋白的降低观察到少量降低，则增加向患者施用的ODC抑制剂的量。在这些实施方案中的一些中，所述方法还包括(i)由确定患者在ODC1基因启动子的至少一个等位基因的位置+316处的基因型的测试获得结果；和(ii)如果所述结果显示患者在ODC1基因启动子的至少一个等位基因的位置+316处的基因型为G，则向患者施用本文提供的组合物。在一些实施方案中，所述方法包括诊断患者的癌症或癌前病况，包括从患者获得样品，和(b)测定样品的选自由let-7非编码RNA、LIN28蛋白和HMGA2蛋白组成的组的至少两种标志物的表达水平，其中如果相对于参照水平，样品中let-7非编码RNA的表达水平降低，或者LIN28蛋白或HMGA2蛋白升高，则患者是被诊断为患有癌症或癌症前病况。在一些实施方案中，将本发明的固定剂量组合施用于具有低细胞或组织let-7水平的患者。在其他方面，将本发明组合物施用于具有高细胞或组织HMGA2水平的患者。在其他方面，将本发明的组合物施用于具有高细胞或组织LIN28水平的患者。参见美国专利公开第US2015/0301060号，其通过引用并入本文。

[0144] 在一些实施方案中，提供了用于预防性或治愈性治疗患者癌症的方法，其包括：(a)由确定患者在至少一个ODC1等位基因的位置+263处的基因型的测试获得结果；和(b)如果所述结果表明患者在ODC1基因的至少一个等位基因的位置+263处的基因型为T，则向患者施用本文提供的组合物。在这些实施方案中的一些中，测试可确定患者ODC1基因的一个等位基因的位置+263处的核苷酸碱基。在一些实施方案中，测试可以确定患者ODC1基因的两个等位基因的位置+263处的核苷酸碱基。在一些实施方案中，所述结果可表明患者在ODC1基因的两个等位基因的位置+263处的基因型是TT。在一些实施方案中，所述结果可表明患者在ODC1基因的两个等位基因的位置+263处的基因型是TG。在这些实施方案的一些中，所述方法进一步可以包括由确定患者在至少一个ODC1等位基因的位置+316处的基因型的测试获得结果，并且仅如果所述结果表明患者在ODC1基因的至少一个等位基因的位置+316处的基因型为G，则向所述患者施用本文提供的组合物。另一方面，提供了用于治疗患者的结肠直肠癌风险因素的方法，包括：(a)由确定患者在至少一个ODC1等位基因的位置+263处的基因型的测试获得结果；和(b)如果所述结果表明患者在ODC1基因的至少一个等位基因的位置+263处的基因型为T，则向所述患者施用本文提供的组合物，其中所述方法预防患者形成新的异常隐窝病灶，新的腺瘤性息肉或伴有发育不良的新腺瘤。另一方面，提供了用于预防处于风险中的患者的癌症发展或复发的方法，包括：(a)由确定患者在至少一个ODC1等位基因的位置+263处的基因型的测试获得结果；和(b)如果所述结果表明患者在ODC1基因的至少一个等位基因的位置+263处的基因型为T，则向所述患者施用本文提供的组合物。参见PCT专利公开WO2015/195120，其通过引用并入本文。

[0145] 在任何上述实施方案的变体中，癌可以是结直肠癌、神经母细胞瘤、乳腺癌、胰腺癌、脑癌、肺癌、胃癌、血癌、皮肤癌、睾丸癌、前列腺癌、卵巢癌、肝癌、食管癌、宫颈癌、头颈癌、非黑素瘤皮肤癌或成胶质细胞瘤。在一些实施方案中，癌可以是结肠直肠癌。在一些实施方案中，结直肠癌可以是I期。在一些实施方案中，结肠直肠癌可以是II期。在一些实施方案中，结直肠癌可以是III期。在一些实施方案中，结肠直肠癌可以是IV期。在任何上述实施方案的变体中，所述方法可以预防患者形成新的晚期结肠直肠瘤。在一些实施方案中，所述方法可以预防形成新的右侧晚期结肠直肠瘤。在一些实施方案中，所述方法可以预防形成新的左侧晚期结肠直肠瘤。

[0146] 在任何上述实施方案的变体中，患者可能已经被鉴定为在结肠、直肠或阑尾中患有一或多个腺瘤性息肉。在一些实施方案中，患者可能已经被鉴定为患有一或多个晚期结肠直肠瘤。在一些实施方案中，患者可能已经被鉴定为患有一或多个左侧晚期结肠直肠瘤。在一些实施方案中，患者可能已经被鉴定为患有一或多个右侧晚期结肠直肠瘤。在一些实施方案中，患者可能已经被诊断患有家族性腺瘤性息肉病。在一些实施方案中，患者可能已经被诊断患有林奇综合征(Lynch syndrome)。在一些实施方案中，患者可能已经被诊断为患有X型家族性结肠直肠癌。在一些实施方案中，患者可满足阿姆斯特丹标准(Amsterdam Criteria)或阿姆斯特丹标准II。在一些实施方案中，患者可具有切除一或多个结肠直肠腺瘤的历史。在一些实施方案中，患者可患有上皮内瘤变或与ODC活动过度相关的癌前病变。在一些实施方案中，患者可患有上皮内瘤变或癌前病变并且细胞内多胺水平升高。

[0147] 在任何上述实施方案的变型中，患者是人。

[0148] VII.定义

[0149] 本说明书所用的“a(一种/个)”或“an(一种/个)”可以表示一种/个或多种/个。如权利要求书中所使用的,当与单词“包含”一起使用时,单词“a(一种/个)”或“an(一种/个)”可以表示一个/种或多于一个/种。

[0150] 贯穿本申请,术语“约”用于指示值包括用于确定所述值的装置、方法的误差的固有变化或存在于研究受试者之间的变化。

[0151] 本文所用的术语“生物利用度”表示在给药后药物或其他物质变得可用于靶组织的程度的手段。在本发明的上下文中,术语“合适的生物利用度”旨在表示,与施用普通片剂(plain tablet)中的活性物质后获得的生物利用度相比,施用本发明的组合物将导致改善的生物利用度;或与施用含有相同量的相同活性物质的市售产品后获得的生物利用度相比,生物利用度至少相同或获得改善。特别是,期望获得活性化合物的更快和更大和/或更完全的摄取,并由此提供给药剂量的降低或每日给药次数的降低。

[0152] 术语“组合物”、“药物组合物”、“制剂(formulation)”和“制剂(preparation)”在本文中同义使用并且可互换使用。

[0153] 术语“包含(comprise)”,“具有(have)”和“包括(include)”是开放式连接动词。这些动词中的一个或多个动词的任何形式或时态,诸如“包含(comprises)”、“包含(comprising)”、“具有(has)”、“具有(having)”、“包括(includes)”和“包括(including)”,也是开放式的。例如,“包括(comprises)”、“具有(has)”或“包括(includes)”一个或多个步骤的任何方法不限于仅具有那些一个或多个步骤,并且还涵盖其他未列出的步骤。

[0154] 术语“其衍生物”是指任何化学修饰的多糖,其中至少一个单体糖单元通过原子或分子基团或键的取代而被修饰。在一个实施方案中,其衍生物是其盐。盐为例如与合适的无机酸例如氢卤酸、硫酸或磷酸的盐,例如盐酸盐、氢溴酸盐、硫酸盐、硫酸氢盐或磷酸盐,与以下的盐:合适的羧酸例如任选地羟基化的低级链烷酸,例如乙酸、乙醇酸、丙酸、乳酸或新戊酸,任选地羟基化和/或氧取代的低级链烷二元酸,例如草酸、琥珀酸、富马酸、马来酸、酒石酸、柠檬酸、丙酮酸、苹果酸、抗坏血酸,还有与芳族、杂芳族或芳脂族羧酸例如苯甲酸、烟酸或扁桃酸的盐,以及与合适的脂肪族或芳族磺酸或N-取代的氨基磺酸的盐,例如甲磺酸盐、苯磺酸盐、对甲苯磺酸盐或N-环己基氨基磺酸盐(环己氨基磺酸盐(cyclamates))。

[0155] 本文所用的术语“崩解”是指其中药物口服固定剂量组合通常借助流体分裂成分开的颗粒并分散的过程。当固体口服剂型处于这样一种状态时,完成崩解,在所述状态中,除了不溶性包衣或胶囊壳的片段(如果存在的话),保留在测试设备的筛网上的固体口服剂型的任何残余物都是软质,不具有符合USP<701>的明显坚硬的核。用于确定崩解性质的流体是水,如自来水或去离子水。通过本领域技术人员已知的标准方法测量崩解时间,参见药典USP<701>和EP 2.9.1和JP中阐述的协调程序。

[0156] 本文所用的术语“溶解”是指将固体物质-在本文中为活性成分-以分子形式分散在介质中的过程。本发明的药物口服固定剂量组合的活性成分的溶解速率的定义为,在液体/固体界面、温度和溶剂组成的标准化条件下,每单位时间进入溶液中的药物物质的量。溶解速率通过本领域技术人员已知的标准方法测量,参见药典USP<711>和EP 2.9.3和JP中阐述的协调程序。出于本发明的目的,用于测量各个活性成分的溶解度的测试按照药典USP<711>在本文针对不同实施方案所述的pH下进行。特别是,使用桨搅拌部件以75rpm(每分钟转数)进行测试。溶出介质优选为缓冲液,通常为磷酸盐缓冲液(例如,pH 7.2)。缓

冲液的摩尔浓度优选为0.1M。

[0157] “活性成分”(AI)(也称为活性化合物、活性物质、活性剂、药剂(pharmaceutical agent)、药剂(agent)、生物活性分子或治疗性化合物)是药物或杀虫剂中具有生物活性的成分。类似的术语活性药物成分(API)和原料药(bulk active)也用于药学中,并且术语活性物质可用于杀虫剂制剂。

[0158] “药物(pharmaceutical drug)”(也称为药品(pharmaceutical)、药物制剂(pharmaceutical preparation)、药物组合物(pharmaceutical composition)、药物制剂(pharmaceutical formulation)、药物产品(pharmaceutical product)、医药产品(medicinal product)、药(medicine)、药物(medication)、药物(medicament)或简单而言药物(drug))是用于诊断、治愈、治疗或预防疾病的药物。活性成分(AI)(定义如上)是药物或杀虫剂中具有生物活性的成分。类似的术语活性药物成分(API)和原料药也用于药物中,术语活性物质可用于杀虫剂制剂。一些药物和杀虫剂产品可以含有多种活性成分。与活性成分相比,非活性成分在制药背景中通常称为赋形剂。

[0159] 在说明书和/或权利要求中使用的术语“有效”是指足以实现期望的、预期的或有意的结果。当在用化合物治疗患者或受试者的背景下使用时,“有效量”、“治疗有效量”或“药学有效量”是指当将化合物施用于受试者或患者以治疗或预防疾病时足以实现这种疾病的治疗或预防的所述化合物的量。

[0160] “预防(prevention)”或“预防(preventing)”包括:(1)抑制可能处于风险中和/或易患疾病但尚未经历或表现出所述疾病的任何或全部病理或症候的受试者或患者的疾病发作,和/或(2)减缓可能处于风险中和/或易患疾病但尚未经历或表现出所述疾病的任何或全部病理或症候的受试者或患者的病理或症状的发作。

[0161] “治疗(treatment)”或“治疗(treating)”包括(1)抑制经历或表现出疾病的病理或症候的受试者或患者的疾病(例如阻止所述病理和/或症候进一步发展),(2)改善正经历或表现出疾病的病理或症候的受试者或患者的疾病(例如逆转病理和/或症候),和/或(3)在正在经历或表现出疾病的病理或症候的受试者或患者中实现疾病的任何可测量的减少。

[0162] 根据本发明,“前药”是指可在体内代谢转化为抑制剂的化合物。对于给定的靶蛋白而言,前药自身可以具有或不具有活性。例如,包含羟基的化合物可以作为通过体内水解转化为羟基化合物的酯而施用。可以在体内转化为羟基化合物的合适酯包括乙酸酯、柠檬酸酯、乳酸酯、磷酸酯、酒石酸酯、丙二酸酯、草酸酯、水杨酸酯、丙酸酯、琥珀酸酯、富马酸酯、马来酸酯、亚甲基-二-β-羟基萘甲酸酯、龙胆酸酯、羟乙基磺酸酯、二-p-甲苯酰基酒石酸酯、甲磺酸酯、乙磺酸酯、苯磺酸酯、p-甲苯磺酸酯、环己基氨基磺酸酯、奎尼酸酯、氨基酸的酯等。类似地,可以将包含胺基的化合物作为通过体内水解转化为胺化合物的酰胺而施用。

[0163] “赋形剂”是与药物、药物组合物、制剂或药物递送系统的活性成分一起配制的药学上可接受的物质。例如,赋形剂可用于稳定组合物、使组合物膨松(因此在用于此目的时通常称为“疏松剂”、“填充剂”或“稀释剂”),或赋予最终剂型中活性成分治疗增强,例如促进药物吸收、降低粘度或提高溶解度。赋形剂包括抗粘附剂、粘合剂、包衣、颜料、崩解剂、调味剂(flavor)、助流剂、润滑剂、防腐剂、吸着剂、增甜剂和媒介(vehicle)的药学上可接受形式。用作运送活性成分的介质的主要赋形剂通常称为载体。除了辅助体外稳定性,例如在

预期保质期内防止变性或聚集，赋形剂还可以用于制造过程中，以例如帮助处理活性物质，例如通过促进粉末流动性或非粘性质。赋形剂的适用性通常取决于给药途径、剂型、活性成分以及其他因素而变化。

[0164] 当用作化合物的改性剂时，术语“水合物”是指该化合物具有与每个化合物分子例如固体形式的化合物结合的少于一个水分子(例如半水合物)、一个水分子(例如一水合物)或多个水分子(例如二水合物)。

[0165] 当单独使用时，术语“依氟鸟氨酸”是指2,5-二氨基-2-(二氟甲基)戊酸的任何形式，包括非盐形式和盐形式(例如依氟鸟氨酸HCl)，非盐形式和盐形式的无水和水合形式(例如依氟鸟氨酸盐酸盐一水合物)，非盐形式和盐形式的溶剂合物，其对映异构体(R和S形式，其也可以称为d和l形式)以及这些对映异构体的混合物(例如外消旋混合物或相对于另一个富含一种对映异构体的混合物)。依氟鸟氨酸的具体形式包括依氟鸟氨酸盐酸盐一水合物(即CAS ID:96020-91-6;MW:236.65)、依氟鸟氨酸盐酸盐(即CAS ID:68278-23-9;MW:218.63)和游离的依氟鸟氨酸(即,CAS ID:70052-12-9;MW:182.17)。必要时，已进一步指定了依氟鸟氨酸的形式。在一些实施方案中，本公开的依氟鸟氨酸为依氟鸟氨酸盐酸盐一水合物(即,CAS ID:96020-91-6)。术语“依氟鸟氨酸”和“DFMO”在本文中可互换使用。依氟鸟氨酸和DFMO的其他同义词包括:α-二氟甲基鸟氨酸、2-(二氟甲基)-DL-鸟氨酸、2-(二氟甲基)鸟氨酸、DL-α-二氟甲基鸟氨酸、N-二氟甲基鸟氨酸、二氟甲基鸟氨酸(ornidyl)、αδ-二氨基-α-(二氟甲基)戊酸和2,5-二氨基-2(二氟)戊酸。

[0166] 如本文所用，就特定组分而言，“基本上不含”在本文中用于表示指定组分未被有目的地配制到组合物中和/或指定组分仅以污染物或痕量存在。因此，由组合物的任何意外污染导致的指定组分的总量远低于0.05％，优选低于0.01％。最优选的组合物是其中用标准分析方法检测不到指定量组分的量。

[0167] 术语“固定剂量组合”或“FDC”是指存在于单一剂量单位(例如片剂或胶囊)中并作为单一剂量单位施用的两种药物或活性成分的确定剂量的组合;如本文进一步所用，“游离剂量组合”是指同时但作为两种不同剂量单位施用的两种药物或活性成分的组合。

[0168] “制粒”是指将粉末颗粒聚集成含有活性药物成分的较大颗粒的过程。“干法制粒”是指所包含步骤中无将液体加入粉末状起始原料、搅拌和干燥以产生固体剂型的任何方法。可以将所得的粒状药物产品进一步加工成各种最终剂型，例如胶囊、片剂、薄片剂、凝胶、锭剂等。

[0169] 权利要求中术语“或”的使用用于表示“和/或”，除非明确指出仅择一方案或所选择方案是相互排斥的，尽管本公开支持仅涉及择一方案和“和/或”的定义。如本文所用，“另一个”可以表示至少又一个或更多个。

[0170] 如本文所用，术语“患者”或“受试者”是指有生命的哺乳动物生物体，例如人、猴、牛、绵羊、山羊、狗、猫、小鼠、大鼠、豚鼠或其转基因物种。在某些实施方案中，患者或受试者是灵长类。人类患者的非限制性实例是成人、青少年、婴儿和胎儿。

[0171] 如本文通常所用，“药学上可接受的”是指在合理的医学判断范围内适合用于与人和动物的组织、器官和/或体液接触而无过量毒性、刺激、过敏反应或具有合理的益处/风险比的其他问题或并发症的那些化合物、材料、组合物和/或剂型。

[0172] “药学上可接受的载体”、“药物载体”或简单地“载体”是与活性成分药物一起配

制，参与携带、递送和/或运输化学药剂的药学上可接受的物质。药物载体可用于改善药物的递送和效力，包括例如调节药物生物利用度、降低药物代谢和/或降低药物毒性的控释技术。一些药物载体可以增加药物递送到特定靶位点的效力。载体的实例包括脂质体、微球体(例如由聚(乳酸-共-乙醇酸)制备)、白蛋白微球体、合成聚合物、纳米纤维、纳米管、蛋白-DNA复合物、蛋白缀合物、红细胞、病毒颗粒和树枝状聚合物。

[0173] 如本文所定义的术语“物理上分离的”是指包含组分a)和b)的药物口服固定剂量组合，组分a)和b)被配制为并未在相同载体中彼此混合，而是分离的。这种分离有助于使两个组分之间的相互作用最小化，特别是在它们释放时。通常，物理分离意味着两种组分a)和b)存在于不同的隔室例如层中，或者以制剂的不同实体例如微粒(particulate)或颗粒(granulate)的存在。两个组分a)和b)不需要通过额外的层或包衣进一步分开，尽管这在不同情况下可能是适当的。两种组分a)和b)在一种剂型中的这种物理分离可以通过本领域已知的各种手段来实现。在一个实施方案中，这通过将各组分a)和b)配制成单独的层来实现，例如多层或双层制剂。本文描述了这种制剂的具体实例。

[0174] 术语“粘连(sticking)”是指颗粒附着到压片机冲头的面，包括在冲头面上的字母、标识或图案内。

[0175] 术语“加帽”是指片剂的帽或顶部从片剂的主体分开或断裂。加帽可由当压缩过程中空气被挤出时迁移的不可压缩的细小颗粒而引起。

[0176] 术语“脆性”在本文中是指压缩后片剂碎裂、粉碎或断裂的倾向。这可由许多因素造成，包括片剂设计差(边缘太锋利)、含水量低、粘合剂不足等等。在一些方面，片剂样品的脆性是以重量损失百分比(即重量损失表示为原始样品重量的百分比)给出。通常，对于大多数片剂来说，最大重量损失不超过1％视为是可接受的。

[0177] 本文所用的术语“释放”是指使药物口服固定剂量组合与流体接触，并且流体将药物输送到剂型外进入围绕所述剂型的流体中的过程。可以将给定剂型在患者中表现出的递送速率和递送持续时间的组合描述为其体内释放曲线。剂型的释放曲线可表现出不同的释放速率和释放持续时间，并且可以是连续的。连续释放曲线包括其中一种或多种活性成分以恒定或可变速率连续释放的释放曲线。当将具有不同释放曲线的两种或更多种组分组合于一种剂型中时，所得的两种组分的单独释放曲线可以与仅具有一种组分的剂型相同或不同。因此，这两个组分可以影响彼此的释放曲线，从而导致每种单独组分的不同释放曲线。

[0178] 双组分剂型可以显示出彼此相同或不同的两种组分的释放曲线。每种组分具有不同的释放曲线的双组分剂型的释放曲线可以描述为“异步的”。这种释放曲线包括(1)不同的连续释放，其中优选组分b)以低于组分a)的速率释放，和(2)组分a)和b)中的一种，优选组分b)为连续释放的，并且组分a)和b)中的另一种，优选组分a)经改性为延时连续释放的释放曲线。对于一种药物来说，两种释放曲线的组合也是可能的(例如50％的药物是连续的，而50％的相同药物是延迟连续的)。

[0179] 速释：对于本申请的目的而言，速释制剂是表现出没有通过特殊的制剂设计或制造方法有意改性的活性物质释放的制剂。

[0180] 改良释放：对于本申请的目的而言，缓释制剂是表现出通过特殊的制剂设计或制造方法有意改性的活性物质释放的制剂。通常，可以通过延迟一种或两种组分，优选组分a)的释放时间来获得这种改良释放。通常对于本发明的目的而言，改良释放是指释放超过5小

时，例如超过3小时或甚至更短。本文所用的改良释放意味着包括两种组分随时间的不同连续释放，或其中一种组分，优选组分a)仅在滞后时间后释放的延迟释放。这种改良释放形式可以通过将改良释放包衣，例如扩散包衣施加于药物物质或施加于包含药物物质的核心，或者通过产生包埋药物物质的释放改良基质来产生。

[0181] 术语“片剂”是指呈任何形状的小的、基本上固体片形式的药理学组合物。片剂形状可以是圆柱形、球形、矩形，囊状或不规则形状。术语“片剂组合物”是指包含在片剂中的物质。“片剂组合物成分”或“片剂成分”是指包含在片剂组合物中的化合物或物质。除了低熔点化合物和水溶性赋形剂之外，这些还可以包括但不限于活性成分和任何赋形剂。

[0182] 上述定义取代通过引用并于本文的任何参考文献中的任何有冲突定义。但是，某些术语被定义的事实不应视为表示任何未定义的术语都是不确定的。相反，所使用的所有术语被认为以本领域普通技术人员能够理解本发明的范围和实践的方式来描述本发明。

[0183] 本文中使用的单位缩写包括平均结果(ar)、千克重(kp)、千牛顿(kN)、基于重量的重量百分比(％w/w)、每平方英寸磅数(psi)、RH(相对湿度)、色差ΔE(dE)和每分钟转数(rpm)。

[0184] VIII.实施例

[0185] 包括以下实施例以说明本发明的优选实施方案。本领域技术人员应该理解的是，在下面的实施例中公开的技术代表发明人发现的在本发明的实践中有效发挥作用的技术，并且因此可以认为构成其实践的优选模式。然而，根据本公开内容，本领域技术人员应该理解，可以对所公开的具体实施方案做出许多改变，并且在不背离本发明的精神和范围的情况下仍然获得相似或类似的结果。

[0186] 实施例1-依氟鸟氨酸HCl和舒林酸组合片剂的开发

[0187] 在包含依氟鸟氨酸HCl和舒林酸的固定剂量组合(FDC)片剂的开发过程中，测试了几种制剂(表1)。测试的参数包括片剂崩解时间、片剂硬度和片剂脆性百分比。

[0188] 首先通过在1夸脱v-搅拌机中混合1/3的硅化MCC(PROSOLV®)和依氟鸟氨酸HCl，将制剂I制造成900mg片剂。接下来，将舒林酸和1/3的硅化MCC(PROSOLV®)在聚乙烯(PE)袋中预混合，并与胶体二氧化硅(CARBOSIL®)和预明胶化玉米淀粉(STARCH 1500®)一起加入到所述搅拌机中。用剩余的1/3硅化MCC(PROSOLV®)漂洗PE袋，并加入到所述搅拌机中。在加入手工筛选的硬脂酸镁之前，将混合物在约25rpm下混合10分钟，然后再混合3分钟。发现该制剂在冲头表面上有一些粘连，并导致粗糙的片剂表面。因此，对于制剂II，将硬脂酸镁从0.5％增加到1％，并且将硅化MCC从38.57％降低到38.07％。

[0189] 通过在PE袋中预混合CARBOSIL®、STARCH 1500®以及舒林酸，将制剂II制造成900mg片剂。接下来，将1/2的PROSOLV®和依氟鸟氨酸HCl加入到8夸脱v-搅拌机中，并预混合。剩余的％PROSOLV®用于漂洗PE袋，并加入到所述搅拌机中。将混合物在约25rpm下混合10分钟。然后将混合物从搅拌机中取出，并用Comill 039R筛网去团块(delump)，然后再回到v-搅拌机中再混合10分钟。接着，通过手工混合将用30目(即590μm)筛网手工筛选的硬脂酸镁加入到v-搅拌机中，并将将混合物在约25rpm混合3分钟。将混合物在Key Model BBTS 10工位上压成片剂。经测定所得片剂的崩解时间为约29-32秒，4分钟时的脆性为0.077％，8分钟时脆性为0.17392％，硬度为约28kp(表1)。然后利用O'Hara Labcoat，12”锅，用2.913重量％的OPADRY®黄(Colorcon)将片剂进行薄膜包衣，产生

927mg的片剂。薄膜包衣的片剂的硬度为约36.0-42.1kp，崩解时间为1分27秒至1分53秒。

[0190] 通过在PE袋中预混合CARBOSIL®、第二部分PROSOLV®以及舒林酸，将制剂III制造成片剂。接下来，将第一部分PROSOLV®的1/2和依氟鸟氨酸加入到8-夸脱v-搅拌机，同时预混合。第一部分PROSOLV®的剩余1/2用于漂洗PE袋，并加入到v-搅拌机中。将该混合物在约25rpm下混合10分钟。然后将混合物从搅拌机中取出并用Comill 039R筛网去团块，然后再回到v-搅拌机中再混合10分钟。接着，通过手动混合将用30目(即590μm)筛网手工筛选的硬脂酸镁加入到v-搅拌机中，并将混合物在约25rpm下混合3分钟。将混合物在Key Model BBTS 10工位压成片剂。经测定所得片剂的崩解时间为约51-57秒，4分钟时的脆性为0.2607%-0.3373%，8分钟时的脆性为0.8988%-1.008%，硬度约为13kp。然后使用0' Hara Labcoat，12”锅，将片剂用2.913重量%的OPADRY®黄(Colorcon)进行薄膜包衣，产生669.5mg的片剂。薄膜包衣的片剂的硬度为约36.0-42.1kp，崩解时间为约1分27秒至1分53秒。这种制剂的重量从900mg减少至650mg，并用PROSOLV®替换STARCH 1500®以增加片剂的强度。然而，在脆性测试以及薄膜包衣的过程中观察到加帽。

[0191] 使用与制剂III相同的方法，将制剂IV制备成700mg片剂。经测定所得片剂的崩解时间为1分10秒至1分34秒，4分钟时脆性为0.1424%-0.1567%，8分钟时的脆性为0.3186%-0.5166%，硬度为约20kp。然后使用0'Hara Labcoat 12”锅，将片剂用2.913重量%的OPADRY®黄(Colorcon)进行薄膜包衣，产生721mg片剂。薄膜包衣的片剂的崩解时间为1分43秒至2分7秒。在此制剂中，增加了PROSOLV®的用量，并且片剂重量从650mg增加到700mg。尽管在脆性测试过程中没有观察到加帽，但是三种片剂在薄膜包衣过程中确实具有加帽。

[0192] 表1:依氟鸟氨酸HCL和舒林酸固定剂量组合片剂的制剂I-Iv

[0193]

	制剂 I		**制剂 II**		**制剂 III**		**制剂 IV**	
成分	单位重量(mg)	% W/W	单位重量(mg)	% W/W	单位重量(mg)	% W/W	单位重量(mg)	% W/W
依氟鸟氨酸 HCl 一水合物外消旋物	375	41.67	375	41.67	375	57.69	375	53.571
舒林酸	75	8.33	75	8.33	75	11.54	75	10.714
硅化 MCC (第一部分)	347.13	38.57	342.63	38.07	149.5	23.0	199.6	28.514
硅化 MCC (第二部分)	0	0	0	0	41.075	6.32	41.075	5.868
预胶化玉米淀粉	96.12	10.68	96.12	10.68	0	0	0	0
胶体二氧化硅	2.25	0.25	2.25	0.25	1.625	1.625	1.625	0.232
硬脂酸镁	4.5	0.5	9	1	7.8	7.8	7.7	1.1
未包衣的片剂重量	**900**	**100**	**900**	**100**	**650**	**100**	**700**	**100**
OPADRY®黄 03B92557			**27.0**		19.5		21.0	
包衣的片剂重量			**927.0**		**669.5**		**721.0**	

[0194]

片剂特征		
	制剂 II	**制剂 IV**
压缩力	NR	85psi
硬度(kp)	平均 28	平均 20
崩解时间	平均 30s	平均 1min 30s (秒)
脆性(4 min (分钟)) (%)	0.08	0.16
脆性(8 min) (%)	0.17	0.52 (一加帽片剂)

[0195] 表2:依氟鸟氨酸HCL和舒林酸固定剂量组合片剂的示例性制剂

[0196]

成分	**单位重量(mg)**	**% (w/w)**
依氟鸟氨酸 HCl 一水合物外消旋物	375.00	52.011
舒林酸	75.00	10.402
硅化微晶纤维素	237.87	32.992
胶体二氧化硅	1.63	0.226
硬脂酸镁	10.50	1.456
核心片剂重量	700.00	
OPADRY®黄	21.00	2.913
薄膜包衣的片剂重量	721.00	100

[0197] 表3:示例性的片剂制造参数

[0198]

变量	**7107/2 R3bis**	**7107/2 R4**	**7107/3**	**7107/5 R2**	**7107/5 R3**
混合机	Turbula	Turbula	Turbula	Turbula	Turbula
混合时间	70 个循环	70 个循环	70 个循环	70 个循环	70 个循环
硬脂酸镁	1.50%	1.50%	1.50%	1.50%	1.50%
压片机(Press)	Korsch	Korsch	Korsch	Ronchi	Ronchi

[0199]

变量	7107/2 R3bis	7107/2 R4	7107/3	7107/5 R2	7107/5 R3
工具尺寸 工具涂层 雕刻顶部 雕刻底部 评分	17.5x8 铬/RC02 414C 波纹标识 无	17.5x8 铬/RC02 414C 波纹标识 无	17x9 R6 铬 中立 中立 可分裂	16.5x7 铬 4141 标识 可分裂	16.5x7 铬 4141 标识 可分裂
压缩力	37 kN 或 30 kN	37 kN	30 kN	37 kN	25 kN
预压缩力	2.1 kN	2.5 kN	2.0 kN	3.7 kN	2.5 kN
测试结果					
分裂(Cleavage)	无	无	无	无	无
粘连	无	无	无	无	无
顶部雕刻强度	通过	通过	通过	通过	通过
底部雕刻强度	通过	通过	通过	通过	通过
硬度	NA	12.80 kp	8.14 kp	18.46 kp	16.62 kp
分裂能力	NA	是	是	否	否
崩解时间	NA	1 分 15"至 1 分 25"	40 秒至 45 秒	2 分 15"至 2 分 32"	1 分 39"至 1 分 53"
4 分时的脆性	NA	0.08%	0.17%	0.21%	0.37%
30 分时的脆性	NA	1.15%	1.19%	1.80%	2.85%
片剂破裂/分裂	NA	无	无	无	无

[0200] 表4:用于实施例1所述制剂的材料

[0201]

材料	供应商
依氟乌氨酸 HCl 一水合物	Scino Pharm
舒林酸	ZACH
硅化微晶纤维素(MCC) (PROSOLV®)	NF EP
淀粉 1500 (部分预胶化玉米淀粉)	Colorcon Limited
胶体二氧化硅(CARBOSIL®)	IMCD France SAS
硬脂酸镁	Mallinkroot-Tyco
OPADRY®黄	Colorcon Limited
设备	
PK blend master V-搅拌机(1 夸脱和 8 夸脱)	
Quadro Comill 197S 型，筛网 0.039″	
Key Model BBTS 10 工位压片机	
O'Hara Labcoat 12″ 锅，0.8 mm 喷嘴	

[0202] 实施例2—制剂IV的开发

[0203] 基于实施例1,进一步测试制剂IV来确定可以改变哪些参数以防止加帽和粘连。测试的第一个参数是压缩力和以约5-15%的压缩力加入预压缩力(表5)。为了评估使制剂IV 700mg片剂达到约20kp的硬度时的压缩力和预压力,进行了几个试验。在第一个试验中,利

用设备C制造制剂IV 700mg片剂的最终共混物(表9)。制造工艺包括在PE袋中预混合CARBOSIL®、第二部分PROSOLV®以及舒林酸。接下来,将第一部分PROSOLV®的1/2和依氟鸟氨酸加入到10夸脱v-搅拌机,同时预混合。第一部分PROSOLV®的剩余1/2用于漂洗PE袋,并加入v-搅拌机中。将混合物在约7rpm下混合35分钟。然后将混合物从搅拌机中取出并用Frewitt TC1501.0mm筛网去团块,然后再回到v-搅拌机中再混合35分钟。接下来,用500μm筛网手工筛选硬脂酸镁,并通过手动混合将其加入到v-搅拌机中,用于在7rpm下进行10分钟的最终混合。在配备有五个17.5×8mm雕刻和镀铬冲头的Courtoy Modul P压片机上进行压缩步骤。设置参数,以便获得17.0-22.5kp之间的硬度。发现在没有预压力的情况下,观察到加帽。然而,预压力的使用增加了硬度并避免了加帽(表10)。此外,用预压力形成的片剂更耐磨损(即更低的脆性)。另外,实施例1所用的Key BBTS 10工位压片机的16.5×8mm冲头似乎更容易磨损。

[0204] 表5:针对制剂IV测试的压缩参数

[0205]

	初始设置	7107/01 设置#3	7107/01 设置 g#2
冲头形状	16.5x8 mm 光滑	17.5x8 mm 雕刻	17.5x8 mm 雕刻
压缩力	85 psi	34 kN	35 kN
预压力	无	无	有(3 kN)
硬度(kp)	ar (平均) 20	ar 13 (*)	ar 17
崩解时间	ar 1min30s	ar 1min20sec	ar 2min
脆碎度(4min) (%)	0.16	0.07	0.03
脆碎度(8min) (%)	0.52 (1 个加帽片剂)	NA	NA
脆碎度(10min) (%)	NA	0.27	0.13
脆碎度(30min) (%)	NA	1.79 (1 个加帽片剂)	0.54 (无加帽片剂)
厚度(mm)	ar 6.1	ar 5.5	ar 5.4

[0206] NA:未施加

[0207] (*)在未预压缩的情况下可以达到的最大硬度。

[0208] 在第二个试验中,改变冲头表面以确定其对制剂IV片剂的影响(表11)。在这个试验中,使用设备B制造制剂IV 700mg片剂的最终共混物。制造工艺包括在PE袋中预混合CARBOSIL®、第二部分PROSOLV®和舒林酸。接下来,将第一部分PROSOLV®的1/2和依氟鸟氨酸的加入10夸脱v-搅拌机中,同时预混合。第一部分PROSOLV®的剩余1/2用于漂洗PE袋,并加入到v-搅拌机中。该混合物以约30个循环/分钟混合8分钟30秒。然后将混合物从搅拌机中取出,并用CMA 1.0mm筛网去团块,然后再返回v-搅拌机再混合8.5分钟。接下来,用500μm筛网手动筛选硬脂酸镁,并通过手动混合加入到v-搅拌机中,用于以30循环/分钟进行2分钟20秒的最终混合。在配备有两个17.5×8mm雕刻和防粘连镀铬冲头的Korsch XL100压片机上进行压缩步骤。将预压力设定为30kN左右的主压缩力的5-10%。还测试了几种不同的冲头表面,包括铬、碳、钨和特氟龙VS不锈钢。在一些实施例中,可以使用特氟龙来减少粘连。

[0209] 为了避免粘连,测试了几个另外的变量,并且在压缩一开始施加了高约束。无论是用1.1%硬脂酸镁进行润滑还是将润滑时间从70转增加到140转都不能防止粘连(表11和12)。然而,将硬脂酸镁的比例率加到1.5%确实防止了粘连(表12),并且片剂硬度略微降低

约20％，但是4分钟后脆性仍然很低，低于0.1％。使用配备有不同类型破裂线(17×9mm和16.5×7mm)的两种冲头，可破碎性结果符合所测试的两种冲头。因此，将硬脂酸镁增加至1.5％可防止粘附，并且预压缩防止制剂IV加帽。

[0210] 表6：试验1和试验2中制剂IV的批重量

[0211]

成分	单位重量(mg)	试验1重量(g)	试验2重量(g)
依氟鸟氨酸HCl	375	1339.500	1340.000
舒林酸	75	268.100	268.027
硅化MCC(第一部分)	199.6	712.800	712.000
硅化MCC(第二部分)	41.075	146.700	146.648
胶体二氧化硅	1.625	5.796	5.8043
硬脂酸镁	7.7	27.515	27.504
片剂重量	700.0	2500.411	2499.983

[0212] 表7：制剂Iv的不同硬脂酸镁量

[0213]

	1.1%硬脂酸镁配方		1.3%硬脂酸镁配方(*)		1.5%硬脂酸镁配方(*)	
成分	单位重量 (mg)	w/w (%)	单位重量 (mg)	w/w (%)	单位重量 (mg)	w/w (%)
依氟鸟氨酸 HCl	375.000	53.571	374.227	53.461	373.457	53.351
舒林酸	75.000	10.714	74.851	10.693	74.704	10.672
硅化 MCC (第 1 部分)	199.598	28.514	199.192	28.456	198.793	28.399
硅化 MCC 第 2 部分)	41.075	5.868	40.992	5.856	40.908	5.844
胶体二氧化硅	1.625	0.232	1.624	0.232	1.617	0.231
硬脂酸镁	7.700	1.100	9.100	1.300	10.500	1.500
片剂重量	**700.0**	**100.00**	**700.0**	**100.00**	**700.0**	**100.00**

[0214] (*)在稀释以增加硬脂酸镁百分比后获得的配方。因此API浓度稍微低于靶标。

[0215] 表8：制剂IV的包衣

[0216]

成分	单位重量(mg)	批重量(g)
未包衣的片剂	700.00	600.00
OPADRY®黄 03B92557	21.00	53.995
纯化水	154.00	395.99
包衣的片剂重量	**721.00**	**653.995**

[0217] 表9：用于开发制剂IV的设备

[0218]

设备 A	设备 B	设备 C
PK 混合大师 V-搅拌机 1 夸脱和 8 夸脱	Turbula T10A 搅拌机 10L 容器	Servolift 搅拌机 10L 容器
Quadro Comill 197S 0.039″筛网	CMA T1 锥形球磨机 1.00 mm 筛网	Frewitt TC150 锥形球磨机 1.00 mm 筛网
	0.500 mm 筛分网	0.500 mm 筛分网
Key BBTS 10 工位压片机	Korsch XL100 压片机	Courtoy Modul P 压片机
O'Hara Labcoat 12″锅	Mini Glatt 涂布机	

[0219]

表 10：用于测试预压缩力对制剂 IV 的影响的第一次试验的参数和结果

压缩参数					
		7107/01 设置#3	**7107/01 设置#5**	**7107/01 设置#2**	**7107/01 设置#4**
速度(tpm)		50	50	50	50
预压力(kN) / %主压力		0.11 / 0%	1.43 / 5%	3.25 / 10%	4.68 / 15%
压缩力 (kN)		33.58	32.23	34.07	33.03
冲头(数量)		5	5	5	5
冲头形状		17.5x8 mm 雕刻	17.5x8 mm 雕刻	17.5x8 mm 雕刻	17.5x8 mm 雕刻
冲头表面处理		防粘连镀铬	防粘连镀铬	防粘连镀铬	防粘连镀铬
结果					
测试	**取样**	**7107/01 设置#3**	**7107/01 设置#5**	**7107/01 设置#2**	**7107/01 设置#4**
重量(mg) RSD (%)	20 个片剂	702.28 1.18	699.14 1.00	703.5 1.06	701.17 0.73
硬度(kp)	10 个片剂	11.5-14.4 (平均值：13.2)	15.2-17.9 (平均值：16.6)	15.8-18.4 (平均值：17.3)	17.0-18.7 (平均值：17.9)
脆性(%) 4min 10min 30min	根据药典	 0.07/无加帽 0.27/无加帽 1.79/ ***1 个加帽***	 0.07/无加帽 0.20/无加帽 0.67/无加帽	 0.03/无加帽 0.13/无加帽 0.54/无加帽	 0.08/无加帽 0.19/无加帽 0.59/无加帽
崩解时间(min)	3 个片剂	1min(分)08sec(秒) 至 1min40sec	1min39sec 至 2min17sec	1min51sec 至 2min11sec	1min41sec 至 1min 57sec
厚度(mm)	10 个片剂	5.4 - 5.6	5.4 - 5.5	5.4 - 5.5	5.4 - 5.5
粘连		有些粘连	有些粘连	有些粘连	有些粘连

[0220]

表 11：用于测试冲头表面对制剂 IV 的影响的第二次试验的参数和结果

最终共混物						
		7107/02 设置#2	7107/02 设置#5	7107/02 设置#6	7107/02 设置#7	7107/02 设置#8
硬脂酸镁的比例(%)		1.1	1.1	1.1	1.1	1.1
最终混合(转数)		140	140	140	140	140
压缩参数						
速度(tpm)		40	40	40	40	40
预压力(kN)		2.5	2.2	2.2	2.1	2.1
压缩力 (kN)		30	30	30	30	30
冲头(数量)		2	2	2	2	2
冲头形状		17.5x8 mm 雕刻	17.5x8 mm 雕刻	17.5x8 mm 雕刻	17.5x8 mm 雕刻	17.5x8 mm 雕刻
冲头表面处理		铬 RC-02 防粘连	碳 RB-01 防粘连	钨 RD-03 防粘连	特氟龙 RF-03 防粘连	钢(无防粘连镀层)
结果						
测试	**取样**	7107/02 设置#2	7107/02 设置#5	7107/02 设置#6	7107/02 设置#7	7107/02 设置#8
重量(mg) / RSD (%)	20 个片剂	697.12 / 0.38	NA	NA	NA	NA
硬度(kp)	5 个片剂	15.4 - 16.3	NA	NA	NA	NA
脆性(%) 4min 10min 30min	根据药典	 0.02/无加帽 0.04/无加帽 0.69/无加帽	NA	NA	NA	NA
崩解时间(min)	3 个片剂	0min58sec 至 1min00sec	NA	NA	NA	NA
厚度(mm)	10 个片剂	5.5 至 5.5	NA	NA	NA	NA
粘连	10 个片剂	有些粘连	有些粘连	有些粘连	非常轻度粘连	有些粘连

[0221]

表 12：用于测试最终混合持续时间和硬脂酸镁对制剂 IV 的影响的第二次试验的参数和结果

		7107/02 设置#1	7107/02 设置#2	7107/02 设置#3	7107/02 设置#4	7107/02 设置#10
最终共混物						
硬脂酸镁的比例 (%)		1.1	1.1	***1.5***	1.5	***1.3***
最终混合持续时间 (转数)		70	***140***	70	70	***140***
压缩参数						
速度(tpm)		40	40	40	40	40
预压力(kN)		3.5	2.5	2.1	2.5	2.2
压缩力 (kN)		>>30	30	30	37	37
冲头(数量)		2	2	2	2	2
冲头形状		17.5x8 mm 雕刻	17.5x8 mm 雕刻	17.5x8 mm 雕刻	17.5x8 mm 雕刻	17.5x8 mm 雕刻
冲头表面处理		防粘连镀铬	防粘连镀铬	防粘连镀铬	防粘连镀铬	防粘连镀铬
结果						
测试	**取样**					
重量(mg) / RSD (%)	20 个片剂	704.01 (*) / 0.23	697.12 / 0.38	695.19 / 0.38	702.61 / 0.39	703.24 / 0.29
硬度(kp)	5 个片剂	17.3 - 17.9	15.4 - 16.3	12.4 - 13.4	11.9 - 14.1	13.4 - 14.7
脆性(%) 4min 10min 30min	根据药典	 NA NA NA	 0.02/无加帽 0.04/无加帽 0.69/无加帽	 0.03/无加帽 0.15/无加帽 1.01/无加帽	 0.08/无加帽 0.11/无加帽 1.15/无加帽	 0.05/无加帽 0.19/无加帽 1.02/无加帽
崩解时间(min)	3 个片剂	1min15sec 至 1min20sec	0min58sec 至 1min00sec	1min00sec 至 1min 10sec	1min15sec 至 1min 25sec	1min30sec 至 1min45sec
厚度(mm)	10 个片剂	5.3 - 5.4	5.5 - 5.5	5.5 - 5.5	5.5 - 5.5	5.5 - 5.6
粘连	10 个片剂	粘连降低，一些较低的冲头是干净的	粘连降低	在较高的冲头上有非常轻度的粘连。在较低的冲头上没有粘连	硬度测试过程中无粘连，但倾向于裂开	在上部的冲头上略微粘连

(*) 对 10 个片剂

[0222]

表 13：用于测试压缩参数对制剂 IV 的影响的试验参数和结果

		7107/03 设置#1	7107/05 设置#1	7107/05 设置#2	7107/05 设置#3
最终共混物					
硬脂酸镁的比例(%)		1.5	1.5	1.5	1.5
压缩参数					
速度(tpm)		40	40	40	40
预压力(kN)		2.0	5.0	3.7	2.5
压缩力 (kN)		30	24	37	25
冲头(数量)		2	2	2	2
冲头形状		17x9R6mm 易碎	16.5x7mm 易碎	16.5x7mm 易碎	16.5x7mm 易碎
冲头表面处理		防粘连铬	防粘连铬	防粘连铬	防粘连铬
结果					
测试	**取样**	**7107/03 设置#1**	**7107/05 设置#1**	**7107/05 设置#2**	**7107/05 设置#3**
重量(mg) / RSD (%)	20 个片剂	700.20 (*) / 0.49	705.29 / 0.59	709.62 / 0.64	700.08 / 0.54
对一半进行的断裂测试 RSD (%)	30 个片剂	0.97	NA	3.19	2.91
硬度(kp)	5 个片剂	7.6-8.8 (**)	18.6-19.7	17.3-19.3	16.1-16.9
脆性(%) 4min 10min 30min	根据药典	0.17/无加帽 0.32/无加帽 1.19/无加帽	0.12/无加帽 0.47/无加帽 1.52/无加帽	0.21/无加帽 0.51/无加帽 1.80/无加帽	0.37/无加帽 1.04/无加帽 2.85/无加帽
崩解时间(min)	3 个片剂	0min40sec 至 0min45sec	1min38sec 至 1min43sec	2min15sec 至 2min32sec	1min39sec 至 1min53sec
厚度(mm)	10 个片剂	5.3-5.3	6.6-6.7	6.5-6.7	6.6-6.7
粘连	10 个片剂	无粘连	无粘连	无粘连	无粘连

(*) 对 30 个片剂 (**)对 10 个片剂 NA：未施加

[0223] 测试了制剂IV组合片剂、依氟鸟氨酸单一片剂和舒林酸单一片剂的稳定性。在6个月时使用用于测定水含量的卡尔费休滴定法进行制剂IV片剂的稳定性分析(图1)。图1表明，与依氟鸟氨酸单一片剂相比，制剂IV的组合片剂在六个月中具有较低的水吸收。水能够影响药物效力和药物溶解；例如，水能够增加药物通过水解而降解的速率(Gerhardt，

2009)。因此,在一些实施方案中,本文提供的组合片剂比单一活性剂片剂中的一种或两种更稳定。

[0224] 最后,还测试了制剂IV的溶出曲线。使用桨搅拌部件以75rpm(USP＜711＞溶解仪II(桨法))在pH 7.2的50mM磷酸钠缓冲液介质中进行溶解研究(图2A-2B)。对于依氟鸟氨酸(elfonithine)和舒林酸的溶解,该方法被验证II级。没有观察到活性药物成分依氟鸟氨酸和舒林酸在它们之间的干扰,与溶解介质、与磷酸盐缓冲溶液或与赋形剂的干扰。令人惊讶的是,据观察,与单药剂片剂相比,制剂IV的固定剂量组合具有重叠的体外溶出曲线。

[0225] 实施例3-药物赋形剂和包衣相容性

[0226] 进行依氟鸟氨酸HCl/舒林酸组合片剂的非cGMP药物赋形剂相容性研究。使用一系列样品评估外观、HPLC测定和XRPD性质。所测试的赋形剂包括PVP、HPMC、乳糖、EXPLOTAB™、Ac-Di-Sol® 、PROSOLV®、STARCH 1500®以及OPADRY®黄。为赋形剂相容性而制备的样品全部为API与赋形剂的1:1物理混合物,除了依氟鸟氨酸HCl:舒林酸制剂为5:1,依氟鸟氨酸HCl:舒林酸:H_2O制剂为约6:1:0.3。大多数样品的总质量约为750mg。制备包括称取成分至20cc闪烁小瓶中,关闭并涡旋大约30秒。然后将样品在40℃/75%RH的稳定箱中储存四周。小瓶上的盖子松散地固定,并避光储存在箱中。

[0227] 通过目视检查为HPLC分析制备的小瓶进行外观观察。用缓冲液(50mM磷酸盐缓冲液,pH2.55)中的50%乙腈提取赋形剂相容性样品。仅含舒林酸的样品通过称取部分(约150mg)样品来制备,并以预定体积提取,使得依氟鸟氨酸和舒林酸的终浓度分别为9.5mg/mL和0.1mg/mL。剩余的相容性样品通过使用预定体积的提取溶剂的定量转移来制备,使得依氟鸟氨酸和舒林酸的终浓度与上述大致相同。使用能够检测依氟鸟氨酸和舒林酸这两种活性物质的方法分析赋形剂相容性样品(图4A)。该方法采用梯度反相HPLC和195nm紫外(UV)检测。

[0228] 使用CuKα辐射在具有Bragg-Brentano配置的Bruker AXS D8 Advance系统上进行XRPD分析。使用以下参数在室温下分析样品:40kV,40mA,1°发散和防散射狭缝,以2-40°2θ的连续模式测量的方法,具有以0.05°步长和1秒/步长时间。在9位自动取样器附件中,使用顶部填充钢的旋转式样品架分析3-25mg样品。使用可追溯标准校准该系统。结果如图4B-4C所示。

[0229] 样品中具有PVP K30的依氟鸟氨酸HCl在样品中显示水分,其在2周样品中开始并且在4周时变成液体。具有PVPK30的舒林酸在2周时表现出样品粘连,并在4周时持续。PVPK30赋形剂仅在2周样品中开始表现出水分,并在4周时变成液体。用依氟鸟氨酸HCl样品观察到相同的行为,但用舒林酸样品没有观察到相同的行为。测试的大多数样品的HPLC测定结果在不同时间点并没有显示出不同的趋势(增加或减少)。尽管许多样品的测定值异常低,但测定水平在4周内表现出更多的增加趋势或保持相对恒定。观察到舒林酸/依氟鸟氨酸ProSolv SMCC90样品测定结果的最高可变性。4周时间点的测定值比最初的测定结果高10.0%。这种可变性可能归因于在不同时间点时方法(未验证)和样品一致性。虽然验证方法的可接受的随机分析误差为2%,但该方法的可变性是未知的。除一些样品外,在不同时间点测试的每个样品的测定值在分析方法的通常可接受的2%随机误差内。在测试的加压条件下,API即依氟鸟氨酸和舒林酸没有不同的趋势。这项研究的结果表明两种API(依氟鸟氨酸HCl/舒林酸)都与潜在的赋形剂相容。

[0230] 通过XRPD分析进行药物赋形剂相容性研究，以确定API与用于依氟鸟氨酸HCl/舒林酸组合产品的潜在制剂赋形剂的结晶度。XRPD结果显示，在四周后，在40℃/75％RH下，API与赋形剂之间没有相互作用。这表明两种API(依氟鸟氨酸HCl/舒林酸)都与潜在赋形剂相容。

[0231] 对片剂进行包衣试验，以确定在1个月和3个月时在25℃/60％RH或40℃/75％RH的水分含量下对稳定性的影响。包衣包括3重量％或4重量％增重的OPADRY®黄(Colorcon，03B92557)、OPADRY®白(Colorcon Y-1-7000)、OPADRY®II白(Colorcon 85F18422)以及OPADRY® Clear(无色)(Colorcon YS-3-7413)。进行颜色目测，以评估处于稳定状态的片剂与初始包衣片剂之间的总颜色差或DE。

[0232] 使用Datacolor Spectraflash 600系列分光光度计测试片剂颜色。使用国际照明委员会(CIE)L*a*b*系统分析数据。在L*a*b*系统中，颜色被表示为三维空间中的坐标。亮度和黑度绘制在L*轴上，L＝100代表纯白色，L＝0代表纯黑色。a*和b*轴分别表示两个互补的颜色对，即红/绿和蓝/黄。通过以几何方式绘制颜色，可以通过使用以下等式计算两点之间的距离来确定两种颜色之间的差异(总色差＝E*)。

[0233] DE*＝[(L*1-L*2)2+(a*1-a*2)2+(b*1-b*2)2]1/2

[0234] 使用Datacolor，在各种包衣制剂的每种增重下分析每种片剂。DE值越接近零，测试的片剂颜色越接近颜色标准(初始样品)。白色包衣的卡乐康(Colorcon)标准规格(通过QC测试)的DE值小于1.5。所有具有白色薄膜包衣的稳定性样品超过该1.5DE，因此不能通过卡乐康标准QC测试(表14)。透明包衣片剂也远高于1.5的值。

[0235] 表14：包衣片剂稳定性的DE值

[0236]

	3%增重 Y-1-7000 (白色)	4%增重 Y-1-7000 (白色)	3%增重 85F18422 (白色)	4%增重 85F18422 (白色)	3%增重 03B92557 (黄色)	4%增重 03B92557 (黄色)	3%增重 YS-3-7413 (透明)
1 mo 25/60	1.81	1.64	2.56	2.8	0.27	0.32	1.15
3 mo 25/60	1.97	1.94	2.96	2.31	0.35	0.22	1.1
1 mo 40/75	1.91	2.47	3.58	2.39	0.3	0.29	4.29
3 mo 40/75	2.71	2.66	2.72	3.31	0.64	0.58	7.6

[0237] 在用黄色制剂包衣的片剂中观察到最佳DE结果。DE值远低于1.5。DE值(总色差)等于或小于1视为是人眼难以察觉的。用于黄色包衣的卡乐康典型内部规格倾向于DE值为2.5-3。因此，使用OPADRY®黄来对组合片剂进行包衣。

[0238] 实施例4—固定的共配制依氟鸟氨酸/舒林酸的生物等效性研究

[0239] 在正常健康受试者中在空腹条件下进行初步研究，以相比于单独服用或共施用的含有依氟鸟氨酸或舒林酸的单独片剂，比较口服施用含有依氟鸟氨酸/舒林酸的共配制片剂后血浆中依氟鸟氨酸、舒林酸、舒林酸硫化物和舒林酸砜的药代动力学参数。本研究的第二个目的是，相比于单独服用或共施用的单独片剂，确定依氟鸟氨酸/舒林酸共配制片剂在正常健康受试者中的安全性和耐受性。

[0240] 该研究包括至少18岁但不超过60岁的十二名男性或女性受试者。主要的入选标准是：轻度吸烟者、非吸烟者或已戒烟者；体重指数(BMI)≥18.50kg/m^2且＜30.00kg/m^2；在进行的12导联EGG中没有发现临床显著性异常(在ECG之前个体必须仰卧10分钟，并且在进行

所有要求的抽血之前进行ECG)；女性受试者的阴性妊娠试验；并根据病史、全面健康体检(包括生命体征)和实验室检查(一般生化，血液学和尿分析)是健康的。

[0241] 受试者在四个治疗组中进行治疗，所述治疗组包括：

[0242] • 治疗1：750/150mg单剂量的共配制依氟鸟氨酸375mg/舒林酸75mg片剂(2 x 375/75mg片剂)

[0243] • 治疗2：750mg单剂量的依氟鸟氨酸250mg片剂(3 x 250mg片剂)

[0244] • 治疗3：150mg单剂量的舒林酸150mg片剂(1 x 150mg片剂)

[0245] • 治疗4：同时施用150mg单剂量的舒林酸150mg片剂(1 x 150mg片剂)，和750mg单剂量的依氟鸟氨酸250mg片剂(3x 250mg片剂)

[0246] 每个受试者被指定在28天内接受4种不同的治疗。在每个研究期间在空腹条件下施用指定治疗的口服单剂量。通过7个公历目的淘汰(wash-out)分开治疗管理(treatment administration)。每个受试者分80个场合收集总计120份血液样品。在给药前收集第一份血液样品，而其他样品则分别在给药后0.25小时、0.5小时、0.75小时、1小时、1.5小时、2小时、2.5小时、3小时、3.5小时、4小时、5小时、6小时、8小时、10小时、12小时、16小时、24小时、36小时和48小时收集。通过HPLC和MS/MS检测测量分析物。依氟鸟氨酸的测定范围为35.0ng/mL至35000.0ng/mL，舒林酸的测定范围为30.0ng/mL至15000.0ng/mL，舒林酸砜和舒林酸硫化物的测定范围为10.0ng/mL至8000.0ng/mL。通过评估不良事件(AE)、标准实验室评估、生命体征和EGG，评估安全性。

[0247] 药代动力学参数的数学模型和统计学方法：主要吸收和配置参数使用具有对数线性末期假设的非房室方法来计算。梯形法则用于评估曲线下的面积。末期评估基于最大化决定系数。该试验的药代动力学参数为C_{max}($C_{最大}$)、T_{max}($T_{最大}$)、AUC_{0-T}、$AUC_{0-\infty}$、$AUC_{0-T/\infty}$、λ_z以及T_{half}($T_{-半}$)。统计分析基于药代动力学参数的参数ANOVA模型；C_{max}、AUC_{0-T}以及$AUC_{0-\infty}$的几何平均值比率的双侧90％置信区间基于ln转换数据；T_{max}进行了秩数转换。ANOVA模型使用顺序、周期和治疗的固定因素；随机因素是嵌套在顺序中的受试者。

[0248] 药代动力学参数包括C_{max}(观察到的最大血浆浓度)、T_{max}(观察到最大血浆浓度的时间；如果它发生在多于一个时间点，则T_{max}被定义为具有该值的第一时间点)，T_{LQC}(最后观察到可定量血浆浓度的时间)、AUC_{0-T}(使用线性梯形法从0-T_{LQC}计算的血浆浓度时间曲线下的累积面积)、$AUC_{0-\infty}$(外推至无穷大的血浆浓度时间曲线下的面积，计算为$AUC_{0-T}+C_{LQC}/\lambda z$，其中$C_{LQC}$是在时间$T_{LQC}$时的估计浓度)、$AUC_{0-T/\infty}$(相对于$AUC_{0-\infty}$，$AUC_{0-T}$的相对百分比)、$T_{LIN}$(对数线性消除相开始时候的时间点)、$\lambda z$(表观消除速率常数，通过对数浓度-时间曲线的末端线性部分的线性回归估算)以及T_{half}(末端消除半衰期，计算为ln(2)/λz)。

[0249] 表15：依氟鸟氨酸的药代动力学参数

[0250]

参数	治疗-1 (n=12)		治疗-2 (n=12)		治疗-4 (n=12)	
	平均值	C.V. (%)	平均值	C.V. (%)	平均值	C.V. (%)
C_{max}(ng/mL)	10643.8	(21.6)	10234.6	(19.9)	10012.8	(25.5)
ln (C_{max})	9.2525	(2.2)	9.2134	(2.3)	9.1822	(2.8)
T_{max} (小时)*	3.25	(2.00-6.00)	3.50	(2.00-5.00)	4.50	(2.50-5.00)
AUC_{0-T} (ng·h/mL)	71459.8	(20.4)	68962.3	(20.2)	69914.9	(18.3)
ln (AUC_{0-T})	11.1562	(1.9)	11.1229	(1.8)	11.1407	(1.6)
$AUC_{0-\infty}$ (ng·h/mL)	71839.3	(20.3)	69301.2	(20.0)	70326.0	(18.1)

[0251]

ln ($AUC_{0-\infty}$)	11.1619	(1.9)	11.1281	(1.8)	11.1468	(1.6)
$AUC_{0-T/\infty}$ (%)	99.44	(0.3)	99.48	(0.2)	99.39	(0.3)
λ_Z (小时$^{-1}$)	0.1453	(25.0)	0.1642	(21.5)	0.1630	(26.3)
T_{half} (小时)	5.07	(27.3)	4.43	(24.9)	4.65	(39.0)

[0252] *中位数(范围)

[0253] 表16:舒林酸的药代动力学参数

[0254]

参数	治疗-1 (n=12)**		治疗-3 (n=12)**		治疗-4 (n=12)***	
	平均值	C.V. (%)	平均值	C.V. (%)	平均值	C.V. (%)
C_{max} (ng/mL)	4553.4	(31.6)	5236.1	(39.2)	5188.5	(42.9)
ln (C_{max})	8.3788	(3.7)	8.4946	(4.7)	8.4562	(5.7)
T_{max} (小时)*	1.54	(0.75-5.00)	1.50	(1.00-2.50)	1.50	(0.75-5.00)
AUC_{0-T} (ng·h/mL)	11268.3	(32.2)	11569.7	(31.4)	11340.8	(43.9)
ln (AUC_{0-T})	9.2823	(3.5)	9.3114	(3.4)	9.2621	(4.2)
$AUC_{0-\infty}$ (ng·h/mL)	11579.4	(39.9)	12687.8	(34.9)	12023.7	(49.3)
ln ($AUC_{0-\infty}$)	9.2896	(4.2)	9.3924	(3.9)	9.3019	(4.8)
$AUC_{0-T/\infty}$ (%)	96.73	(4.9)	98.14	(1.2)	97.58	(1.6)
λ_Z (小时$^{-1}$)	0.2810	(48.0)	0.3408	(45.9)	0.2034	(58.0)
T_{half} (小时)	4.97	(142.9)	2.88	(83.5)	4.61	(55.3)

[0255] *中位数(范围)

[0256] **对于$AUC_{0-\infty}$、λ_Z和T_{half}而言n＝7

[0257] ***对于$AUC_{0-\infty}$、λ_Z和T_{half}而言n＝8

[0258] 生物等效性标准:依氟鸟氨酸的统计推断将基于生物等效性方法,所述方法使用从治疗1与治疗2之间、治疗2与治疗4之间以及治疗1与治疗4之间的差异指数、针对ln转换参数C_{max}、AUC_{0-T}和$AUC_{0-\infty}$计算的几何LS平均值(LSmean)的比率和相应的90％置信区间,全部与80.00-125.00％的范围进行比较。舒林酸的统计推断基于生物等效性方法,所述方法使用从治疗1与治疗3、治疗3与治疗4以及治疗1与治疗4之间的差异指数、针对ln转换参数C_{max}、AUC_{0-T}和$AUC_{0-\infty}$计算的几何LS平均值的比率和相应的90％置信区间,全部与80.00至125.00％的范围进行比较。将相同的标准应用于舒林酸硫化物和舒林酸砜,将结果作为可比较治疗结果的支持性证据呈现。

[0259] 安全性结果:共有12名受试者进入该研究,并且所有受试者接受了正在研究的4种治疗。对在这项研究中登记的任何受试者而言,没有报告严重不良事件(SAE)和死亡。为安全起见,调查人员没有使任何受试者退出。在参与本研究的12名受试者中,4名受试者(33％)报告了总共4次治疗紧急不良事件(TEAE)。在这些事件中,2次发生在在治疗1给药后,1次发生在治疗3给药后,余下的1次发生在治疗4给药后。用治疗2给药的受试者没有报告任何TEAE。在研究过程中经历的一半的TEAE被认为与药物给药有关。

[0260] 本研究中经历的TEAE发生率低,每个治疗组有1个受试者(8％)经历了TEAE。在治疗4给药后报告了口干,在治疗3给药后报告了上呼吸道感染,并且在治疗1给药后各报告了血管穿刺部位瘀伤和头痛。

[0261] 对于用治疗3和治疗4给药的受试者(8％)而言,TEAE发生率相同,并且略低于用治疗1给药的受试者(17％)的发生率。对用治疗1和治疗4给药的受试者(8％)报告了发生率相同的药物相关TEAE,而用治疗-3剂量给药的个体没有经历药物相关TEAE。在研究期间经历的TEAE在强度上被认为是轻微的(3/4,75％)和适度的(1/4,25％)。在研究期间没有受试者经历严重的TEAE。

[0262] 所有异常的临床实验室值都略高于或低于它们的参考范围,没有一个被研究者认为是临床显著的。此外,在本研究受试者的生命体征和ECG中没有临床显著性异常。所有体检都被判断为正常。总体而言,所测试的药物通常是安全的,并且被本研究所包括的受试者良好耐受。

[0263] 治疗1和治疗2之间依氟鸟氨酸比较:药代动力学结果表明,依氟鸟氨酸的C_{max}、AU_{C0-T}和$AUC_{0-\infty}$的几何LS平均值比率和相应的90％置信区间均包括在80.00％-125.00％的范围内。该比较的结果表明,当在空腹条件下施用治疗1和治疗2时,符合生物等效性标准,并表明依氟鸟氨酸生物利用度在含有依氟鸟氨酸/舒林酸的共配制片剂与仅含依氟鸟氨酸的片剂之间相当。

[0264] 表17:与治疗2相比,治疗1中依氟鸟氨酸统计分析摘要

[0265]

参数	受试者内 C.V. (%)	几何 LS 平均值*		比率 (%)	90%置信限 (%)	
		治疗-1 (n=12)	治疗-2 (n=12)		下限	上限
C_{max}	16.8	10430.9	10030.8	103.99	92.42	117.01
AUC_{0-T}	13.5	69998.7	67701.4	103.39	94.03	113.69
$AUC_{0-\infty}$	13.4	70395.4	68056.2	103.44	94.17	113.61

[0266] *C_{max}的单位为ng/mL，AUC_{0-T}和$AUC_{0-\infty}$的单位为ng·h/mL。

[0267] 治疗2和治疗4之间的依氟鸟氨酸比较：药代动力学结果表明，依氟鸟氨酸C_{max}、AUC_{0-T}和$AUC_{0-\infty}$的几何LS平均值比率和相应的90％置信区间均包括在80.00％-125.00％的范围内。该比较的结果表明，当在空腹条件下施用治疗2和治疗4时满足生物等效性标准，并表明舒林酸与依氟鸟氨酸单独片剂的共同给药不影响单独给药时依氟鸟氨酸的生物利用度。

[0268] 表18：与治疗4相比，治疗2中依氟鸟氨酸的统计分析摘要

[0269]

参数	受试者内 C.V. (%)	几何 LS 平均值*		比率 (%)	90%置信限(%)	
		治疗-2 (n=12)	治疗-4 (n=12)		下限	上限
C_{max}	16.8	10030.8	9722.7	103.17	91.69	116.09
AUC_{0-T}	13.5	67701.4	68916.4	98.24	89.34	108.02
$AUC_{0-\infty}$	13.4	68056.2	69338.0	98.15	89.36	107.81

[0270] *C_{max}的单位为ng/mL，AUC_{0-T}和$AUC_{0-\infty}$的单位为ng·h/mL

[0271] 治疗1和治疗4之间的依氟鸟氨酸比较：药代动力学结果表明，依氟鸟氨酸C_{max}、AUC_{0-T}和$AUC_{0-\infty}$的几何LS平均值比率和相应的90％置信区间均包括在80.00％-125.00％的范围内。该比较的结果表明，当空腹条件下施用治疗1和治疗4时满足生物等效性标准，并表明对于含有依氟鸟氨酸/舒林酸的共配制片剂和各自含有依氟鸟氨酸或舒林酸的单独片剂的共同给药，依氟鸟氨酸的生物利用度是相似的。

[0272] 表19：与治疗4相比，治疗1中依氟鸟氨酸的统计分析摘要

[0273]

参数	受试者内 C.V. (%)	几何 LS 平均值*		比率(%)	90%置信限(%)	
		治疗-1 (n=12)	治疗-4 (n=12)		下限	上限
C_{max}	16.8	10030.8	9722.7	107.28	95.35	120.72
AUC_{0-T}	13.5	67701.4	68916.4	101.57	92.37	111.68

[0274]

$AUC_{0-\infty}$	13.4	68056.2	69338.0	101.53	92.43	111.51

[0275] *C_{max}的单位为ng/mL，AUC_{0-T}和$AUC_{0-\infty}$的单位为ng·h/mL

[0276] 治疗1与治疗3之间的舒林酸比较：药代动力学结果表明，舒林酸的C_{max}、AUC_{0-T}和

$AUC_{0-\infty}$的几何LS平均值比率和相应的90％置信区间(90CI)并未全部包括在80.00％-125.00％的范围内。C_{max}的90CI的下限低于80.00％限度。由于所有PK参数的比率均在80.00％-125.00％范围内，因此受试者内变异性可以解释C_{max}的下限在BE范围之外。该比较获得结果表明，该初步研究所用的样品量不足以证明共同配制片剂和单独舒林酸的舒林酸生物利用度等效。

[0277] 表20：与治疗3相比，治疗1中舒林酸的统计分析摘要

[0278]

参数	受试者内 C.V. (%)	几何 LS 平均值*		比率 (%)	90%置信限 (%)	
		治疗-1 (n=12)**	治疗-3 (n=12)**		下限	上限
C_{max}	24.6	4353.6	4888.5	89.06	75.04	105.69
AUC_{0-T}	11.9	10746.4	11063.6	97.13	89.34	105.60
$AUC_{0-\infty}$	13.6	12029.4	12743.6	94.40	82.27	108.30

[0279] *C_{max}的单位为ng/mL，AUC_{0-T}和$AUC_{0-\infty}$的单位为ng·h/mL

[0280] **对于$AUC_{0-\infty}$而言，n＝7

[0281] 基于所述数据，包含所有比较之间的变化性的受试者内变化对于C_{max}而言约为24.6％，对于AUC_{0-T}而言约为12％。统计学上，考虑到几何LS平均值的期望的治疗1与治疗3的比率在90％-110％之间，估计欲满足统计事前功效(priori power)为至少80％的80.00-125.00％生物等效性范围，受试者数目对未来的关键研究而言应为约54。包含60个受试者应该足以说明退出(drop-out)的可能性和所评估的受试者内CV的变化。

[0282] 治疗3与治疗4之间的舒林酸比较：药代动力学结果证明，舒林酸C_{max}、AUC_{0-T}和$AUC_{0-\infty}$的几何LS平均值比率和相应的90％置信区间都包括在80.00％-125.00％的范围内。该比较的结果表明，当在空腹条件下施用治疗3和治疗4时满足生物等效性标准，并且表明含有依氟鸟氨酸或舒林酸的单独片剂的共施用不影响单独施用时舒林酸的生物利用度。

[0283] 表21：与治疗4相比，治疗3中舒林酸的统计分析摘要

[0284]

参数	受试者内 C.V. (%)	几何 LS 平均值*		比率 (%)	90%置信限 (%)	
		治疗-3 (n=12)**	治疗-4 (n=12)**		下限	上限
C_{max}	24.6	4888.5	4704.2	103.92	87.56	123.32
AUC_{0-T}	11.9	11063.6	10530.9	105.06	96.63	114.22
$AUC_{0-\infty}$	13.6	12743.6	11834.3	107.68	93.31	124.27

[0285] *C_{max}的单位为ng/mL，AUC_{0-T}和$AUC_{0-\infty}$的单位为ng·h/mL

[0286] **对于$AUC_{0-\infty}$而言，n＝7

[0287] 治疗1与治疗4之间的舒林酸比较：药代动力学结果表明，舒林酸C_{max}、AUC_{0-T}和$AUC_{0-\infty}$的几何LS平均比率和相应的90％置信区间(90CI)并未全部包括在80.00％-125.00％的范围内。C_{max}的90CI的下限低于80.00％限度。由于所有PK参数的比率均在80.00％-125.00％范围内，因此受试者内变化性可以解释C_{max}的下限在BE范围之外。该比较获得的结果表明，该初步研究所用的样品量不足以证明共配制片剂和含有依氟鸟氨酸或舒林酸的单

独片剂的共施用的舒林酸生物利用度的生物等效性。

[0288] 表22:与治疗4相比,治疗1中舒林酸的统计分析摘要

[0289]

参数	个体内 C.V. (%)	几何 LS 平均值*		比率 (%)	90%置信限 (%)	
		治疗-1 (n=12)**	治疗-4 (n=12)**		下限	上限
C_{max}	24.6	4353.6	4704.2	92.55	77.98	109.83
AUC_{0-T}	11.9	10746.4	10530.9	102.05	93.86	110.94
$AUC_{0-\infty}$	13.6	12029.4	11834.3	101.65	88.09	117.30

[0290] *C_{max}的单位为ng/mL,AUC_{0-T}和$AUC_{0-\infty}$。的单位为ng·h/mL

[0291] **对于$AUC_{0-\infty}$而言,n=8

[0292] 基于数据,包含所有比较之间的变化性的受试者内变化对于C_{max}而言为约24.6%,对于AUC_{0-T}而言为约12%。统计学上,考虑到几何LS平均值的期望的治疗1与治疗4的比率在92.5和107.5%之间,估计欲满足统计事前功效(priori power)为至少80%的80.00-125.00%生物等效性范围,受试者的数目对于未来的关键研究而言应为约36。40个受试者的入选应该足以说明退出的可能性和所评估的受试者内CV的变化。

[0293] 根据本公开,在不需过度实验的情况下可以制备和实施本文公开和要求保护的所有组合物和方法。虽然已经根据优选实施方案描述了本发明的组合物和方法,但是对于本领域技术人员显而易见的是,可以在不背离本发明的构思、精神和范围的情况下,对本文所描述的方法、所述方法的步骤或者所述方法的步骤顺序进行改变。更具体而言,显而易见的是,化学上和生理学上相关的某些试剂可以代替本文所述的试剂,而获得相同或相似的结果。对于本领域技术人员显而易见的所有这些类似的替代和修改被认为是落入所附权利要求限定的本发明的精神、范围和构思内。

[0294] 参考文献

[0295] 专门通过引用将下述参考文献并入本文,并入程度为他们提供与本文所阐述的那些补充性的示例性程序性上的详情或其他详情。

[0296] U.S.Patent 3,647,858

[0297] U.S.Patent 3,654,349

[0298] U.S.Patent 4,330,559

[0299] U.S.Patent 4,413,141

[0300] U.S.Patent 5,814,625

[0301] U.S.Patent 5,843,929

[0302] U.S.Patent 6,258,845

[0303] U.S.Patent 6,428,809

[0304] U.S.Patent 6,702,683

[0305] U.S.Patent 8,329,636

[0306] U.S.Patent 9,121,852

[0307] U.S.Patent Publication US2013/0217743

[0308] U.S.Patent Publication US2015/0301060

[0309] PCT Patent Publication WO2014/070767

[0310] PCT Patent Publication WO2015/195120

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产品	条件	封装	T6 M	T0
依氟鸟氨酸/舒林酸 375mg/75mg	25°C/75% HR	密闭瓶	6,0	6,1
	30°C/65% HR	密闭瓶	6,0	
	40°C/75% HR	打开的瓶子	6,4	
	40°C/75% HR	密闭瓶	6,2	
依氟鸟氨酸 250mg	25°C/75% HR	密闭瓶	6,9	6,5
	30°C/65% HR	密闭瓶	7,1	
	40°C/75% HR	打开的瓶子	7,5	
	40°C/75% HR	密闭瓶	7,3	
舒林酸 150mg	25°C/75% HR	密闭瓶	2,9	2,9
	30°C/65% HR	密闭瓶	3,2	
	40°C/75% HR	打开的瓶子	3,5	
	40°C/75% HR	密闭瓶	3,2	

图1

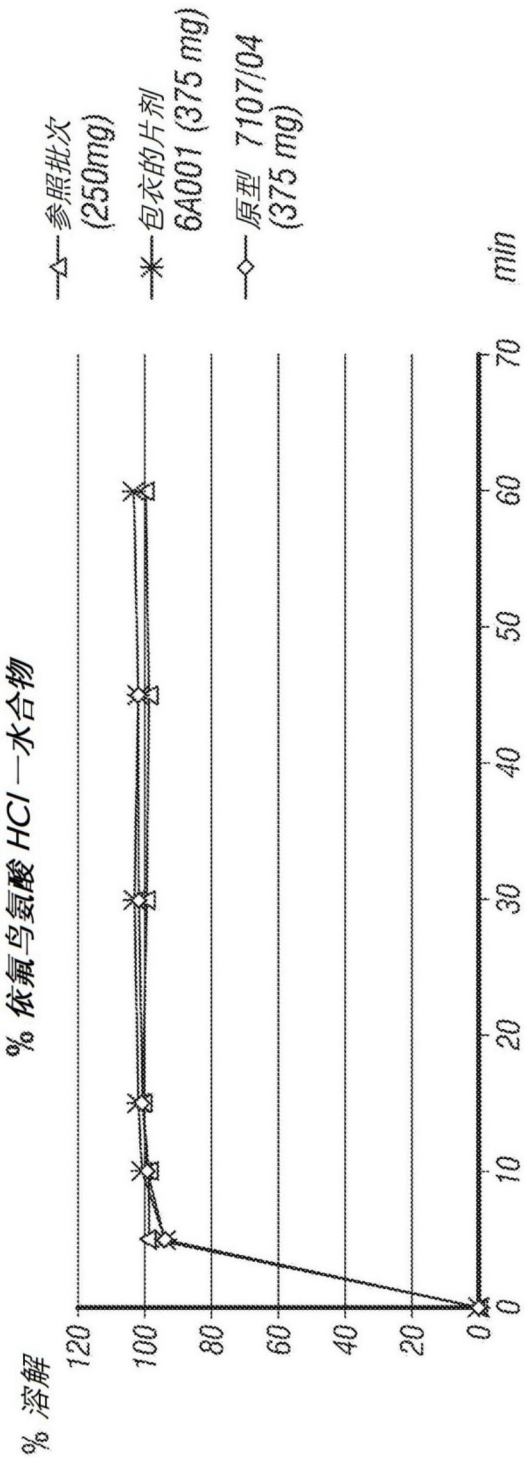
参照批次 (250mg)
包衣的片剂 6A001 (375 mg)
原型 7107/04 (375 mg)
% 依氟乌氨酸 HCl 一水合物
% 溶解
min
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70

图2A

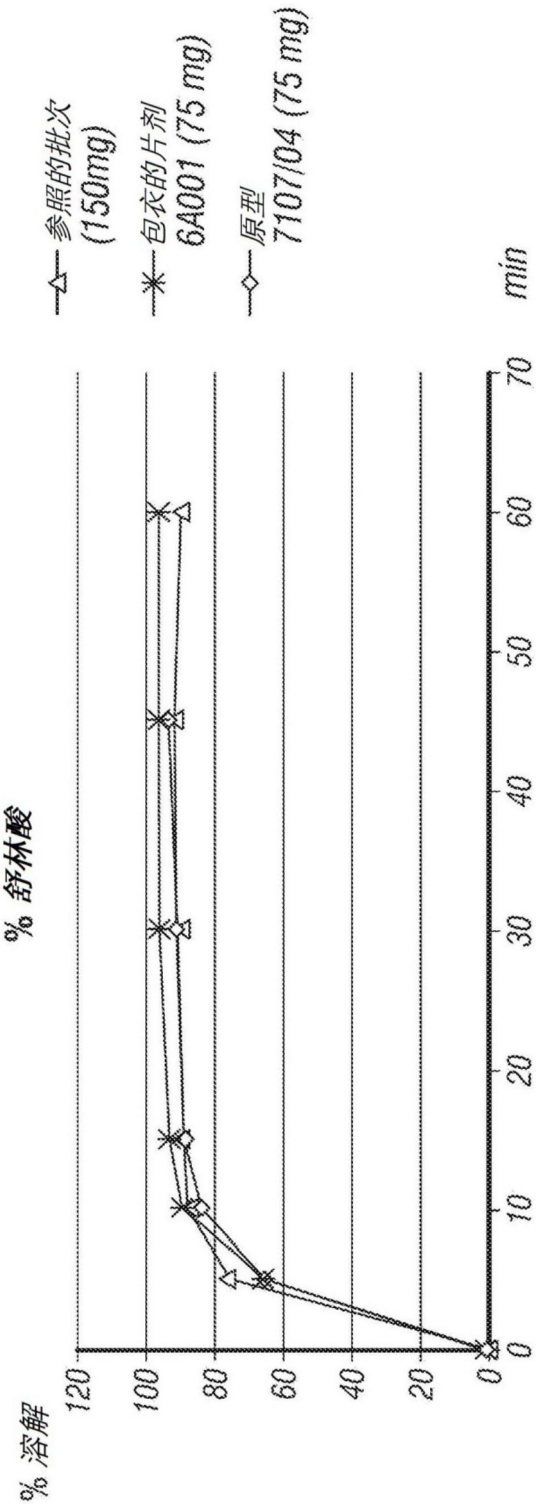
参照的批次
(150mg)
包衣的片剂
6A001 (75 mg)
原型
7107/04 (75 mg)
min
% 舒林酸
% 溶解
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70

图2B

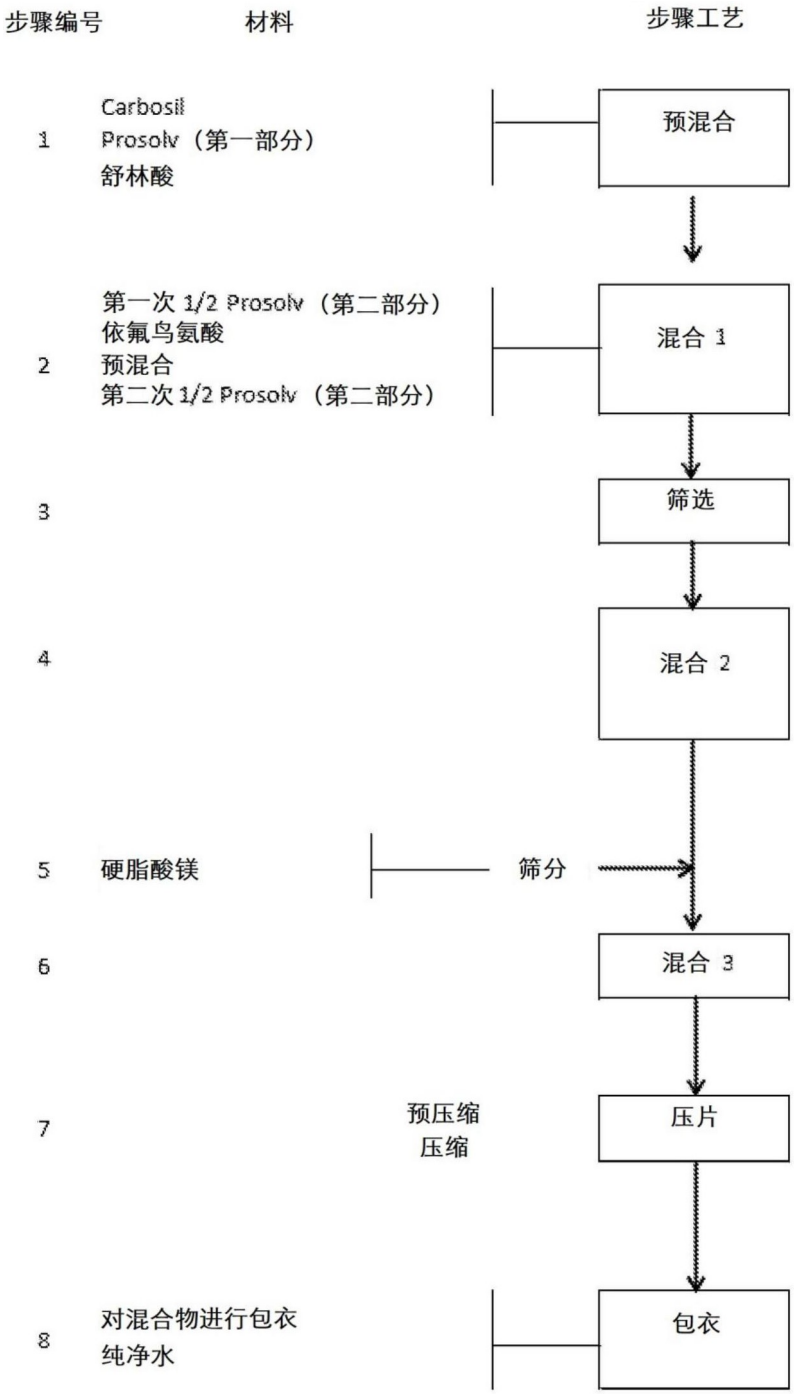
步骤编号
材料
步骤工艺
1
Carbosil
Prosolv（第一部分）
舒林酸
预混合
2
第一次 1/2 Prosolv（第二部分）
依氟鸟氨酸
预混合
第二次 1/2 Prosolv（第二部分）
混合 1
3
筛选
4
混合 2
5
硬脂酸镁
筛分
6
混合 3
7
预压缩
压缩
压片
8
对混合物进行包衣
纯净水
包衣

图3

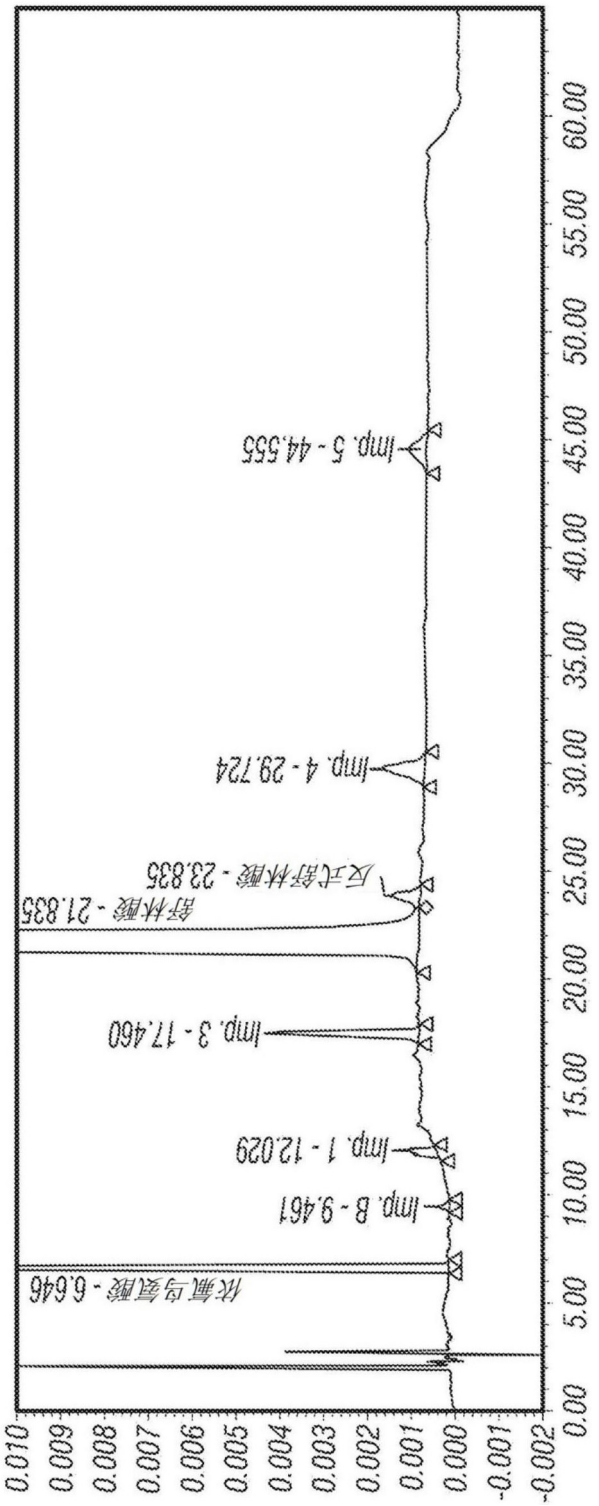
0.010
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
-0.001
-0.002
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00
依氟乌氨酸 - 6.646
Imp. 8 - 9.461
Imp. 1 - 12.029
Imp. 3 - 17.460
舒林酸 - 21.835
反式舒林酸 - 23.835
Imp. 4 - 29.724
Imp. 5 - 44.555

图4A

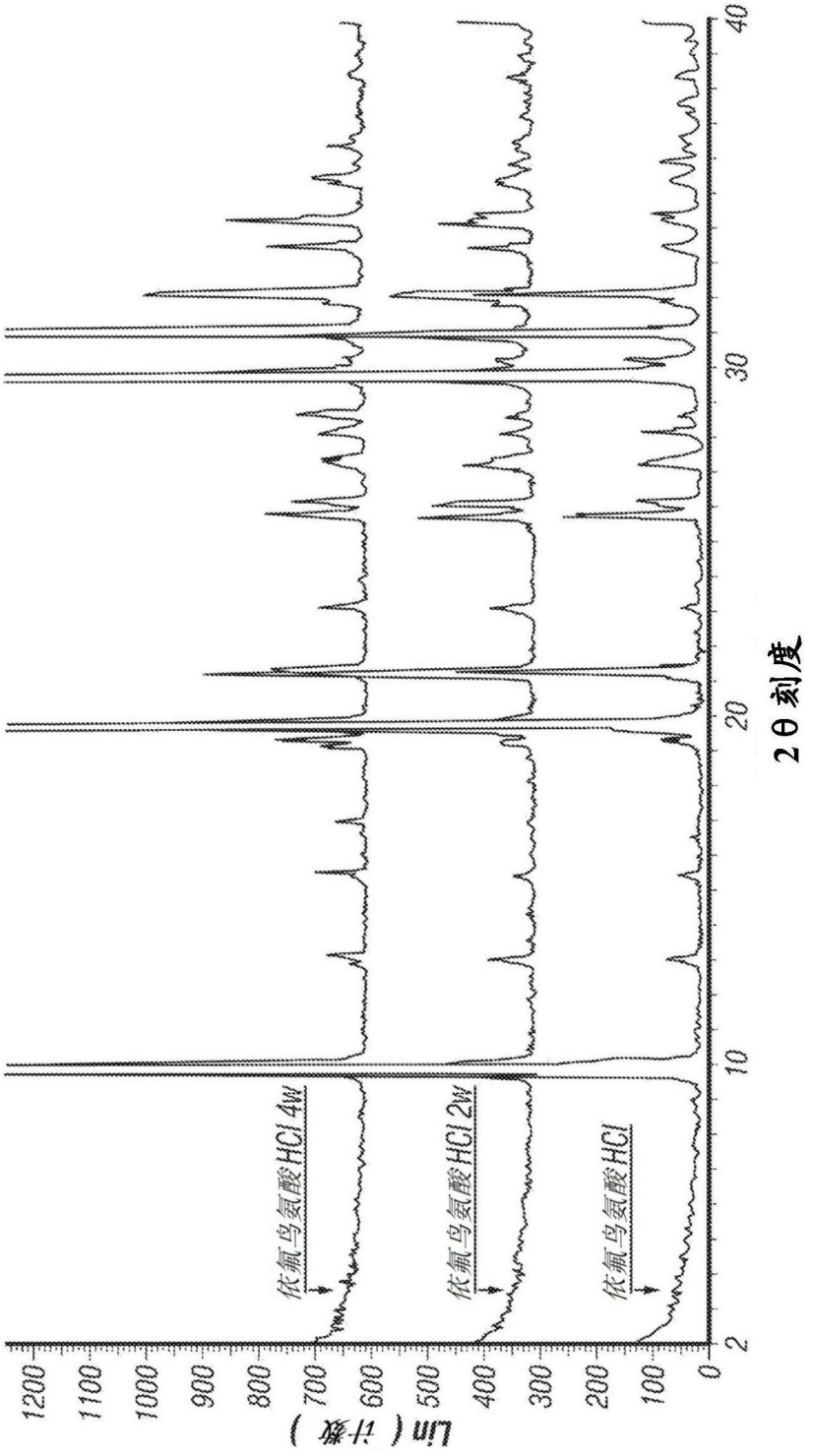
依氟虫氨酸HCl 4w
依氟虫氨酸HCl 2w
依氟虫氨酸HCl
Lin (计数)
2θ刻度
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
2
10
20
30
40

图4B

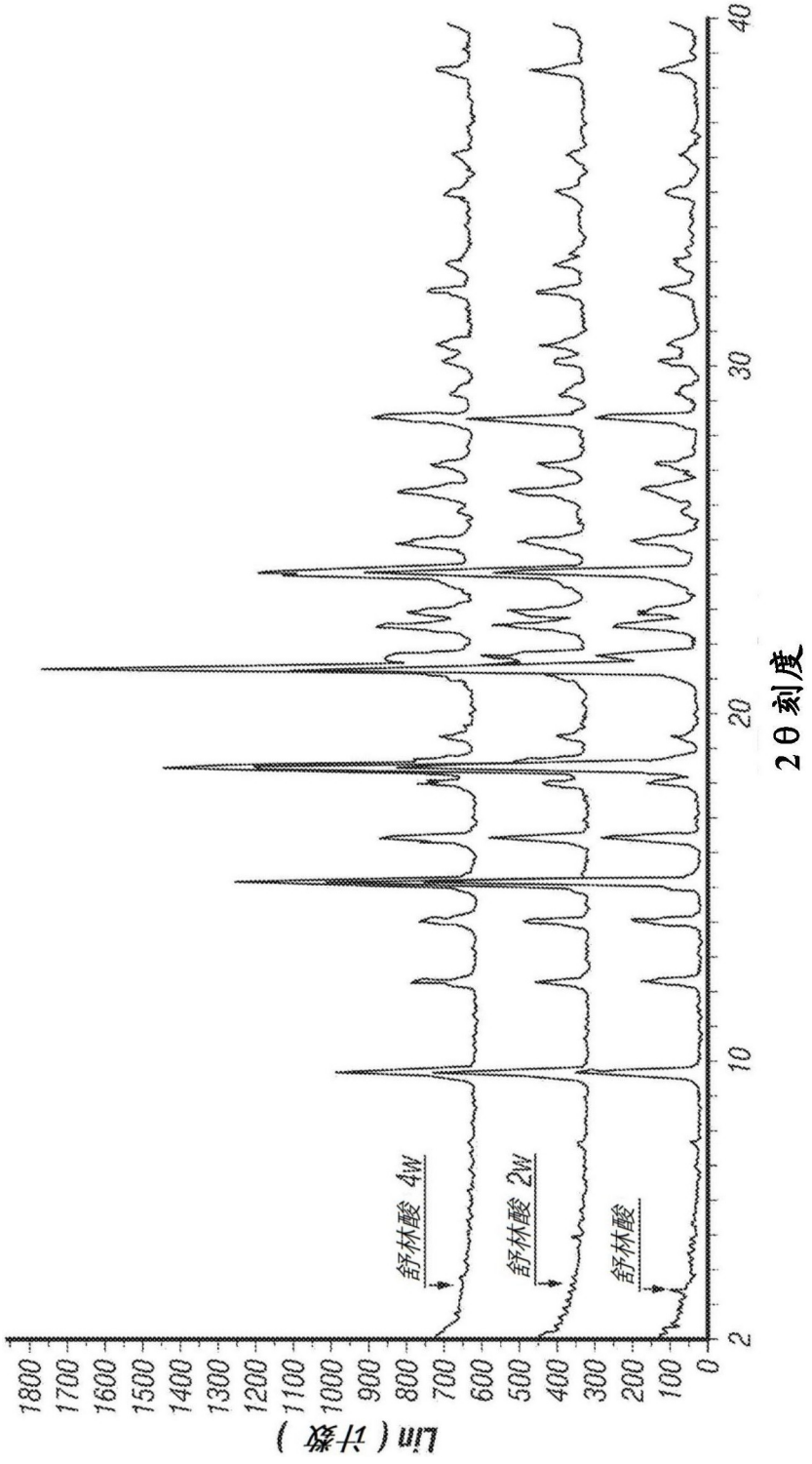

图4C