NOVEL FORMS OF CDDO METHYL ESTER

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application claims priority from US Provisional Application 60/955,939, filed August 15, 2007, incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Triterpenoids are biosynthesized in plants by the cyclization of squalene. Although candidates for medicinal use, these naturally occurring molecules display relatively weak biological activity. Accordingly, chemists have sought to synthesize analogues of enhanced potency (Honda *et al.*, 1997 & 1998).

[0003] Several synthetic analogs are reported to suppress the *de novo* formation of iNOS and COX-2 in macrophages that have been stimulated by IFN-γ or LPS (Suh *et al.*, 1998; Honda *et al.*, 2002). Another synthetic triterpenoid, 2-cyano-3,12-dioxoleana-1,9(11)-dien-28-oate (CDDO), exhibits anti-inflammatory and anti-proliferative activity (Honda *et al.*, 1998 & 2000).

[0004] Studying the methyl ester of CDDO, which is methyl 2-cyano-3,12-dioxoleana-1,9(11)-dien-28-oate (CDDO methyl ester), Bore *et al.* (2002) determined a crystal structure. In that form, which is hydrated, water coordinates interactions that engender a particular crystal packing and structure.

SUMMARY

[004a] In one aspect of the present disclosure, there is provided anhydrous, crystalline methyl 2-cyano-3,12-dioxoleana-1,9(11)-dien-28-oate (CDDO-methyl ester) characterized in that its X-ray powder diffractogram comprises peaks at 8.78° 2θ, 12.94° 2θ, 13.35° 2θ, 14.18° 2θ, and 17.4° 2θ±0.2° 2θ based upon data collected on a diffractometer with Cu K_α radiation.

[004b] In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising a therapeutically effective amount of the CDDO-methyl ester disclosed herein and one or more solid pharmaceutically acceptable carriers.

[004c] In a further aspect of the present disclosure, there is provided the CDDO-methyl ester disclosed herein for use in treating a chronic inflammatory condition in a subject suffering therefrom.

[004d] In another aspect of the present disclosure, there is provided a use of CDDO-methyl ester disclosed herein in the manufacture of a medicament for treating a chronic inflammatory condition in a subject suffering therefrom.

[004e] In a further aspect of the present disclosure, there is provided crystalline CDDO-methyl ester hemibenzenate, characterized in that its X-ray powder diffractogram comprises peaks at 9.25° 2θ, 14.17° 2θ, , 14.62° 2θ, 16.32°2θ, and 17.11° 2θ±0.2° 2θ based upon data collected on a diffractometer with Cu K_α radiation.

[0005] In one embodiment of the present disclosure, a non-hydrous crystalline form of CDDO methyl ester is provided. The non-hydrous crystal form preferably has a space group of $P4_3\ 2_12$ with unit cell dimensions of a=14.2Å, b=14.2Å, and c=81.6Å. The present disclosure also contemplates a pharmaceutical composition in solid dosage form, comprising (i) a therapeutically effective amount of a non-hydrous crystalline form of CDDO methyl ester with (ii) an edible carrier.

[0006] Additionally, the present disclosure is embodied in a glassy solid form of CDDO methyl ester, having an x-ray powder diffraction pattern with a halo peak at approximately 13.5 °2θ, as shown in Figure 2C, and a glass transition temperature (T_g). In particular

embodiments, the T_g can range from about 120°C to about 135°C. In other embodiments, the T_g ranges from about 125°C to about 130°C. The glassy solid form of CDDO-methyl ester can have a PDF spectrum with similar peaks to Figure 28 from about 5 Å to about 20 Å.

[0007] Furthermore, the present disclosure provides a pharmaceutical composition in solid dosage form, comprising (i) a therapeutically effective amount of a glassy solid form of CDDO methyl ester with (ii) an edible carrier. In this regard, the present disclosure contemplates a methodology for treating a cancer patient, comprising: administering such a pharmaceutical composition to a cancer patient. The present disclosure also contemplates administering the glassy form of CDDO-methyl ester in combination with another anti-cancer drug. For example, the anti-cancer drug may be gemcitabine and the cancer may be pancreatic cancer. The present disclosure encompasses as well a methodology for the treatment of diseases or disorders that involve acute or chronic oxidative stress and inflammation, particularly those characterized in part by overexpression of inducible nitric oxide synthase (iNOS) or inducible cyclooxygenase (COX-2).

[0008] In addition, the present disclosure is drawn to a dimethanol solvate form of CDDO-methyl ester, having an x-ray powder diffraction pattern with characteristic peaks as shown in Table 18 and a DSC pattern as shown in Figure 24. Pursuant to the present disclosure, the dimethanol solvate form may be used as an intermediate for the production of a glassy solid form of CDDO methyl ester. A method for the production of the glassy solid form of CDDO-methyl ester, via the dimethanol solvate form, comprises preparing a dimethanol solvate form of CDDO-methyl ester and drying the dimethanol solvate form.

[0009] In accordance with another embodiment, the present disclosure is drawn to a method of growing a crystal of CDDO methyl ester dimethanolate, comprising preparing a solution of purified CDDO methyl ester in warm anhydrous methanol, adding the warm solution to a vessel of chilled methanol, and filtering the resulting crystals.

[0010] In accordance with another embodiment, the present disclosure is drawn to a pharmaceutical composition comprising (i) a therapeutically effective amount of CDDO-methyl ester and (ii) an excipient that is a glass former, such that the composition has a T_g.

[0011] The excipient may be selected, for instance, from the group consisting of (A) a carbohydrate, carbohydrate derivative, or carbohydrate polymer, (B) a synthetic organic polymer, (C) an organic acid salt, (D) a protein, polypeptide, or peptide, and (E) a high

molecular weight polysaccharide. Illustrative of the class of synthetic organic polymer excipients are a hydroxpropyl methyl cellulose, such as hydroxpropyl methyl cellulose phthalate ester, a poly[1-(2-oxo-1-pyrrolidinyl)ethylene or copolymer thereof, such as PVP/VA, and a methacrylic acid copolymer, such as methacrylic acid – ethyl acrylate copolymer (1:1).

[0012] Another exemplary excipient in this regard is copovidone, which is 1-vinyl-2-pyrrolidone - vinyl acetate copolymer (3:2).

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1 depicts the chemical structure of CDDO methyl ester.

[0014] Figure 2 presents the XRPD pattern of Form A (top) and Form B (bottom). From top to bottom: unmicronized Form A; micronized Form A; and Form B.

[0015] Figure 3 shows the DSC and TG curve of CDDO methyl ester (Form A).

[0016] Figure 4 shows the hot stage analysis of Form A – unmicronized.

[0017] Figure 5 shows the dynamic vapor sorption isotherm of Form A – unmicronized.

[0018] Figure 6 shows the SEM images of Form A – unmicronized.

[0019] Figure 7 shows Form A before (top) and after (bottom) stress at 195°C.

[0020] Figure 8 shows the NMR spectrum of Form A - unmicronized.

[0021] Figure 9 shows the MDSC curve of Form B CDDO methyl ester.

[0022] Figure 10 shows Form B CDDO methyl ester before (top) and after (bottom) thermal stress at 200° C/ambient RH for 60 minutes.

[0023] Figure 11 shows the NMR spectrum of Form B CDDO methyl ester.

[0024] Figure 12 shows an ORTEP drawing of a single Form A molecule with labeling. Atoms are represented by 50% probability anisotropic thermal ellipsoids.

[0025] Figure 13 shows an ORTEP drawing of the contents of the asymmetric unit of Form A crystals. Atoms are represented by 50% probability anisotropic thermal ellipsoids.

[0026] Figure 14 shows a packing diagram of Form A crystals viewed down the crystallographic *a* axis.

[0027] Figure 15 shows a packing diagram of Form A crystals viewed down the crystallographic *b* axis.

[0028] Figure 16 shows a packing diagram of Form A crystals viewed down the crystallographic *c* axis.

[0029] Figure 17 shows the calculated X-ray powder pattern of Form A

[0030] Figure 18 shows the experimental XRPD of Form A.

[0031] Figure 19 presents a comparison of the calculated and experimental XRPD patterns for Form A CDDO methyl ester.

[0032] Figure 20 shows a representative plot of the area under the curve for Form A and Form B, following a 4.1 mg/kg oral administration to cynomolgus monkeys. Each datum point represents the mean plasma concentration of CDDO methyl ester in 8 animals. Error bars represent the standard deviation within the sampled population.

[0033] Figure 21 shows a comparison of plasma concentration of Form B CDDO methyl ester versus Form A in Animal #505M (top panel) and animal #507F (bottom panel).

[0034] Figure 22 presents a comparison of plasma concentration of Form B CDDO methyl ester versus Form A between Animal #508F (top panel) and animal #502M (bottom panel).

[0035] Figure 23 depicts thermograms of CDDO methyl ester hemibenzene solvate.

[0036] Figure 24 shows thermograms of CDDO-methyl ester dimethanol solvate.

[0037] Figure 25 depicts TGIR data relating to CDDO methyl ester dimethanol solvate.

[0038] Figure 26 presents XRPD patterns of CDDO methyl ester dimethanol solvate, before (top) and after (bottom) TGIR analysis (up to 140°C).

[0039] Figure 27 is an overlay representation of PDF data for Form A vs. Form B Local order is similar from about 5 to about 20 .

[0040] Figure 28 is an overlay representation of X-ray amorphous patterns for different preparations of Form B, showing substantial uniformity among the preparations.

[0041] Figure 29 is a schematic representation of space group $P4_32_12$ (#96).

[0042] Figure 30 shows the mean blood concentrations of CDDO-methyl ester following single oral administrations of CDDO-methyl ester capsules to male Cynomolgus monkeys (Phases 2 and 3).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0043] As noted, the study of triterpenoids as suppressors of iNOS activity, and specifically in the inhibition of NO production, has demonstrated the high potency of CDDO and CDDO methyl ester (IC_{50} <1nM level). See Honda *et al.* (2000). These studies focused on solubilized CDDO methyl ester, providing little characterization of a solid of CDDO methyl ester. The work of Bore *et al.* (2002) elucidated a structure, the first published for a triterpenoid, of a single, solvated crystalline form of CDDO methyl ester.

[0044] To realize the therapeutic potential of CDDO methyl ester, depicted in Figure 1 (chemical structure) and in Figure 12 (ORTEP drawing), the present inventors investigated other forms of the compound that possessed properties, such as greater aqueous solubility and chemical stability, that are advantageous to development of a medicinal product with suitable pharmacokinetics. Consequently, the inventors discovered two forms of CDDO methyl ester, distinct from the crystal form elucidated by Bore *et al.* (2002), that have such properties and, hence, are candidates for drug development in their own right.

[0045] Inventive "Form A" of CDDO methyl ester is unsolvated (non-hydrous) and is characterized by a distinctive crystal structure, with a space group of $P4_3\,2_12$ (no. 96), shown in Figure 29, unit cell dimensions of a = 14.2 Å, b = 14.2 Å and c = 81.6 Å, and by a packing structure, depicted in Figures 14–16, whereby three molecules are packed in helical fashion down the crystallographic *b* axis. Table 10 below enumerates additional crystal data for Form A, along with crystallographic data-collection parameters.

[0046] The other "Form B" of the invention is in a single phase but lacks such a defined crystal structure. Rather, Form B is typified by an x-ray powder diffraction (XRPD) spectrum differing from that of Form A (see Figure 2, *inter alia*). Moreover, Form B displays a bioavailability that is surprisingly better than that of Form A (see Example 7).

[0047] Methodology for the synthesis of CDDO methyl ester has been published. See U.S. patent No. 6,326,507, Honda *et al.* (1998), and Honda *et al.* (2000). The inventors have discovered that both Form A and Form B of CDDO methyl ester are readily prepared from a variety of solutions of the compound, illustrated by those detailed in Table 3-5, *infra*. In particular, Form B can be prepared by fast evaporation or slow evaporation in MTBE, THF, toluene, or ethyl acetate. By the same token, Form A can be prepared via fast evaporation, slow evaporation, or slow cooling of a CDDO methyl ester solution in ethanol or methanol.

Preparations of CDDO methyl ester in acetone can produce either Form A, using fast evaporation, or Form B, using slow evaporation. Additional preparation methods are described below, including the tables provided there.

[0048] Since it does not have a defined crystal structure, Form B likewise lacks distinct XRPD peaks, such as those that typify Form A, and instead is characterized by a general "halo" XRPD pattern. In particular, the non-crystalline Form B falls into the category of "x-ray amorphous" solids because its XRPD pattern exhibits three or fewer primary diffraction halos (see Figure 10, for instance). Within this category, Form B is a "glassy" material: As shown by the PDF, the nearest neighbor atom-atom interactions match that observed for crystalline Form A, but the notion of an average unit cell does not apply because there is no long-range order manifested.

[0049] Unlike Form A, therefore, samples of Form B show no long-range molecular correlation, *i.e.*, above roughly 20 Å (see Figure 27). Moreover, thermal analysis of Form B samples reveals a glass transition temperature (T_g). In contrast, a disordered nanocrystalline material, does not display a T_g but instead only a melting temperature (T_m), above which crystalline structure becomes a liquid.

[0050] The present description also characterizes a CDDO-methyl ester dimethanol solvate form that can be used to prepare form B (see Example 9). Also characterized here is a CDDO-methyl ester hemibenzenate form (see Example 8).

[0051] Although micronization of other crystalline materials has been found to affect XRPD spectra, XRPD analysis of micronized Form A results in a spectrum similar to unmicronized Form A. See Figure 2 for a side-by-side comparison of unmicronized Form A, micronized Form A, and Form B CDDO methyl ester.

[0052] Various means of characterization can be used together to distinguish Form A and Form B CDDO methyl ester from each other and from other forms of CDDO methyl ester. Illustrative of the techniques suitable for this purpose are solid state Nuclear Magnetic Resonance (NMR), X-ray powder diffraction, X-ray crystallography, Differential Scanning Calorimetry (DSC), dynamic vapor sorption/desorption (DVS), Karl Fischer analysis (KF), hot stage microscopy, modulated differential screening calorimetry, FT-IR, and Raman spectroscopy.

[0053] In particular, analysis of the XRPD and DSC data can distinguish Form A, Form B, and hemibenzenate forms of CDDO-methyl ester.

[0054] The properties of the inventive CDDO methyl ester forms are both distinctive, as mentioned above, and conducive to their use as medicinal agents. For example, the bioavailability of Form B and Form A CDDO methyl ester varied in monkeys when the monkeys received equivalent dosages of the two forms orally, in gelatin capsules. See Example 7. In addition, the stability of the newly identified CDDO-methyl ester forms will be useful in the production of pharmaceutical compositions.

In similar manner to distinguishing Form A and Form B CDDO methyl ester from each other and from other forms of CDDO methyl ester, CDDO methyl ester dispersions that retain "x-ray amorphous" character, as described in greater detail below, can be distinguished from dispersions containing crystalline Form A CDDO methyl ester by a variety of techniques, including XRPD and DSC analysis. Thus, dispersions containing Form A crystalline CDDO methyl ester typically display discrete peaks characteristic of the pure Form A CDDO methyl ester, particularly those that occur at approximately 13.35 and 8.78 (°2θ) (for example, see Table 17, *infra*).

[0055] The properties of a CDDO methyl ester polymer excipient dispersion of the invention are both distinctive and conducive to their use as medical agents. For example, the bioavailability of selected CDDO methyl ester dispersions, formulated with additional inactive additives, varied in monkeys when the monkeys received equivalent dosages of the dispersions in gelatin capsules. See Example 7, *infra*, study phases 2 and 3. In several instances, formulations containing CDDO methyl polymer excipient dispersions produced surprising further enhancements in bioavailability, even relative to formulations produced from pure Form B CDDO methyl ester.

[0056] The presence of multiple forms, including polymorphs, in pharmaceutical solids has been previously described, for instance, by Cui (2007). The crystalline and amorphous forms of a compound may exhibit different physical and chemical characteristics. For instance, amorphous forms may have higher solubility relative to the crystalline form. Every compound is unique in this regard, however, and the degree to which an amorphous material will differ from the crystalline state must be investigated on a case-by-case basis and cannot be predicted *a priori*. In addition, some amorphous materials are prone to re-crystallization.

[0057] In the present context, variability in data collection can arise due to a myriad of factors. Accordingly, this description uses the terms "about" or "approximately" to indicate variations in data used to describe the CDDO-methyl ester forms. For example, a melting temperature may vary based on instrumentation or conditions. Regarding the precision of the measurement, the USP <891> states that "In the case of melting, both an "onset" and a "peak" temperature can be determined objectively and reproducibly, often to within a few tenths of a degree." Practical experience indicates this is not true for measuring the T_g of a material. The T_g will depend on many factors: how the sample was prepared, the thermal history of the sample (relaxation), residual solvent that may or may not volatilize prior to T_g, the instrument, sample preparation (sample mass, particle size, packing, diluents), the parameters used to measure T_g (particularly scan rate), the parameters used to determine the location of the T_g (onset temperature, mid-point temperature, inflection point temperature, or offset temperature), whether a relaxation endotherm is present at T_g, and other factors. Some factors will decrease T_g (plasticization due to residual water/solvent), while others will increase T_g (faster scan rate, relaxation) and may do so by as much as 10-15°C. The change in heat capacity at T_g (ΔCp) can be important, as reported by Zhou *et al.*, *J. Pharmaceutical Sciences* 91: 1863–72 (2002).

[0058] The present description speaks of different patterns in terms of their "characteristic" peaks. The assemblage or group of such peaks is unique to a given polymorphic form, within the uncertainty attributable to individual instruments and to experimental conditions, respectively.

[0059] For each of the crystalline forms, a group of five characteristic peaks is listed in Tables 17-19, below. Typical variation can be ± 0.1°2✓, but peak position can vary up to ±0.2°2✓ or more in some experiments.

Table 17 - Form A

Peak Position (°2✓)
13.35
8.78
17.4
12.94
14.18

Table 18 - Dimethanolate

Peak Position (°2✓)
8.87
11.26
16.63
16.9
13.72

Table 19 - Hemibenzenate

Peak Position (°2✓)
14.17
9.25
16.32
14.62
17.11

[0060] The XRPD pattern of the glassy material (Form B) shows a broad halo peak at approximately 13.5 °2✓, which appears to be characteristic of Form B. Other halos are not as well-defined, and the shape/position of this pattern may change as a function of the instrument and experimental conditions. Variation in the position of this broad peak will be larger than that of the characteristic peaks of the respectively crystalline forms. In particular, variability of up to ±1°2✓ for the broad peak of Form B can be expected in certain instruments.

[0061] The XRPD pattern of glassy materials produced as CDDO methyl ester excipient dispersions also show a broad halo peak, typically centered at approximately 13.5 °2θ. These materials likewise display a T_g by modulated Differential Scanning Calorimetry (mDSC). Similar to pure Form B CDDO methyl ester samples, the shape and position of the XRPD pattern for an excipient dispersion may change as a function of the instrument used, the experimental conditions, and the specific excipient employed to produce the dispersions.

[0062] The present invention further relates to the use of Form A, Form B, and glassy, XRPD-amorphous excipient dispersions of CDDO methyl ester, respectively, for treating diseases associated with inflammation, including a cancerous condition and various pathologies affecting the central nervous system. Pursuant to the invention, treatment of these diseases comprises administering to a subject in need thereof an effective amount of the

novel CDDO methyl ester forms enumerated here. These compounds have utility for ameliorating or preventing inflammation involved in the etiology of cancer, Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), rheumatoid arthritis (RA) and other autoimmune diseases, inflammatory bowel disease, and other pathological conditions tied to excessive production of either nitric oxide or prostaglandins.

[0063] As previously noted, the aberrant or excessive expression of either cyclooxygenase-2 (COX-2) or inducible nitric oxide synthase (iNOS) has been implicated in the pathogenesis of many disease processes, including carcinogenesis in the colon. Several synthetic analogs of triterpenoids, including CDDO methyl ester, have been reported to suppress iNOS expression. Related studies have shown triterpenoid suppression of both iNOS and COX-2 expression in macrophages that have been stimulated by IFN-γ or LPS (Suh *et al.*, 1998; Honda *et al.*, 2002). Therefore, treatments administering CDDO methyl ester forms are expected to affect iNOS and COX-2 suppression.

[0064] Overexpression of the gene for COX-2 is an early and central event in colon carcinogenesis (Prescott and White, 1996; Dubois *et al.*, 1996). Mice with defects in the APC (adenomatous polyposis coli) gene develop large numbers of intestinal polyps at an early age, and marked elevations in COX-2 enzyme levels have been found in these polyps. These animal findings correlate with the finding of elevated levels of COX-2 mRNA and protein in many human primary colon cancers and colon cancer cell lines (Prescott and White, 1996), and it is believed that this elevation in COX-2 leads to a suppression of apoptosis, which would ordinarily lead to death of pre-neoplastic cells (Tsujii and DuBois, 1996). The functional relevance of COX-2 to intestinal tumorigenesis has been demonstrated by knockout of the COX-2 gene (Oshima *et al.*, 1996). Mice bearing this knockout were mated with polyp-forming mice bearing lesions in the APC gene; the COX-2 knockout caused a dramatic diminution in the number of polyps in the offspring. Furthermore, treatment of experimental animals with either selective COX-2 inhibitors or non-selective COX-1/COX-2 inhibitors has been reported to be a potent approach to chemoprevention of intestinal cancer (Marnett, 1992; Oshima *et al.*, 1996; Boolbol *et al.*, 1996; Reddy *et al.*, 1996; Sheng *et al.*, 1997). As for the role of iNOS in carcinogenesis, it is clear that NO is a potent mutagen (Tamir and Tannebaum, 1996), and that nitric oxide can also activate COX-2

(Salvemini *et al.*, 1993, 1994). There also is a marked increase in iNOS in rat colon tumors induced by the carcinogen, azoxymethane (Takahashi *et al.*, 1997). Similarly, overexpression of iNOS in human tumors has been reported as a negative prognostic factor (e.g., Ekemekcioglu *et al.*, 2006).

[0065] Inflammatory signaling pathways and other disease-associated signaling pathways, such as are induced by angiotensin II, frequently stimulate excessive production of reactive oxygen or nitrogen species (RONS), including superoxide, hydrogen peroxide, nitric oxide and peroxynitrite. CDDO methyl ester has been shown to be a potent inducer of antioxidant activity and a potent inhibitor of inflammatory processes in many different cell types (Dinkova-Kostova *et al.*, 2005; Liby *et al.*, 2006; Ahmad *et al.*, 2006; Shishodia *et al.*, 2006). Severe, acute inflammation due to a variety of causes including infection, trauma, burns, and chemical exposure can be life threatening and may cause liver failure, kidney failure, respiratory failure, or heart failure. Chronic inflammation and the associated oxidative stress contribute to the pathology of many important diseases including autoimmune diseases (e.g., rheumatoid arthritis, lupus, psoriasis, and multiple sclerosis), cardiovascular diseases (e.g., atherosclerosis and heart failure), diabetes (type I and type II), respiratory diseases (e.g., chronic obstructive pulmonary disease and asthma), chronic kidney disease, renal failure, liver failure, and pain syndromes (e.g., neuropathic pain, fibromyalgia, and migraine). In addition, triterpenoids have been shown to inhibit the replication of HIV-1 in macrophages (Vazquez *et al.*, 2005) and so may be useful in the treatment of viral diseases, particularly those in which significant morbidity is caused by organ or tissue inflammation (e.g., viral hepatitis, influenza, herpes simplex).

[0066] MS is known to be an inflammatory condition of the central nervous system (Williams, Ulvestad and Hickey, 1994; Merrill and Beneviste, 1996; Genain and Nauser, 1997). Inflammatory, oxidative, or immune mechanisms may be involved in the pathogenesis of MS, AD, PD, and ALS (Bagasra *et al.*, 1995; Griffin *et al.*, 1995; McGeer and McGeer, 1995; Good *et al.*, 1996; Simonian and Coyle, 1996; Kaltschmidt *et al.*, 1997). Both reactive astrocytes and activated microglia have been implicated in causation of NDD/NID; there has been a particular emphasis on microglia as cells that synthesize both NO and prostaglandins as products of the respective enzymes, iNOS and COX-2. De novo formation of these enzymes may be driven by inflammatory cytokines such as interferon-

gamma or interleukin-1. In turn, excessive production of NO may lead to inflammatory cascades and/or oxidative damage in cells and tissues of many organs, including neurons and oligodendrocytes of the nervous system, with consequent manifestations in AD and MS, and possibly PD and ALS (Coyle and Puttfarcken, 1993; Goodwin *et al.*, 1995; Beal, 1996; Good *et al.*, 1996; Merrill and Benvenist, 1996; Simonian and Coyle, 1996; Vodovotz *et al.*, 1996). Epidemiologic data indicate that chronic use of NSAID's, which block synthesis of prostaglandins from arachidonate, markedly lowers the risk for development of AD (McGeer *et al.*, 1996; Stewart *et al.*, 1997). Accordingly, Form A and Form B of CDDO methyl ester, as agents that block formation of NO and prostaglandins, should be useful in therapeutic approaches to treating or preventing NDD.

[0067] As described above, in a variety of preclinical studies CDDO-Me has demonstrated an ability to inhibit the expression of COX-2 and iNOS, enzymes associated with both inflammation and carcinogenesis. CDDO-Me also was shown to inhibit the activation of nuclear factor-kappa B (NF-☑B) and Signal Transducer and Activator of Transcription 3 (STAT3), transcription factors associated with inflammation, tumor progression, and tumor resistance to therapy. Initial studies evidenced that CDDO-Me inhibited the growth of many cancer cell lines; the average IC_{50} value for CDDO-Me in the NCI-60 tumor cell line panel was approximately 35 nM. *In vivo* studies confirmed that CDDO-Me effectively inhibited the growth of tumors formed by human tumor cell lines implanted in rodents or syngeneic cancer cell lines implanted in rodents (Table 16). Doses used in these studies were generally in the range of 10 to 100 mg/kg/day, depending on the species, strain, and method of administration.

[0068] Studies detailed below provide human data that reflect the beneficial effect of CDDO-methyl ester on patients suffering from a cancerous condition. See Example 10.

[0069] In light of the foregoing, the present invention encompasses stable, controlled release dosage forms containing a CDDO methyl ester form. A dosage form of the invention can be for once-per-day administration, for delayed release, or for pulsatile release, thereby to optimize therapy by matching pharmacokinetic performance with pharmacodynamic requirements.

[0070] Any of Form B, Form A, and formulations containing excipient dispersions of CDDO methyl ester may be administered orally. The active compound may be coated in a

material to protect the compound from the action of acids and other natural conditions which may inactivate the compound. Other modes of administration, such as topical, subcutaneous, intravenous, and intraperitoneal are also part of the current invention.

[0071] To administer the therapeutic compound, it may be necessary to coat the compound with or to co-administer the compound with a material to prevent its inactivation. Thus, either Form B or Form A CDDO methyl ester may be administered to a subject in an appropriate carrier, such as liposomes, or in a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes. See, e.g., Strejan *et al.*, *J. Neuroimmunol.* 7: 27(1984).

[0072] The therapeutic compound can be administered orally, with inert diluents, additives, or an edible carrier, to form a pharmaceutical composition. To this end, the therapeutic compound of the invention, with other ingredients, may 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, Form A or Form B may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Similarly, an excipient dispersion of the present invention may be presented in a variety of dosage form types, including those described here for Form A or Form B. The percentage of the therapeutic compound in the compositions and preparations may be varied, in accordance with conventional practice, to effect a suitable dosage of the active agent.

[0073] Additionally, the present invention relates to a pharmaceutical composition comprising an effective amount of Form B CDDO methyl ester or Form A, in association with one or more non-toxic, pharmaceutically acceptable carriers and/or diluents and, if desired, other active ingredients. As noted above, the active compound can be produced as a homogeneous excipient dispersion, starting from either Form B or Form A. Such a CDDO methyl ester excipient dispersion is a solid solution and can be viewed as a homogeneous dispersion at the molecular level. Such dispersions can be advantageously formulated together with other pharmaceutically acceptable additives, to stabilize the active compound and, in some instances, to provide further improvements in bioavailability.

[0074] In formulating CDDO methyl ester as an excipient dispersion, the choice of an excipient for the dispersion is guided by the criteria that the excipient be both a good "glass former" and pharmaceutically acceptable. More generally, the excipient should form a stable, homogeneous glassy matrix, which stabilizes the dispersion by affording a T_g that is above typical, ambient temperature storage conditions. An additional criterion in this regard is that the excipient used for the dispersion should be chemically compatible with other additives, such as binders, fillers, lubricants, glidants, and the like, which may be employed in the final formulation to confer desired functional properties.

[0075] In satisfaction of these criteria, an excipient can be selected, pursuant to the invention, from a number of compounds characterized by suitably high T_g values, such as (A) carbohydrates, carbohydrate derivatives, and carbohydrate polymers, (B) synthetic organic polymers, (C) organic acid salts, (D) proteins, polypeptides, and peptides, and (E) high molecular weight polysaccharides such as heparin, which is a sulfated polysaccharide, and hyaluronic acid, a mucopolysaccharide.

[0076] Illustrative of class (A) are: cellulose derivatives, such as hydroxypropyl cellulose (HPC), hydroxpropyl methyl cellulose (HPMC), and ethyl cellulose; polysaccharides, such as raffinose, maltotriose, stachyose, dextrins (including maltodextrins and cyclodextrins, *inter alia*), dextrans, and soluble starch; alditols, such as mannitol, xylitol, and sorbitol; and disaccharides, such as lactose, trehalose, maltose, and sucrose. A preferred excipient from this class is hydroxypropyl methyl cellulose phthalic ester (HPMC-P).

[0077] Class (B) is exemplified by poly[1-(2-oxo-1-pyrrolidinyl)ethylene, *a/k/a* povidone or polyvinylpyrrolidone (PVP) and related co-polymers, such as PVP/VA, of varying molecular weights. Also included in this class is the methacrylic acid family of copolymers, such as methacrylic acid copolymer Type C (USP/NF).

[0078] Class (C) is illustrated by salts, such as sodium, potassium, calcium and magnesium salts, of lactic acid, ascorbic acid, maleic acid, oxalic acid, malonic acid, malic acid, succinic acid, citric acid, gluconic acid, and glutamic acid, respectively. Thus, representative salts in this regard are sodium citrate, sodium lactate, sodium maleate, magnesium gluconate, and sodium ascorbate.

[0079] Exemplary class (D) excipients are: human serum albumin; a polyamino acid, *e.g.*, polyalanine, polyarginine, polyglycine, and polyglutamic acid; casein; collagen; gelatin

and purified gelatin proteins; and certain pharmacologically active compounds, such as insulin.

[0080] Excipients can alter some of the physical characteristics of the pharmaceutical formulations, as noted. For instance, dispersion within the various polymeric excipients may lead to a reduction in the observed T_g of the formulation. Normally, T_g is an additive property based on proportions of materials involved. Accordingly, when utilizing polymers with T_g values that are less than that of the amorphous Form B, there is an expectation of a reduction in observed T_g for the dispersions (mixtures). Additionally, moisture or traces of residual organic solvent often are present, which tends to reduce T_g as well.

For purposes of generating a solid CDDO-Me dispersion, the optimal choice of excipient typically must be determined empirically. For example, attempts to produce glassy, XRPD-amorphous dispersions using the polyethylene glycol (PEG) family of excipients, such as PEG 6000, produced mixtures containing characteristic peaks associated with the presence of Form A CDDO methyl ester. Similar results were obtained using Vitamin E – TPGS, an excipient produced by esterifying d-alpha-tocopheryl acid succinate with polyethylene glycol 1000, as well as using ethylene oxide – propylene oxide copolymers such as Pluronic®. As the examples below illustrate, certain polymeric excipients used to form dispersions with CDDO-Me, pursuant to the invention, display surprising improvements in oral bioavailability relative to the pure Form B drug substance.

[0081] Methods can vary for preparing homogenous, glassy, X-ray amorphous dispersions of CDDO-Me with pharmaceutically acceptable excipients, and the examples presented here utilize spray drying to generate such dispersions. Other methods of manufacture may be used to produce dispersions of the invention with equivalent properties and utility. See Repka *et al.*, 2002, and references cited therein. Such other methods include but are not limited to solvent evaporation and extrusion, such as hot melt extrusion.

[0082] In addition to an excipient, other additives may be included to aid in stability of the active ingredient, to adjust the pH (*i.e.*, a buffering agent), to improve dispersibility, to aid in providing uniformity of delivery, and to achieve other characteristics desired for a pharmaceutical formulation.

[0083] The administered quantity of the compound or composition of the present invention will vary, depending on the patient and the mode of administration, and can be any effective amount.

[0084] A given treatment regimen for the administration of a composition of the present invention can be developed by way of normal and routine pre-clinical and clinical testing, the details of which are a function of the therapeutic indication, among other factors. The quantity of the active agent administered may vary over a wide range, thereby to provide, in a unit dosage, a pharmacologically effective amount based upon the body weight of the patient per day, to achieve the desired effect. The desired dosage may also vary according to the condition being treated. For example, treatment of acute cancer may require a significantly higher dose than treatment of an inflammatory condition such as arthritis.

[0085] In particular, a composition of the present invention is presented as a unit dose and taken preferably from 1 to 3 times daily, most preferably once daily to achieve the desired effect.

[0086] In addition, a composition of the current invention may be taken every two days, every three days, every four days,, every five days, every six days, or once a week.

[0087] The compositions of the current invention also may be administered alone or in combination with other drugs based on the particular needs of a patient. In particular, the compositions of the current invention may be administered with anti-cancer agents as part of a treatment regimen. For example, CDDO-methyl ester may be administered with gemcitabine, or other agents, during the treatment of a cancer, such as pancreatic cancer.

[0088] In general, pharmaceutical compositions of the invention are prepared using conventional materials and techniques, such as mixing, blending and the like. Moreover, a medicament containing Form A or Form B also can contain other components, including but not limited to suitable adjuvants, carriers, excipients, and stabilizers, etc. A therapeutic formulation of the invention is preferably a solid but, in principle, could be a liquid, such as a suspension or emulsion.

[0089] Pursuant to the invention, the oral maintenance dose typically is between about 0.1 mg and about 1000 mg, preferably given once daily. The dosage may be varied or keyed to a subjects weight. Typical dosages may be from about 0.01 mg/kg to 100 mg/kg, with the preferred unit dosage forms including tablets and capsules.

[0090] The following examples are illustrative only and are not intended to limit the present invention. The materials and methods employed in the examples are outlined below:

a. Materials

[0091] Solvents and other reagents were purchased from commercial suppliers and were either HPLC or ACS grade.

b. Experimental Methods

i. Approximate Solubility – Solvent Addition Method

[0092] A weighed sample was treated with aliquots of the test solvent at room temperature. Complete dissolution of the test material was determined by visual inspection. Solubility was estimated based on the total solvent used to provide complete dissolution. The actual solubility may be greater than the value calculated because of the use of solvent aliquots that were too large or due to a slow rate of dissolution. The solubility is expressed as "less than" if dissolution did not occur during the experiment. If complete dissolution was achieved as a result of only one aliquot addition, the solubility is expressed as "greater than."

ii. Polymorph Screen

[0093] Both thermodynamic and kinetic crystallization techniques were employed. These techniques are described in more detail below. Once solid samples were harvested from crystallization attempts, they were either examined under a microscope for morphology or observed with the naked eye. Any crystalline shape was noted, but sometimes the solid exhibited unknown morphology, due to small particle size. Solid samples were then analyzed by XRPD, and the patterns were compared to each other to identify new crystalline or non-crystalline forms.

(i) Cold Precipitation (CP)

[0094] Solutions were prepared in various solvents at elevated temperature. The solutions were then filtered through a 0.2-μm nylon or PTFE filter into an antisolvent at sub-room temperature. The presence or absence of solids was noted. If there were no solids present, or if the amount of solids was judged too small for XRPD analysis, the vial was placed in a freezer. The resulting solids were isolated by filtration and dried prior to analysis.

(ii) Fast Evaporation (FE)

[0095] Solutions were prepared in various solvents and sonicated between aliquot additions to assist in dissolution. Once a mixture reached complete dissolution, as judged by

visual observation, the solution was filtered through a 0.2-μm nylon filter. The filtered solution was allowed to evaporate at room temperature in an uncapped vial. The solids that formed were isolated and analyzed.

(iii) Freeze Dry (FD)

[0096] 1,4-dioxane solutions were prepared, filtered through a 0.2-μm nylon filter, and frozen using dry ice. The frozen sample was lyophilized using an FTSsystems Flexi-Dry. The lyophilization temperature was not controlled.

(iv) Micronization

[0097] Micronization of materials can be accomplished in fluid energy mills and can reduce particle size to 1 to 20 microns. Further description of these processes can be found in PERRY'S CHEMICAL ENGINEERS' HANDBOOK, 7th ed. (McGraw Hill, 1998).

(v) Grinding

[0098] A solid sample was placed into a stainless steel milling rotor with a small metal ball. Some samples had a small amount of water added (wet grinding). The sample was then ground at 30 Hz on a Retesh type MM220 mixermill for approximately 20 minutes. The resulting solids were isolated and analyzed.

(vi) Cryogrinding

[0099] A solid sample was placed into a stainless steel grinding jar with a grinding rod. The sample was then ground at 15 Hz on a SPEX Certiprep model 6750 cryomill for a set amount of time. The grinding jar was submerged in a bath of liquid nitrogen during the experiment. The solids were isolated and analyzed.

(vii) Melt/Quench

[0100] A solid sample was placed on a glass microscope slide and leveled. The slide was then placed on a hot plate at a set temperature until the solid melted. Upon melting, the slide was removed from the hot plate and placed on a cold counter top to cool quickly. The resulting solids were dried under nitrogen and analyzed.

(viii) Rotary Evaporation

[0101] Solutions were prepared in various solvents and filtered through a 0.2-μm nylon filter. The sample was placed on the rotary evaporator and removed when dry. The resulting solids were isolated and analyzed.

(ix) Slow Evaporation (SE)

[0102] Solutions were prepared in various solvents and sonicated between aliquot additions to assist in dissolution. Once a mixture reached complete dissolution, as judged by visual observation, the solution was filtered through a 0.2-μm nylon filter. The filtered solution was allowed to evaporate at room temperature or under nitrogen in a vial covered with aluminum foil perforated with pinholes. The solids thus formed were isolated and analyzed.

(x) Slow Cool (SC)

[0103] Saturated solutions were prepared in various solvents at approximately 60°C and filtered through a 0.2-μm nylon filter into an open vial while still warm. The vial was covered and allowed to cool slowly to room temperature. The presence or absence of solids was noted. If there were no solids present, or if the amount of solids was judged too small for XRPD analysis, the vial was placed in a refrigerator. Again, the presence or absence of solids was noted and, if there were none, the vial was placed in a freezer. Solids that formed were isolated by filtration and allowed to dry prior to analysis.

(xi) Slurry Experiments

[0104] Solutions were prepared by adding enough solids to a given solvent so that excess solids were present. The mixture was then agitated in a sealed vial at room temperature. After either 7 or 10 days, the solids were isolated by vacuum filtration and analyzed.

(xii) Stress Experiments

[0105] Solids were stressed under different temperature and/or relative humidity (RH) environments for a measured time period. Specific RH values were achieved by placing the samples inside sealed chambers containing saturated salt solutions or inside ESPEC temperature and humidity cabinets. The salt solutions were selected and prepared following an ASTM standard procedure. Samples were analyzed by XRPD immediately after removal from the stress environment.

iii. Single Crystal Structure Determination

(i) Sample Preparation

[0106] A saturated solution of CDDO methyl ester was prepared in methanol at ~60°C and filtered through a 0.2-μm filter into an open vial while still warm. The vial was covered

and allowed to cool slowly to room temperature. The presence of pyramidal tablets was observed after 1 day.

(ii) Data Collection

[0107] A colorless plate of CDDO-Me ($C_{32}H_{43}NO_4$) having approximate dimensions on two sides of 0.01 x 0.01 mm, was mounted on a glass fiber in random orientation. Preliminary examination and data collection were performed with Mo K_α radiation (λ = 0.71073 Å) on a Nonius KappaCCD diffractometer equipped with a graphite crystal, incident beam monochromator. Refinements were performed on a LINUX PC using SHELX97 (Sheldrick, 1997) [1].

[0108] Cell constants and an orientation matrix for data collection were obtained from least-squares refinement using the setting angles of 46742 reflections in the range $2° < \theta < 22°$. The refined mosaicity from DENZO/SCALEPACK [2] was 0.32° indicating good crystal quality. The space group was determined by the program XPREP [3]. From the systematic presence of the following conditions: *h*00 *h=2n*, 00*l* *l=4n*, and from subsequent least-squares refinement, the space group was determined to be $P4_32_12$ (no. 96).

[0109] The data were collected to a maximum 2θ value of 44.43°, at a temperature of 150 ± 1 K.

(iii) Data Reduction

[0110] Frames were integrated with DENZO-SMN [2]. A total of 46742 reflections were collected, of which 9168 were unique. Lorentz and polarization corrections were applied to the data. The linear absorption coefficient is 0.074 mm^{-1} for Mo K_α radiation. An empirical absorption correction using SCALEPACK [2] was applied. Transmission coefficients ranged from 0.9995 to 0.9999. Intensities of equivalent reflections were averaged. The agreement factor for the averaging was 9.3% based on intensity.

(iv) Structure Solution and Refinement

[0111] The structure was solved by direct methods using SHELXS97 [1]. The remaining atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were included in the refinement but restrained to ride on the atom to which they are bonded. The structure was refined in full-matrix least-squares by minimizing the function:

$$\sum w\left(|F_o|^2 - |F_c|^2\right)^2$$

The weight w is defined as $1/[\sigma^2(F_o^2) + (0.0176P)^2 + (0.0000P)]$, where $P = (F_o^2 + 2F_c^2)/3$.

[0112] Scattering factors were taken from the "International Tables for Crystallography" [5]. Of the 9168 reflections used in the refinements, only the reflections with $F_o^2 > 2\sigma(F_o^2)$ were used in calculating R. A total of 5421 reflections were used in the calculation. The final cycle of refinement included 1024 variable parameters and converged (largest parameter shift was < 0.01 times its estimated standard deviation) with unweighted and weighted agreement factors of:

$$R = \sum |F_o - F_c| / \sum F_o = 0.051$$

$$R_w = \sqrt{\left(\sum w\left(F_o^2 - F_c^2\right)^2 / \sum w\left(F_o^2\right)^2 \right)} = 0.085$$

The standard deviation of an observation of unit weight was 1.05. The highest peak in the final difference Fourier had a height of 0.22 e/Å3. The minimum negative peak had a height of -0.25 e/Å^3.

(v) Calculated X-ray Powder Diffraction (XRPD) Pattern

[0113] A calculated XRPD pattern was generated for Cu radiation using PowderCell 2.3 [6] and the atomic coordinates, space group, and unit cell parameters from the single crystal data.

(vi) ORTEP and Packing Diagrams

[0114] The ORTEP diagram was prepared using ORTEP III [7][9]. Atoms are represented by 50% probability anisotropic thermal ellipsoids. Packing diagrams were prepared using CAMERON [8] modeling software. Additional figures and BFDH morphology predictions were generated using Mercury 1.4.1 [4].

c. Instrumental Techniques

i. Differential Scanning Calorimetry (DSC)

[0115] Analyses were carried out on a TA Instruments differential scanning calorimeter 2920 or Q1000. The instrument was calibrated using indium as the reference material. The sample was placed into a standard aluminum DSC pan with a crimped lid configuration, and the weight accurately recorded. The sample cell was equilibrated at 25 °C and heated under a nitrogen purge at a rate of 10 °C/min, up to a final temperature of 250 °C.

ii. Dynamic Vapor Sorption/Desorption (DVS)

[0116] Moisture sorption/desorption data were collected on a VTI SGA-100 Vapor Sorption Analyzer. Sorption and desorption data were collected over a range of 5% to 95%

relative humidity (RH) at 10% RH intervals under a nitrogen purge. Samples were not dried prior to analysis. Equilibrium criteria used for analysis were less than 0.010 % weight change in 5 minutes, with a maximum equilibration time of 3 hours if the weight criterion was not met. Data were not corrected for the initial moisture content of the samples. Sodium chloride and polyvinypyrrolidine were used as calibration standards.

iii. Karl Fischer (KF)

[0117] Coulometric Karl Fischer (KF) analysis for water determination was performed using a Mettler Toledo DL39 Karl Fischer titrator. Approximately 24-32 mg of sample was placed in the KF titration vessel containing Hydranal – Coulomat AD. The sample was then titrated by means of a generator electrode which produces iodine by electrochemical oxidation:

2 I- => I_2 + 2e. Three replicates were obtained to ensure reproducibility.

iv. Hot Stage Microscopy

[0118] Hot stage microscopy was performed using a Linkam hot stage (model FTIR 600) mounted on a Leica DM LP microscope. Samples were observed using a 20× objective (obj.) with cross polarizers (CP) and lambda (λ) compensator. Samples were placed on a coverslip. A second coverslip was then placed over the sample. Each sample was visually observed as the stage was heated. Images were captured using a SPOT Insight™ color digital camera with SPOT Software v. 4.5.9. The hot stage was calibrated using USP melting point standards.

v. Modulated Differential Scanning Calorimetry (MDSC)

[0119] Modulated differential scanning calorimetry data were obtained on a TA Instruments differential scanning calorimeter equipped with a refrigerated cooling system (RCS). The sample was placed into an aluminum DSC pan, and the weight accurately recorded. The pan was covered with a lid and crimped. MDSC data were obtained using a modulation amplitude of +/- 0.8°C and a 60 second period with an underlying heating rate of 2°C/min from -25 to 250°C. The temperature and the heat capacity were calibrated using indium metal and sapphire as the calibration standards, respectively. The reported glass transition temperature is obtained from the inflection of the step change in the reversible heat flow versus temperature curve.

vi. Nuclear Magnetic Resonance (NMR)

[0120] The solution phase ^{1}H NMR spectra were collected by Spectra Data Services, Inc. Acquisition parameters are printed on each spectrum. Spectra were referenced to internal tetramethylsilane at 0.0 ppm.

vii. Optical Microscopy

[0121] Observations made by optical microscopy were collected on a Wolfe polarizing optical microscope at a magnification of 4x. Crossed polarizers (CP) were used to observe birefringence in the samples.

viii. Scanning Electron Microscopy (SEM)

[0122] Scanning electron microscopy (SEM) was performed using a FEI Quanta 200 scanning electron microscope. Under high vacuum mode, a solid state backscatter (Etd) detector was used. Beam voltage was 5.0 kV. Samples were sputter coated using a Cressington 108auto Sputter Coater at ~20 mA and ~0.13 mbar (Ar) with Au/Pd for 75 seconds. Samples were prepared for analysis by placing a small amount on carbon double-stick tape fixed to an aluminum sample mount. The instrument was calibrated for magnification using NIST standards. Data was collected using xTm (v. 2.01), build number 1564 and analyzed using XT Docu (v. 3.2). Magnifications reported on the SEM images were calculated upon the initial data acquisition. The scale bar reported in the lower portion of each image is accurate upon resizing the images and should be utilized when making size determinations.

ix. Thermogravimetry (TG)

[0123] Analyses were carried out on a TA Instruments 2950 thermogravimetric analyzer. The calibration standards were nickel and Alumel™. Each sample was placed in an aluminum sample pan and inserted into the TG furnace. Samples were first equilibrated at 25°C, then heated under a stream of nitrogen at a heating rate of 10°C/min, up to a final temperature of 350°C unless specified otherwise.

x. X-Ray Powder Diffraction (XRPD)

(i) Inel XRG-3000

[0124] X-ray powder diffraction analyses were also performed on an Inel XRG-3000 diffractometer, equipped with a curved position-sensitive detector with a 2θ range of 120°. Real time data was collected using Cu Kα radiation at a resolution of 0.03°2θ. The tube

voltage and amperage were set to 40 kV and 30 mA, respectively. Patterns are displayed from 2.5 to 40°2θ to facilitate direct pattern comparisons. Samples were prepared for analysis by packing them into thin-walled glass capillaries. Each capillary was mounted onto a goniometer head that is motorized to permit spinning of the capillary during data acquisition. Instrument calibration was performed daily using a silicon reference standard.

d. Additional Calculation Techniques

i. PDF

[0125] One technique used in the computational analysis of x-ray amorphous data is the Pair Distribution Function (PDF). As the name suggests, the PDF is made up from a linear sum of all the coherent atom-atom interactions within the material. Defective (disordered) materials will exhibit the same atom-atom interactions as the crystalline phase, but over a reduced length scale. Therefore, such materials can be compared to parent crystalline materials by examining the peaks in the PDF over the first few nanometers. Comparisons between spectra in the range of 0 Å to about 5 Å are difficult due to artifacts in this region.

[0126] On the formation of glassy material, the PDF peaks will exhibit some movement from the crystalline peak positions as the molecules relax their positions. This is similar to a thermal expansion/contraction event where, although the PDF peaks move slightly, the relative peak intensities should still be recognizable as being related to the original crystalline material. As the material enters the thermodynamic amorphous state, some of the point group symmetry relationships will be lost giving a PDF of reduced complexity. There also will be some peak movement. The glassy/amorphous materials will exhibit PDFs that will rapidly fall to zero over 2 to 3 nearest neighbor (NN) distances.

[0127] Measurement conditions are used to minimize the background in the x-ray patterns and algorithms are used to calculate the PDF from measured x-ray data. The PDFs were calculated using PatternMatch v2.2.1, using the entire range of measured data for all samples.

Example 1 – Solubility Estimates

[0128] Approximate solubilities were determined in various solvents at room temperature, with the results as provided in Table 2. CDDO methyl ester exhibits high solubility in the majority of the organic solvents used. The solubility in water appears to be less than 0.1 mg/mL.

Example 2 – Polymorph Screen Results

[0129] Approximately 50 polymorph screen experiments were performed. Form A was observed from approximately 50% of the samples. The formation of Form A was not limited to a particular crystallization condition and was prepared from a variety of different experiments and solvents. Form B material was prepared from lyophilization, melt/quench, and several evaporation experiments.

[0130] Polymorph screen samples are listed alphabetically in Tables 3-5 by the solvent used. Representative XRPD patterns of Form A and Form B materials are compared in Figure 2. Characterization data of the forms are summarized in the examples below.

Example 3 – Characterization of CDDO-methyl ester – Form A (unmicronized)

[0131] Form A is unsolvated (Table 6). The single crystal structure of Form A was determined based on methods described above. Crystals of CDDO-methyl ester were grown and submitted for single crystal structure analysis. The crystal structure was determined by single crystal X-ray diffraction. The proposed structure of CDDO-methyl ester is shown in Figure 1.

[0132] The tetragonal cell parameters and calculated volume are: a = 14.21620(10) Å, b = 14.21620(10) Å, c = 81.5875(12) Å, $\alpha \subseteq 90.00°$, δ = 90.00°, γ = 90.00°, V = 16488.9(3) Å^3. The molecular weight of CDDO methyl ester is 505.70 g/mol with Z = 24 resulting in a calculated density of 1.222 g cm^{-3}. The space group was determined to be $P4_32_12$ (no. 96). A summary of the crystal data and crystallographic data collection parameters is provided in Table 10.

[0133] The quality of the structure obtained is high, as indicated by the R-value of 0.051 (5.1%). Usually R-values in the range of 0.02 to 0.06 are quoted for the most reliably determined structures.

[0134] An ORTEP drawing of a single CDDO methyl ester molecule is shown in Figure 12. The asymmetric unit shown in Figure 13 contains three CDDO methyl ester molecules. The molecules are the same as the proposed structure from Figure 1.

[0135] Packing diagrams viewed along the a, b, and c crystallographic axes are shown in Figures 14-16, respectively. With no hydrogen bonds, the crystal structure includes numerous van der Waals interactions. The view down the crystallographic b axis (Figure 15) highlights the helical nature of the packing arrangement of the tetragonal screw axis and the

predicted BFDH morphology. The predicted morphology is in good agreement with the observed habit of the single crystal used in the data collection.

[0136] The calculated XRPD pattern of CDDO methyl ester, generated from the single crystal data, is provided in Figure 17. The experimental XRPD pattern of CDDO methyl ester is shown in Figure 18. Characteristic peaks for the Form A XRPD pattern are provided in Table 17. A comparison of the calculated and experimental XRPD patterns (Figure 19) reveals all peaks in the experimental patterns are represented in the calculated XRPD pattern, indicating the bulk material is likely a single phase. The slight consistent shifting observed in peak location is likely due to the fact that the experimental powder pattern was collected at room temperature, and the single crystal data were collected at 150°K. Low temperatures are used in single crystal analysis to improve the quality of the structure.

[0137] In summary, the single crystal structure of CDDO methyl ester Form A was determined to confirm to the proposed molecular structure. The space group was determined to be $P4_32_12$ (no. 96). The structure of CDDO methyl ester consists of three molecules packed in a helical nature down the crystallographic *b* axis. All peaks in the experimental patterns are represented in the calculated XRPD pattern, indicating the bulk material is likely a single phase.

[0138] The thermal data for Form A are shown in Figure 3. The DSC curve shows a baseline shift at approximately 157°C and an endotherm with an onset temperature of approximately 222°C (signal maximum at ~224°C). The event at ~224°C was confirmed as the melt by hot stage microscopy (Figure 4). The thermogravimetry (TG) curve exhibits a negligible weight loss of 0.34% up to 150°C, followed by a weight loss of 1.2% from 150 to 210°C. Karl Fischer data shows the material to contain approximately 0.38% residual water, consistent with the initial weight loss observed by TG.

[0139] The DVS data indicate that Form A is not hygroscopic (Figure 5). The material shows a negligible weight change throughout the experiment. The resulting material was analyzed by XRPD and is Form A.

[0140] SEM images are shown in Figure 6. Several habits are observed, including pyramidal, tablet, and plate-like.

[0141] The physical stability of Form A at various conditions was investigated (Table 7). Samples stressed at either 25°C/60% RH or 40°C/ 75% RH for 7 days both showed a

negligible weight change (0.6% loss and 0.2% gain, respectively) suggesting that Form A is not hygroscopic. Two samples were ground on a ball mill for approximately 20 minutes, one dry and one with a small amount of water. All samples were reanalyzed by XRPD and remained Form A. A sample was stressed at 195°C for 15 minutes and showed a 2% weight loss. The XRPD of the resulting material is similar to that of Form A; however, an increase in the baseline noise is evident (Figure 7).

[0142] The solution NMR spectrum is shown in Figure 8. The spectrum is consistent with the structure of CDDO methyl ester. Peaks at approximately 1.6 and 7.3 ppm are assigned to water and chloroform (due to exchange), respectively.

[0143] Form A is unsolvated and not hygroscopic, therefore, and it melts at approximately 228°C, based on observations of analyst during hot stage microscopy.

Example 4 – Characterization of CDDO-methyl ester – Form A (micronized)

[0144] Micronized Form A CDDO methyl ester was determined to be Form A by XRPD (Figure 2, Table 1). Micronized material can be produced by conventional methodology, well known to the field, such as air jet milling. These findings appear to indicate that micronization does not affect Form A in order to alter its XRPD pattern.

Example 5 – Characterization of CDDO-methyl ester – Form B

[0145] Form B material can be prepared from lyophilization, melt/quench, and several other evaporation experiments, as provided in Table 3.

[0146] The modulated DSC (MDSC) data are shown in Figure 9. The reversible curve shows a glass transition temperature (Tg) at approximately 125°C. The non-reversible curve shows an exotherm with signal maximum at 195°C and an endotherm with signal maximum at 223°C. The non-reversible events are most likely due to crystallization of the Form B material (exotherm) followed by the melt of the crystallized material (endotherm).

[0147] The physical stability of Form B material at various conditions was investigated (Table 9). Samples stressed at 22°C/97% RH, 40°C/ 75% RH, 80°C/0% RH, and 195°C/ambient RH remained Form B. Stressing the material at 200°C/ambient RH for 60 minutes produced Form A plus minor Form B material (Figure 10).

[0148] The solution NMR spectrum is shown in Figure 11. The spectrum is consistent with the structure of CDDO methyl ester. Peaks at approximately 1.6, 5.3, and 7.3 ppm are assigned to water, dichloromethane, and chloroform, respectively.

[0149] Form B is not hygroscopic, crystallizes to Form A at approximately 200°C, and has a glass transition temperature (Tg) of approximately 125°C - 130°C.

[0150] In summary, Form B is not hygroscopic. The MDSC data indicates that the glass transition temperature (Tg) of Form B is approximately 125°C – 130°C. Form B material crystallizes to Form A when stressed at approximately 200°C.

Example 6 – Stability Studies for Form B CDDO-methyl ester and CDDO-methyl ester polymer excipient dispersions

(i) Purified Form B Studies

[0151] Form B CDDO-methyl ester was subjected to varying stress conditions. Table 15 provides some of the results from these studies. Form B CDDO-methyl ester produced by a useful but less preferred embodiment of this invention, involving the use of ethyl acetate as solvent, shows considerable stability. Nevertheless, testing of Form B samples produced in the presence of ethyl acetate revealed the formation of Form A after 28 days of storage at temperatures of 60°C and above. By contrast, all samples produced in accordance with a preferred embodiment of this invention, Example 11 below being illustrative thereof, retained amorphous characteristics after stress testing under particularly rigorous conditions (see Table 15). These studies show the surprising stability of the Form B material, especially when it is prepared in accordance with the aforementioned, preferred embodiment.

[0152] In addition, Form B samples prepared under varying conditions were analyzed to determine whether they have similar chemical properties. As described above, that is, Form B samples were prepared by cryogrinding, melt quench, and spray drying methods. In addition, unmicronized Form B was micronized to produce micronized Form B. PDF analysis was performed on the samples (Figure 28), which were determined to be glassy in nature.

(ii) Studies with CDDO-Me dispersion preparations

[0153] Studies were performed to compare the performance of different Form B :polymer solid dispersions produced by spray drying. The product attributes studied included stability and drug dissolution profile.

[0154] Different polymers were used at three active pharmaceutical ingredient (API) to polymer ratios (20:80; 40:60 and 60:40 %w/w).

[0155] The following three polymers were selected:

Methacrylic Acid - Ethyl Acrylate Copolymer (1:1)
Copovidone [1-vinyl-2-pyrrolidone - vinyl acetate copolymer (3:2)]
Hypromellose Phthalate.

[0156] Solutions of CDDO-Me, either Form A crystalline material or Form B material, and the polymer chosen for study were dissolved in a suitable solvent at the appropriate weight ratios, typically to afford from 10-20% by weight of solids in solution. The solvent typically employed was acetone. The resulting solutions were spray dried by means of a laboratory scale spray dryer (BÜCHI, model B-290), equipped with a two-fluid nozzle, using nitrogen as a drying and carrier gas. Carrier gas inlet temperatures of 65-85°C and outlet temperatures of 50-60°C typically were employed. The solids were collected and the spray dried powders were post-dried under vacuum, further to reduce the levels of the organic solvents.

[0157] The spray dried powders were analyzed for the level of residual organic solvents, glass transition temperature (T_g) and bulk density. After post-drying the powders were also analyzed for purity, water content, average particle size, absence of crystalline material by X-ray powder diffraction (XRPD) and dissolution profile.

[0158] The physiochemical characteristics of the dispersions were evaluated after short term stressing (after 5 days, 40°C / 75% RH) by XRPD and modulated differential scanning calorimetry (mDSC).

[0159] These studies found that the glass transition temperature, T_g, decreased during short term stressing, probably due to the uptake of moisture over the course of stressing. The decrease was more pronounced with the formulations having low CCDO methyl ester : polymer ratios. For samples before the short-term stressing, one or two endothermic transitions were observed at higher temperatures, although these temperatures are somewhat lower than the ones observed initially. The enthalpy associated with these transitions decreased as polymer content decreased. This is an indication that this transition is most probably related with the polymer and not with the melting of a crystalline form. In fact, for dispersions produced with PVP/VA, the temperature of this endothermic transition was similar to the one observed for pure excipient, as received from the manufacturer. In

each case, the XRPD profile after stressing continued to be the characteristic halo pattern centered around 13.5 °2θ, and no peaks associated with the crystalline form were detected.

[0160] Further spray drying studies on two formulation were conducted, using larger scale spray drying equipment. In these cases, a Niro pilot scale dryer model PSD-1 (mobile minor 2000) was employed. Also employed were equivalent nozzle and spray drying conditions to those described above. Tables 20 and 21 summarize the solutions prepared for spray drying and their characteristics following spray drying. The formulations showed a lower T_g, relative to pure Form B, due to the formulation including polymers with a lower T_g than Form B.

Example 7 – Administration of CDDO methyl ester:
Form B versus Form A in Cynomolgus Monkeys

[0161] In phase 1 of this study, a quantity of hard gelatin capsules, containing pure micronized Form A CDDO-Me or pure micronized Form B CDDO-Me, were prepared by (i) adding an appropriately weighed quantity of the pure form of the drug substance to a size 1 hard gelatin capsule and then (ii) closing the capsule. No additional excipients were employed. Either CDDO methyl ester Form B or Form A was administered orally in a gelatin capsule to Cynomolgus monkeys (dose = 4.1 mg/kg in all cases).

[0162] Administration of the CDDO methyl ester Form B provided a median exposure which was approximately 520% greater than the equivalent dose of CDDO methyl ester Form A in monkeys. Table 11 compares the individual animal drug exposures. A crossover design was implemented in this study to increase the 'n' value and to enhance data reliability. A wash-out period of 1 week was implemented during the crossover. Figure 20 demonstrates the achieved plasma concentrations of both forms of CDDO methyl ester over time in the sampled population. Figure 21 represents comparative CDDO methyl ester plasma concentrations of animals #505M and #507F. Figure 22 represents comparative CDDO methyl ester plasma concentrations of animals #508F and #502M.

[0163] To assess further the comparative bioavailability of the oral dosage forms of CDDO-methyl ester, including those containing CDDO-methyl ester excipient dispersions, two additional study phases 2 and 3 were conducted, as detailed below. These studies included some of the CDDO- Me Form B – polymer dispersions described in Example 6. All formulations included commonly used formulation additives.

Phase 2:

[0164] An aqueous suspension of nanocrystalline CDDO-Me was produced, starting from a sample of micronized Form A material. A Retsch® Planatory Ball Mill model PM 400, containing zirconia balls of 2 mm average size, was charged with 25 gm of micronized CDDO-Me (average particle size distribution of 6.1 uM), 5 gm of docusate sodium, 1 gm of Tween 80, and 68.3 gm of water. Grinding was initiated at approximately 400 RPM and was continued for 2 hours. A particle size distribution (PSD) determination using a laser light granulometer indicated an average PSD of 0.37 μM was obtained. To this thick suspension were added 1 gm of microcrystalline cellulose and 0.2 gm of xantham gum, with brief mixing, and the suspension was stored refrigerated.

[0165] The ball milled nano suspension was spray coated onto a dry excipient blend in a laboratory scale Aeromatic Strea 1 fluid bed, with the top spray assembly having a pray nozzle size of 0.4 mm. The inlet temperature was set at 55°C. The exhaust temperature range during spraying was 32 to 35°C. The resulting granulation was dried for approximately 5 minutes, until the exhaust temperature reached 38°C (Attachment 3-5). The composition of the coated materials is given below.

Theoretical Composition of the nanocrystalline 30 mg CDDO-Me Formulation

Item#	Ingredient	%	mg/unit
1	CDDO-Me	5.7	19
2	Tween 80	0.12	0.4
3	DOS	1.14	3.8
4	Microcrystalline Cellulose in suspension	0.21	0.7
5	Xanthan Gum	0.03	0.1
6	Microcrystalline Cellulose in granulation	24.4	81.55
7	Starch Pregel	58.5	195.3
8	Povidone K29/32	0.5	1.75
9	Crospovidone	8.4	28
10	Colloidal Silicon Dioxide	0.5	1.75
11	Magnesium Stearate	0.5	1.75
	Total:	100.0	334.1

[0166] The resulting dry granulation was submitted for HPLC analysis to determine the assay of active ingredient. That assay was determined to be 14.4 % (wt/wt), substantially

higher than the theoretical value (5.7%). Based on the HPLC analysis, capsules were filled so as to afford a net CDDO-Me content of 30 mg.

[0167] The crystalline micronized Form A and amorphous, micronized Form B formulations were produced by a conventional dry powder blending process, using, as additives, microcrystalline cellulose, pregelatinized starch, crospovidone (functioning as a disintegrant), colloidal silicon dioxide, and vegetable grade magnesium stearate. Micronized CDDO-Me Form A of average PSD 6.1 μM was used for the Form A formulation, while micronized CDDO-Me Form B of average PSD 10.8 μM was employed for the corresponding CDDO-Me Form B formulation. The table below presents the quantitative composition of both formulations.

Form A and Form B 30 mg capsule Quantitative Composition

Identity	% w/w	mg per capsule
CDDO-Me (Micronized)	18.18	30.0
Microcrystalline Cellulose	18.55	30.6.
Pregelatinized Starch	53.45	88.2.
Crospovidone	8.72	14.4
Colloidal Silicon Dioxide	0.55	0.9
Magnesium Stearate (Vegetable Grade)	0.55	0.9
Total Capsule Contents	100.00	165 mg

[0168] Each of 15 male Cynomolgus monkeys, Macaca fascicularis, received a single oral administration of CDDO-methyl ester (3 different formulations, 5 monkeys per formulation) at a target dose level of 10 mg/kg. The monkeys ranged from 1-3 years in age and 2.5-3.5 kg in size. Blood samples were collected up to 72 hours post-dose.

Phase 3:

[0169] The CDDO-Me excipient dispersions described in Example 6 were further formulated by a conventional dry powder blending process, using microcrystalline cellulose, lactose monohydrate, crospovidone (functioning as a disintegrant), and sodium lauryl sulfate as additives. The quantitative composition of each formulation appears below.

Composition of CDDO-Me excipient dispersions blended with addities in capsule formulation

Component	40% CDDO-Me / 60% Methacrylic acid copolymer Type C	60% CDDO-Me/ 40% PVP/VA	60% CDDO-Me/ 40% HPMC-P
CDDO-Me Dispersion	60.0%	50.0%	50.0%
Microcrystalline Cellulose	12.8%	16.0%	16.0%
Lactose monohydrate	20.0%	25.0%	25.0%
Crospovidone	6.4%	8.0%	8.0%
Sodium lauryl sulfate	0.8%	1.0%	1.0%
Total Capsule Contents	100%	100%	100%
Fill mass	125mg	100mg	100mg

[0170] After a suitable wash-out period (7 to 10 days), the same 15 male Cynomolgus monkeys used in Phase 2 each received a single oral administration of CDDO-mehtyl ester (3 different formulations, 5 monkeys per formulation) at a target dose level of 10 mg/kg. Blood samples were collected up to 72 hours post-dose.

[0171] The below chart summarizes each phase of the study.

Phase number	**Route of administration**	**CDDO-Me formulation**	**Target dose level**	**Number of animals (males)**
2	Oral (capsule)	Nanocrystalline Form A	30 mg/per animal (10 mg/kg)	5
		Amorphous micronized Form B		5
		Crystalline micronized Form A		5
3	Oral (capsule)	PVP/VA Form B	30 mg/per animal (10 mg/kg)	5
		HPMC-P Form B		5
		Methacrylic acid copolymer Type C-Form B		5

[0172] The mean intravenous and oral doses received by male Cynomolgus monkeys and mean concentrations of CDDO-methyl ester are summarized below:

Phase number	Formulation	Mean body weight (kg)	Mean dose (mg/kg)
2	PO (nanocrystalline Form A)	2.89 ± 0.267	10.5 ± 0.971
	PO (amorphous micronized Form B)	2.87 ± 0.177	10.5 ± 0.635
	PO (crystalline micronized Form A)	2.91 ± 0.202	10.3 ± 0.702
3	PO Form B (PVP/VA)	2.98 ± 0.311	10.2 ± 1.06
	PO Form B (HPMC-P)	2.93 ± 0.183	10.3 ± 0.627
	PO Form B (Methacrylic acid copolymer Type C)	2.93 ± 0.142	10.2 ± 0.486

PO Oral administration by capsule.

[0173] Gelatin capsule size 2 was used to deliver the formulations in phase 2 and gelatin capsule size 1 was used for delivery in phase 3. The net drug content in each capsule was 30 mg, corresponding to a 10 mg/kg dosage of drug, based on an assumption that each monkey weighed 3 kg. The capsule was attached to a gavage, the animal was gavaged, and the capsule was released from the end of the gavage by air pressure from an empty syringe. A small amount of water (approximately 10 mL) was given orally after the administration of the last capsule.

[0174] Serial blood samples (approximately 1 mL) were removed from the femoral vein or artery of each animal and transferred into tubes containing K_2-EDTA at each of the following time points (actual times were recorded):

Phase 2 Pre-dose, 1, 2, 4, 8, 16, 24, 48 and 72 h post-dose

Phase 3 Pre-dose, 1, 2, 4, 8, 16, 24, 48 and 72 h post-dose

[0175] All samples were thoroughly mixed following collection and placed on wet ice, prior to being refrigerated at approximately 4°C. CDDO-methyl ester concentrations in blood were analysed by HPLC-MS/MS.

[0176] The results are provided in Table 22 and in Figure 30. For phase 2, Form B showed significantly better bioavailability to the two Form A formulations tested. Phase 3 results show that each of the CDD)-Me : polymer dispersion-based formulations had much higher bioavailability than either the micronized Form A or nanocrystalline Form A formulations. The methacrylic acid copolymer Type C and HPMC-P formulations showed the greatest bioavailability in the subject monkeys.

Example 8- Characterization of Hemibenzenate Form of CDDO-Methyl Ester

[0177] Various experiments, replicating the last recovery step from the synthesis of CDDO methyl ester, were performed. Honda *et al.*, 2000. The objective was the isolation of crystalline material from a solution mixture of (10:1) benzene/acetone.

[0178] Approximately 100 mg of CDDO-methyl ester was dissolved in 300 μL of benzene/acetone (10:1) and filtered through a 0.2-μm nylon filter. The solution was then sonicated using an ultrasonic processor for 10 minutes and allowed to evaporate at room temperature in an uncapped vial overnight. A clear gel formed and 100 μL of benzene/acetone (10:1) was added. The solution was submitted to sonication on an ultrasonic processor for approximately 30 minutes. A white precipitate formed. The solids were allowed to air dry.

[0179] In other experiments, approximately 200 mg of CDDO-methyl ester was dissolved in 0.8 mL of benzene/acetone (10:1) and filtered through a 0.2-μm nylon filter. The solution was then evenly divided into two 1-dram vials. Samples A and B were then allowed to fast evaporate at room temperature for a few hours. Sample A was capped and placed in a freezer. After the sample froze, the sample was allowed to thaw at room temperature. A small scratch was introduced using a spatula and the sample was allowed to evaporate at room temperature. White solids formed and were allowed to air dry. Sample B was capped, left at room temperature and was a clear solution after sitting at room temperature overnight. A small scratch was introduced using a spatula and the sample was allowed to evaporate at room temperature. White solids formed and were allowed to air dry.

[0180] Crystalline material, determined to be a hemibenzenate, was obtained from several of these experiments. As described above, minor disturbances, such as sonication or merely introducing a small scratch within the recovery vessel, will facilitate the crystallization of the benzene solvate (Table 12).

[0181] Characterization data of the hemibenzenate are summarized in Table 13. Characteristic peaks for the hemibenzenate XRPD pattern are provided in Table 19. The DSC curve exhibits a broad endotherm near 133°C, associated with ~7.0 % of weight loss in the TG thermograph Figure 23. The weight loss is likely due to the volatilization of benzene (see NMR discussion below), and corresponds to 0.5 moles of benzene for each mole of

CDDO-methyl ester. The DSC endotherm observed near 223°C most likely results from the melt of desolvated material.

[0182] These data distinguish previously isolated forms of CDDO-methyl ester from the present invention.

Example 9 - Characterization of Novel Dimethanolate Form of CDDO-Methyl Ester

[0183] A CDDO-methyl ester dimethanol solvate was prepared according to the below procedure. Approximately 500 mg of CDDO-methyl ester was dissolved in 20 mL of methanol at 60°C. The solution was then slowly added to 20 mL of cold methanol at -10°C with agitation. White solids were collected by vacuum filtration and then stored in a freezer.

[0184] Characterization data are summarized in Table 14. Characteristic peaks for the dimethanolate XRPD pattern are provided in Table 18.

[0185] The DSC curve shows a broad endotherm near 102°C, associated with ~11% of weight loss in the TG thermograph (Figure 24). The TGIR data confirms the weight loss is due to volatilization of ~2.0 moles of methanol (Figure 25). The resulting material from the TGIR experiment was recovered and was amorphous by XRPD (Figure 26). A baseline shift at approximately 130°C, a broad exotherm near 203°C followed by a sharp endotherm (onset: 223°C) are also observed in the DSC curve. These events are most likely indicative of the Tg of the amorphous material (Form B) obtained through the desolvation of the dimethanol solvate followed by crystallization of the amorphous material to Form A and melting of that crystalline material.

[0186] The solution proton NMR spectrum was obtained. The chemical assignments were not performed; however it appears consistent with the chemical structure of CDDO-methyl ester. The peaks at ~3.51 ppm are assigned to methanol and correspond to ~1.7 moles. This result is consistent with the thermal data above.

Example 10 – Clinical Studies with CDDO-methyl ester

[0187] CDDO-Me, formulated using micronized Form A, was selected for clinical development and first tested in a Phase I safety-oriented study in patients with advanced cancer who had failed to respond adequately to prior therapies. In this Phase I dose-escalation trial, CDDO-Me was administered to 21 adult patients with various forms of advanced (metastatic) cancer. Patients were administered daily doses of CDDO-Me capsules

at doses ranging from 5 to 900 mg/day (specifically 5, 10, 20, 40, 80, 150, 300, 600, or 900 mg/day). CDDO-Me was administered in "cycles" which were repeated until the patient experienced unacceptable toxicity or showed evidence of disease progression. In this study, one cycle of CDDO-Me consisted of 21 consecutive days of dosing followed by a 7-day rest period after which the patient was eligible to start the next cycle.

[0188] Both the safety and anti-tumor activity of CDDO-Me were reviewed. In addition, the biological effects of CDDO-Me were characterized. CDDO-Me was very well tolerated in these patients, with no significant drug-related adverse events reported. Several patients (approximately 75% of evaluable patients) were considered to have stable disease (based on standard radiological and clinical criteria) at the first evaluation point following completion of the second treatment cycle. Patients who were found to have evidence of progressive disease before completing the second cycle were not formally evaluated, and were not included in the group of evaluable patients. Five patients, including patients with melanoma and renal cell cancer, continued to show stable disease, some with evidence of regression of individual tumor lesions, after four cycles of treatment. Four patients were considered to have stable disease after at least six cycles of treatment. No new metastases developed in any patient receiving a dose of at least 40 mg CDDO-Me per day according to the prescribed schedule.

[0189] Based on the known anti-inflammatory properties of CDDO-Me, circulating inflammatory cytokines were evaluated in patients in the Phase I trial. At doses as low as 5 mg/day, there was a reduction of several circulating pro-inflammatory cytokines and chemokines including MMP-9, TNFα, IL-8, and VEGF. In particular, TNFα, which is known to play a significant role in the inflammatory process of diseases such as rheumatoid arthritis, was reduced substantially or to below detectable limits in 3 patients with elevated baseline TNF levels (one patient each at treatment doses of 10, 20, and 40 mg per day). Unlike anti-TNF monoclonal antibodies, which bind to and render targets inactive, CDDO-Me reduces production of TNFα and resultant circulating levels of TNFα.

[0190] Additionally, phase 2 gene products, which include antioxidant and detoxification enzymes, have been monitored in peripheral blood mononuclear cells of patients in the Phase I study. Significant induction of NQO1 (NAD(P)H:quinone oxidoreductase), a marker of phase 2 transcriptional activity, has been seen at doses of 10 mg/day and above.

[0191] Tumor biopsy data from several patients, taken after two cycles of treatment with CDDO-Me, indicated pronounced reductions in tumor tissue levels of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and phosphorylated STAT3 (pSTAT3). High levels of expression of each of these proteins are known to be correlated with tumor progression and poor clinical outcomes. Tumor biopsy data in several patients also indicated a pronounced degree of tumor cell death after two cycles of treatment with CDDO-Me. Levels of serum creatinine were significantly lower on day 21, compared to the pre-treatment baseline level, in more than 80% of the patients in this study. A number of patients who continued on treatment for multiple cycles showed continuing reductions in serum creatinine. Since serum creatinine is a widely used indicator of renal function, these observations indicate that treatment with CDDO-Me improves kidney function.

[0192] These studies provide data in human cancer patients showing the beneficial effect of CDDO-methyl ester on patients suffering from cancer. The data further indicate that CDDO-Me is likely to have clinically useful effects in patients suffering from other inflammation-related diseases, including renal dysfunction.

Example 11 – Large Scale Production of Form B
Using a Dimethanol Solvate Intermediate

[0193] One kilogram of Form A CDDO-Me was dissolved in 60 ± 5°C methanol to afford a complete solution. The resulting hot solution of CDDO-Me was added to a vessel containing cold -5°C to -15°C methanol, while maintaining agitation and a temperature of -5°C to -15°C throughout the addition. The resulting suspension of crystalline dimethanol solvate of CDDO-Me was filtered. The resulting solids, which displayed an XRPD pattern consistent with that presented in Figure 26 (prior to TGIR analysis), were dried in an oven at 70 ± 5°C. Drying was continued until the XRPD profile displayed no reflections characteristic of crystalline substance. The resulting XRPD amorphous CDDO-Me solids were passed through a sieve and packaged. Product recovery ranges from 65-95%.

Example 12 – Cryoground Form A and Form B

[0194] Form A was cryoground and analyzed. The measured x-ray data of the sample obtained through cryogrinding (2 hours) showed some broadening in the peak at approximately 13.5°2✓. PDF analysis of cryoground Form A produced results similar to the

Form B analysis. These results suggest that the cryoground Form A is a glassy material and that cryogrinding can provide an alternative method for producing Form B.

[0195] Form B was cryoground and analyzed. The measured x-ray data of the sample obtained through cryogrinding (1 hour) was similar to the starting Form B material. These results indicate that Form B is stable and does not change Form due to cryogrinding.

Table 1 – Sample Information

SAMPLE INFORMATION	DESCRIPTION	XRPD RESULT
Unmicronized	White powder	A
micronized	–	A

Table 2 – Approximate Solubilities of CDDO methyl ester

SOLVENT	SOLUBILITY (mg/mL)[a]
Acetone	70
ACN (acetonitrile)	66
DCM (dichloromethane)	> 194
1,4-Dioxane	21
EtOH (ethanol)	4
EtOAc (ethyl acetate)	36
Hexanes	< 1
(10:1) Isopropanol/water	4
MeOH (methanol)	8
MTBE (tert-butyl methyl ether)	4
THF (tetrahydrofuran)	97
Toluene	38
Water	< 0.1

[a] Solubilities are calculated based on the total solvent used to give a solution; actual solubilities may be greater because of the volume of the solvent portions utilized or a slow rate of dissolution. Solubilities are rounded to the nearest mg/mL.

Table 3 – Crystallization Experiments on CDDO methyl ester

SOLVENT	CONDITIONS[a]	HABIT/DESCRIPTION	XRPD RESULT
Acetone	FE	White chunks	A
	SE	White broken glass	Form B
(3:10) Acetone/hexanes	SC	No solid	–
ACN	FE	White broken glass	A + Form B
	SE	White chunks	A
(7:5) ACN/water	SC	Small white blades	A
DCM	Rotovap	White broken glass	Form B
	FE	Broken glass	Form B
	SE	White broken glass	Form B
(1:5) DCM/hexanes	SC	No solid	–
1,4-Dioxane	FD	Fluffy white solid	Form B
EtOH	FE	White chunks	A
	SE	White irregulars	A
	SC	Small white tablets	A
EtOAc	FE	White broken glass	Form B
	SE	White broken glass	Form B
	SC	No solid	–
(10:1) IPA/water	SC	Small white tablets	A
MeOH	FE	White chunks	A
	SE	White tablets, irregulars	A
	SC	Pyramidal tablets	A[b]
MTBE	FE	White broken glass	Form B
	SE	White broken glass	Form B
	SC	No solid	
THF	FE	White broken glass	Form B
	SE	White broken glass	Form B
(1:2) THF/hexanes	SC	No solid	–
Toluene	FE	White chunks	Form B
	SE	White broken glass	Form B
	SC	No solid	–
–	Melt/quench	Clear broken glass	Form B

[a] FE = fast evaporation, SE = slow evaporation, SC = slow cool, temperatures are approximate, FD = freeze dry, rotovap = rotary evaporation
[b] single crystal structure determination

Table 4 – Slurry Experiments of CDDO methyl ester

SOLVENT	TIME (DAYS)	HABIT/DESCRIPTION	XRPD RESULT
(10:1) IPA/water	7	White bits	A
MTBE	10	White chunks	A
MeOH	10	White chunks	A

Table 5 – Cold Precipitation Experiments of CDDO methyl ester

SOLVENT	ANTISOLVENT	HABIT/DESCRIPTION	XRPD RESULT
Acetone	Hexanes	No solid	–
	Water	White solid	A
DCM	Hexanes	No solid	–
THF	Water	White solid	A

Table 6 – Characterization Data of Form A

ANALYTICAL TECHNIQUE	RESULTS[a]
XRPD	A
DSC	Baseline shift 157°C, endotherm 224°C
TGA	0.3% weight loss up to 150°C 1.2% weight loss from 150 to 210°C
KF	0.38%
Hot Stage	28.9: begin of heating 150.1: - 176.4: - 200.2: no changes 225.9: melting 227.9: melt point
MB	Negligible weight change throughout experiment
Post MB XRPD	A
SEM[b]	Pyramidals, tablets, and plates
NMR ($CDCl_3$)	Consistent with structure

[a] all temperatures are in°C, endo = endotherm
[b] results based on images taken

Table 7 – Stress Studies of Form A

CONDITIONS[a]	HABIT/ DESCRIPTION	XRPD RESULT
25°C/60% RH 3 days: 1.5% loss 7 days: 0.6% loss	White powder	A
40°C/75% RH 3 days: 1.4% loss 7 days: 0.2% gain	White powder	A
195°C[b] 15 minutes: 2% loss	White powder	A
20 min grind, dry	White powder	A
20 min wet grind, water	White powder	A

Table 8 – Characterization Data of Form B CDDO methyl ester

ANALYTICAL TECHNIQUE	RESULTS[a]
XRPD	Form B
MDSC	Tg ~125°C
NMR ($CDCl_3$)	Consistent with structure

Table 9 – Stress Studies of Form B CDDO methyl ester

CONDITIONS[a]	HABIT/DESCRIPTION	XRPD RESULT[c]
22°C/97% RH 7 days	Broken glass	Form B
40°C/75% RH 8 days	Broken glass	Form B
80°C/0% RH ~24 hours	White broken glass, clear rounded chunks	Form B
195°C[b] 5 minutes	White fines	Form B
200°C[b] 60 minutes	White birefringent bits	A + Form B (min)

[a] RH = relative humidity, Tg = glass transition temperature
[b] Ambient lab humidity was measured as 74% RH
[c] min = minor

Table 10 – Crystal Data and Data Collection Parameters for Form A

formula	$C_{32}H_{43}NO_4$
formula weight	505.70
space group	*P* 43 21 2 (No. 96)
a, Å	14.21620(10)
c, Å	81.5875(12)
V, Å^3	16488.9(3)
Z	24
d_{calc}, g cm^{-3}	1.222
crystal dimensions, mm	0.01 x 0.01 x 0.00[b]
temperature, K	150.
radiation (wavelength, Å)	Mo K_α (0.71073)
monochromator	graphite
linear abs coef, mm^{-1}	0.074
absorption correction applied	empirical[a]
transmission factors: min, max	0.9995, 0.9999
diffractometer	Nonius KappaCCD
h, *k*, *l* range	-15 to 15 -10 to 10 -86 to 72
2✓ range, deg	4.08-44.43
mosaicity, deg	0.32
programs used	SHELXTL
F_{000}	6576.0
weighting $1/[\sigma^2(F_o^2)+(0.0176P)^2+0.0000P]$ where $P=(F_o^2+2F_c^2)/3$	
data collected	46742
unique data	9168
R_{int}	0.093
data used in refinement	9168
cutoff used in *R*-factor calculations	$F_o^2>2.0\sigma(F_o^2)$
data with $I>2.0\sigma(I)$	5421
number of variables	1024
largest shift/esd in final cycle	0.00
$R(F_o)$	0.051
$R_w(F_o^2)$	0.085
goodness of fit	1.045

[a] Otwinowski Z. & Minor, W. *Methods Enzymol.*, **1997**, *276*, 307.

[b] The crystal dimensions are approximate

Table 11 – Summary of the area under the plasma concentration (AUC) versus time in all animals studied (n=8)

CDDO METHYL ESTER FORMULATION STUDY - PARENT COMPOUND

Monkey subject #	Form A AUC (ng/ml*hr)	Form B AUC (ng/ml*hr)	% Form B over Form A
501M	37.5	203.4	542
502M	28.3	204.7	723
503F	21.3	107.3	504
504F	N/A	175	N/A
505M	60.4	158.3	262
506M	N/A	N/A	N/A
507F	57.3	164	286
508F	37.3	228	611
Mean	**40.4**	**177.2**	**439**
Median	**37.4**	**175.0**	**523**
Range (min-max)	**21 - 60.4**	**158 - 228**	**262 - 723%**

N/A - not determined.

Table 12 – Crystallization Experiments of CDDO-methyl ester.

SOLVENT	CONDITIONS[a]	DESCRIPTION	XRPD RESULT
10:1 Benzene/acetone	RE, room temperature	White solids	X-ray amorphous
10:1 Benzene/acetone	FE followed by drying at 60°C	Clear gel	–
10:1 Benzene/acetone	FE w/capillaries	Clear film	–
10:1 Benzene/acetone	FE, capped and put in a freezer over night, scratched the vial using a metal spatula and left to evaporate at room temperature	White solid	Benzene solvate
10:1 Benzene/acetone	FE, capped and sitting at room temperature over night, scratched the vial using a metal spatula and left to evaporate at room temperature	White solid	Benzene solvate
10:1 Benzene/acetone	FE w/ sonication	White solids	Benzene solvate

[a] RE = rotary evaporation, FE = fast evaporation

Table 13 – Characterization of CDDO-methyl ester Hemibenzene Solvate

ANALYTICAL TECHNIQUE	RESULTS
XRPD	Hemibenzene solvate
DSC, 10°C/min	Endotherm 133°C Endotherm 223°C (onset 221°C)
TG, 10°C/min	7.0% weight loss up to 160°C Corresponds to 0.5 moles of benzene
^{1}H NMR ($CDCl_3$)	Consistent with structure ~0.5 moles of benzene evident

Table 14 – Characterization of CDDO-methyl ester Dimethanol Solvate

ANALYTICAL TECHNIQUE	RESULTS
XRPD	Dimethanol solvate
DSC, 10°C/min	Endotherm 102°C Baseline shift at 130°C Broad exotherm 203°C Endotherm 225°C (onset: 223°C)
TG, 10°C/min	11% weight loss up to 110°C Corresponds to ~2.0 moles of methanol
TGIR, 20°C/min	9.8% weight loss up to 110°C Corresponds to ~1.7 moles of methanol Volatile component identified as methanol
Post TGIR XRPD	X-ray amorphous
IR	Consistent with structure
^{1}H NMR ($CDCl_3$)	Consistent with structure ~1.7 moles of methanol evident

Table 15 – Stress Studies of Micronized Form B CDDO-methyl ester

	FORM B PRODUCED VIA EXAMPLE 11 PROCEDURE		FORM B PRODUCED BY ISOLATION FROM ETHYL ACETATE AND ETHANOL MIXTURES	
Stress Conditions[a]	**XRPD Result**	**KF Result**	**XRPD Result**	**KF Result**
80°C/P_2O_5, 14d	Form B	NP	NP	NP
80°C/P_2O_5, 28 d	Form B	1.10	100% Form A	0.37
60°C/23% RH, 28d	Form B	0.04	~ 50% Form A + Form B	0.02
40°C/48% RH, 28d	Form B	0.00[b]	Form B	0.39
25°C/75% RH, 14d	Form B	NP	NP	NP
25°C/75% RH, 28d	Form B	0.77	Form B	0.58

[a] RH = relative humidity
[b] Actual value was negative

Table 16 – CDDO-Me Single Agent Anticancer Activity

MODEL	AGENT/ROUTE	DRUG ACTIVITY	COMMENTS
NCI-H460 Lung Cancer Xenograft (nude rat)	CDDO-Me, p.o.	78% TGI[1]	Dose-dependent effects. CDDO-Me as effective as radiation
MCF-7/Her-2 Breast Cancer Xenograft (nude mouse)	CDDO-Me, i.v.	15% regression	Superior results achieved in tumors that over-express Her-2 [a]
4T1 Syngeneic Breast Cancer Metastasis Model (mouse) – Early Treatment	CDDO-Me, i.v.	100% suppression	All animals remained tumor-free at sacrifice on Day 85 [b]
4T1 Syngeneic Breast Cancer Metastasis Model (mouse) – Delayed Treatment	CDDO-Me, i.v.	67% suppression	33% of animals tumor-free at sacrifice on Day 95 [2]
L3.6PL Pancreatic Cancer Xenograft (nude mouse)	CDDO-Me, i.v.	51% TGI	CDDO-Me outperformed Gemcitabine
DU-145 Prostate Cancer Xenograft (nude rat)	CDDO-Me, p.o.	77% TGI	Multiple schedules tested – TGI 60-77%

[1]TGI=Tumor Growth Inhibition
Data presented is from doses at or below the MTD (defined as ≤10% mortality and ≤20% weight loss).

Table 20 – Solutions prepared for spray drying

SOLID RATIO	40% Form B, 60% Polymer	60% Form B, 40% Polymer
POLYMER	Methacrylic acid copolymer Type C	HPMCP HP-55
SOLVENT	Acetone	Acetone
CONC. % W/W	10	10
TEMPERATURE°C	-20	-20
OBSERVATIONS	Solution showed some haziness after the charge of the polymer. A sample of the solution was heated up to 35/40°C, but the haziness didn't disappear and some flocculation was observed..	The solution was completely clear after the charge of the polymer. The two runs done used the solution in the same day that it was prepared.

[a] Konopleva, *et al.*, "Synthetic triterpenoid 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid induces growth arrest in HER2-overexpressing breast cancer cells," *Mol Cancer Ther.* 2006, 5: 317-28.

[b] Ling, *et al.*, "The novel triterpenoid C-28 methyl ester of 2-cyano-3, 12-dioxoolen-1, 9-dien-28-oic acid inhibits metastatic murine breast tumor growth through inactivation of STAT3 signaling," *Cancer Res.* 2007, 67: 4210-8.

Table 21 – Characteristics of tested formulations

Polymer	Methacrylic acid copolymer, Type C	HPMCP HP-55
Tests		
Description	White solid	White solid
X-Ray Powder Diffraction	No crystalline peak detected	No crystalline peak detected
Water	1.50% w/w	–
DSC - Tg (inflection point)	106°C	118°C
Assay (by HPLC)	409% w/w (calculated on the anhydrous and solvent free basis)	59.4% w/w (calculated on the anhydrous and solvent free basis)
Total of impurities (HPLC)ti	1.14% area	0.42% area
Particle size (D50)	21 pm	
Bulk density	0.18g/cm^3 (calculated with 4g of product)	0.19 g/cm^3 (calculated with 4g of product)

Table 22 – Mean Concentrations of CDDO-Me in Plasma of Cynomolgus Monkeys in Phase 2 and 3 Samples

Time	Mean concentrations of CDDO-methyl ester (ng/ mL)																	
	PO Form A (nanocrystalline)			**PO Form B (micronized)**			**PO Form A (crystalline micronized)**			**PO Form B (PVP/VA)**			**PO Form B (HPMCP)**			**PO Form B (Eudragit L100-55)**		
Pre-dose	BLQ			BLQ			BLQ			BLQ			BLQ			BLQ		
5 min	NST			NST			NST			NST			NST			NST		
10 min	NST			NST			NST			NST			NST			NST		
15 min	NST			NST			NST			NST			NST			NST		
30 min	NST			NST			NST			NST			NST			NST		
1 h	***1.53***	±	***1.49***	***6.01***	±	***5.67***	***0.619***	±	***0.207***	9.45	±	3.64	***9.94***	±	***4.83***	9.06	±	4.38
2 h	3.00	±	2.02	14.5	±	9.63	***0.505***	±	***0.260***	14.8	±	3.04	24.1	±	13.2	22.2	±	11.7
4 h	3.52	±	1.30	32.0	±	11.6	***1.15***	±	***0.73***	27.3	±	3.79	50.7	±	10.0	50.6	±	19.3
8 h	8.56	±	2.32	33.3	±	8.44	4.31	±	1.35	30.4	±	10.8	40.0	±	15.6	51.1	±	8.72
16 h	7.90	±	2.26	14.2	±	5.68	5.39	±	1.12	13.3	±	3.92	15.4	±	7.41	25.6	±	13.9
24 h	4.40	±	2.33	5.23	±	1.86	3.05	±	1.42	6.39	±	2.02	5.79	±	2.95	7.47	±	4.10
36 h	NST			NST			NST			NST			NST			NST		
48 h	***0.649***	±	***0.342***	***0.576***	±	***0.175***	***0.419***	±	***0.291***	***1.78***	±	***1.61***	***0.983***	±	***0.343***	***1.37***	±	***0.626***
72 h	***0.312***	±	***0.390***	***0.408***	±	***0.368***	***0.0169***	±	***0.0252***	***0.395***	±	***0.319***	***0.448***	±	***0.283***	***0.344***	±	***0.461***

NST No sample taken.
BLQ Below limit of quantification (1.00 ng/mL).
IV Intravenous administration.
PO Oral administration by capsule.

Values in bold italics calculated using all values presented in Appendix 3, consequently the mean may differ from means calculated where values that are BLQ are assumed to be zero.

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WHAT IS CLAIMED IS:

1. Anhydrous, crystalline methyl 2-cyano-3,12-dioxoleana-1,9(11)-dien-28-oate (CDDO-methyl ester) characterized in that its X-ray powder diffractogram comprises peaks at 8.78° 2θ, 12.94° 2θ, 13.35° 2θ, 14.18° 2θ, and 17.4° 2θ±0.2° 2θ based upon data collected on a diffractometer with Cu K_α radiation.

2. The CDDO-methyl ester according to claim 1, wherein the X-ray powder diffractogram is substantially as shown in FIG. 18.

3. The CDDO-methyl ester according to claim 1, further characterized in that its differential scanning calorimetry curve comprises a baseline shift at approximately 157° C. and an endotherm maximum at about 224° C.

4. The CDDO-methyl ester according to claim 3, wherein the differential scanning calorimetry curve is substantially as shown in FIG. 3.

5. A pharmaceutical composition comprising a therapeutically effective amount of the CDDO-methyl ester according to claim 1 and one or more solid pharmaceutically acceptable carriers.

6. The pharmaceutical composition according to claim 5, wherein the CDDO-methyl ester is micronized and the solid pharmaceutically acceptable carriers are edible carriers.

7. The CDDO-methyl ester according to claim 1 for use in treating a chronic inflammatory condition in a subject suffering therefrom.

8. Use of CDDO-methyl ester according to claim 1 in the manufacture of a medicament for treating a chronic inflammatory condition in a subject suffering therefrom.

9. Crystalline CDDO-methyl ester hemibenzenate, characterized in that its X-ray powder diffractogram comprises peaks at 9.25° 2θ, 14.17° 2θ, , 14.62° 2θ, 16.32°2θ, and 17.11° 2θ±0.2° 2θ based upon data collected on a diffractometer with Cu K_α radiation.

10. The crystalline CDDO-methyl ester hemibenzenate according to claim 9, further characterized in that it has a differential scanning calorimetry curve as substantially shown in FIG. 23.

NOVEL FORMS OF CDDO METHYL ESTER

ABSTRACT

A triterpenoid compound, methyl 2-cyano-3,12-dioxoIeana- 1,9(1 l)-dien-28-oate (CDDO methyl ester), has a non-crystalline, glassy solid form and a non-hydrous crystalline form that can prepared, for example, from a saturated methanol solution. The glassy form displays an enhanced bioavailability over the non-hydrous crystalline form. Each form of CDDO methyl ester is a superior candidate for use, typically in solid dosage form, for treating a variety of disease states, generally associated with inflammation.

[Figure 1]