Title: CAFFEIC ACID DERIVATIVES AND USES THEREOF

FIGURE 4

(57) Abstract: The present invention relates to compounds of formula (I): including any stereochemically isomeric form thereof, or pharmaceutically acceptable salts thereof, for the treatment of tuberculosis.
CAFFEIC ACID DERIVATIVES AND USES THEREOF

FIELD OF THE INVENTION

[0001] The invention relates generally to derivatives of caffeic acid useful for the treatment of cancers and for reducing resistance to proteasome inhibitors during cancer treatment. All documents cited to or relied upon below are expressly incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with Government support under P20GM103542, UL1TR001450 and NIH/NCI 1R41CA213488-01. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Proteasome inhibitors (Pis) such as, for example, bortezomib, are cornerstone agents in the treatment of Multiple Myeloma (MM). Despite the initial effectiveness of Pis, resistance is inescapable and represents a significant obstacle to sustained and durable responses in patients. A need exists in the art for new, targeted strategies that restore PI sensitivity to refractory MM cells.

SUMMARY OF THE INVENTION

[0004] The present invention is directed to a compound of formula (I):
wherein:

\( X \) is \(-0-, -(NH)n-, -NH-SO2-\) or \(-CH-\);

\( R_i \) is \( H \) or \( OH \);

\( R_2 \) and \( R_3 \), independently of each other, are \( H \), \( OH \), alkoxy, halogen, \( NH_2 \) or lower alkyl;

\( R_4 \) is \( H \) or \( OH \);

\( R_5 \) is \( H \), \( OH \) or halogen;

\( R_6 \) is \((C_5-C_6)\)alkyl, unbranched or branched, optionally substituted with \( R_7 \);

\( R_7 \) is \(-N_3\), \(-C≡CH\), phenyl or \( OH \);

\( R_8 \) is \( H \) or lower alkyl; and

\( n \) is 1 or 2,

or a pharmaceutically acceptable salt thereof.

[0005] The present invention is also directed to a pharmaceutical composition, comprising a therapeutically effective amount of a compound according to formula I, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.
The present invention is further directed to a method for the treatment of cancer, such as multiple myeloma, and to reducing resistance to Pis, comprising the step of administering to a patient in need thereof a therapeutically effective amount of a compound according to formula 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

**BRIEF DESCRIPTION OF THE DRAWINGS**

**Figure 1** provides data showing PI re-sensitizing characteristics of compound E61. (A) Cell viability data are shown. The effects of E61 alone were insignificant, and thus any separation of the dose response curves is indicative of a superadditive/synergistic drug response. Data was represented using a log scale. (B) An isobologram is shown to further demonstrate synergy between E61 and Btz in PI resistant MM. 1S BzR cells. Cell viability data were collected following treatment with varying concentrations of both agents alone and in combination. The black line indicated the zero effect of the isobole (additive effect). A leftward shift of the curve is indicative of a synergistic drug interaction. (C) PI resistant (MM. 1S BzR) cells were co-treated with E61 (5 µM) and a dose range of the indicated PI for 24 hrs. The effect of combinations on cell viability were measured using a luminescence based assay system. EC50 95% confidence intervals are shown. Data represented using a log scale.

**Figure 2** provides data showing that compound E61 synergizes with Pis via a redox dependent mechanism. (A) PI resistant MM. 1S BzR cells were treated with E61 (5 µM) or vehicle (DMSO) for 2 hrs. ROS levels as measured by DCF fluorescence are shown. (B) PI resistant MM. 1S BzR cells were pretreated with E61 (5 µM) or DMSO then exposed to Btz (25 nM). ROS induction was measured over time by kinetic spectrofluorimetry. Data were normalized to time=0 to account for differences at baseline and to indicate relative levels of ROS induction. (C) PI resistant MM. 1S BzR cells were treated with E61 (5 µM) and Btz (25 nM), alone, in combination, and in the presence or absence of the ROS scavenger N-acetyl cysteine (NAC, 1 mM). Cell viability data are shown.

**Figure 3** provides data showing that compound E61 has anti-multiple myeloma activity in vivo. (A) NSG mice were injected with PI resistant MM. 1S BzR cells via the lateral
tail vein on day 0. On day 21, daily treatments with 50 mg/kg E61 (i.p.) were initiated. Animal survival data are shown for mice treated with vehicle (N=10) and E61 (N=15). (B) Between days 45 and 50, bone marrow from randomly selected mice was harvested and human MM cells were quantified using flow cytometric analysis of the plasma cell marker, CD138 (left panel), and the human HLA-ABC (right panel) antigens. Statistical significance was determined using a student's t-test (N=6). (C) The tolerability of E61 was assessed by monitoring mouse body weight. Body weight data are shown for 21 days of the experiment.

[00010] Figure 4 shows characterization data of certain E61 derivatives. (A) The stability of E61 (an ester) and E62-10 (an amide) were evaluated in vitro by incubating the drugs in 50% mouse plasma for the indicated time points. The amount of remaining compound was quantified by LC-MS. E62-10 was ~50X more stable as it is not a substrate for serum esterases. Likewise, E61-13-KET is predicted to be equally more stable than E61. (B) The single agent activity of E61 and derivatives was evaluated in MM.1S BzR cells. Cells were treated with the indicated dose range of drug over a 72 hour time period. Cell viability data are shown. (C) PI resistant MM.1S BzR cells were co-treated with E61 or derivatives and a dose range of Btz for 24 hrs. Cell viability data are shown. A leftward shift of the dose response curves is indicative of a superadditive/synergistic drug response. Data represented using a log scale. (D) The chemical structure of E61-13-KET is shown.

[00011] Figure 5 shows a comparison of proteasome inhibitor re-sensitizing activity of E61 and E64FlMe.

[00012] Figure 6 provides data showing superior in vitro pharmacology of E64FlMe compared to E61 and E64.

[00013] Figure 7 provides data showing superior pharmacokinetics of E64FlMe in mice compared to E61 and E64.
DETAILED DESCRIPTION OF THE INVENTION

[00014] It is to be understood that the descriptions of the present invention have been simplified to illustrate elements that are relevant for a clear understanding of the present invention, while eliminating, for the purpose of clarity, many other elements found in typical pharmaceutical compositions. Those of ordinary skill in the art will recognize that other elements and/or steps are desirable and/or required in implementing the present invention. However, because such elements and steps are well known in the art, and because they do not facilitate a better understanding of the present invention, a discussion of such elements and steps is not provided herein. The disclosure herein is directed to all such variations and modifications to such elements and methods known to those skilled in the art. Furthermore, the embodiments identified and illustrated herein are for exemplary purposes only, and are not meant to be exclusive or limited in their description of the present invention.

[00015] The inventors identified cellular reduction/oxidation (redox) regulation as a promising target/pathway in PI refractory MM. Using a high throughput drug screening (HTS) platform, the inventors identified a potent PI re-sensitizing drug, E61 (3,4-dihydroxycinnamic acid n-octyl ester), which restores PI sensitivity by enhancing Pi-induced ROS generation and oxidative damage. Compound E61 exhibits strong anti-MM activity in cell culture models and in mice with experimentally induced MM, where it significantly reduces MM tumor burden and prolongs animal survival. But E61 is metabolically unstable with a short half-life in blood due to the activity of serum esterases. Thus, the inventors synthesized more stable - and therefore more potent - derivatives of E61 and evaluated their preclinical efficacy and tolerability using a mouse model of MM.

[00016] Technical and scientific terms used herein have the meaning commonly understood by one of skill in the art to which the present invention pertains, unless otherwise defined. Reference is made herein to various methodologies and materials known to those of skill in the art. Standard reference works setting forth the general principles of pharmacology include Goodman and Gilman's *The Pharmacological Basis of Therapeutics*, 10th Ed., McGraw Hill
Companies Inc., New York (2001). Any suitable materials and/or methods known to those of skill can be utilized in carrying out the present invention. However, preferred materials and methods are described. Materials, reagents and the like to which reference are made in the following description and examples are obtainable from commercial sources, unless otherwise noted.

[00017] A compound according to the invention is inherently intended to comprise all stereochemically isomeric forms thereof. The term "stereochemically isomeric forms" as used hereinbefore or hereinafter defines all the possible stereoisomeric forms which the compounds of formula (I), and their N-oxides, pharmaceutically acceptable salts or physiologically functional derivatives may possess. Unless otherwise mentioned or indicated, the chemical designation of compounds denotes the mixture of all possible stereochemically isomeric forms. In particular, stereogenic centers may have the R- or S-configuration; substituents on bivalent cyclic (partially) saturated radicals may have either the cis- or trans-configuration. Compounds encompassing double bonds can have an E (entgegen) or Z (zusammen)-stereochemistry at said double bond. The terms cis, trans, R, S, E and Z are well known to a person skilled in the art.

[00018] Stereochemically isomeric forms of the compounds of formula (I) are obviously intended to be embraced within the scope of this invention. Of special interest are those compounds of formula (I) which are stereochemically pure.

[00019] Following CAS-nomenclature conventions, when two stereogenic centers of known absolute configuration are present in a molecule, an R or S descriptor is assigned (based on Cahn-Ingold-Prelog sequence rule) to the lowest-numbered chiral center, the reference center. The configuration of the second stereogenic center is indicated using relative descriptors [R*,R*] or [R*,S*], where R* is always specified as the reference center and [R*,R*] indicates centers with the same chirality and [R*,S*] indicates centers of unlike chirality. For example, if the lowest-numbered chiral center in the molecule has an S configuration and the second center is R, the stereo descriptor would be specified as s—[R*,S*]. If "a" and "β" are used: the position of the highest priority substituent on the asymmetric carbon atom in the ring system having the lowest
ring number, is arbitrarily always in the "a" position of the mean plane determined by the ring
system. The position of the highest priority substituent on the other asymmetric carbon atom in
the ring system relative to the position of the highest priority substituent on the reference atom is
denominated "a", if it is on the same side of the mean plane determined by the ring system, or
"β", if it is on the other side of the mean plane determined by the ring system.

[00020] When a specific stereoisomeric form is indicated, this means that said form is
substantially free, i.e. associated with less than 50%, preferably less than 20%, more preferably
less than 10%, even more preferably less than 5%, further preferably less than 2% and most
preferably less than 1% of the other isomer(s). Thus, when a compound of formula (I) is for
instance specified as (R,S), this means that the compound is substantially free of the (S,R)
isomer.

[00021] The compounds of formula (I) may be synthesized in the form of mixtures, in particular
racemic mixtures, of enantiomers which can be separated from one another following art-known
resolution procedures. The racemic compounds of formula (I) may be converted into the
Corresponding diastereomeric salt forms by reaction with a suitable chiral acid. Said
diastereomeric salt forms are subsequently separated, for example, by selective or fractional
crystallization and the enantiomers are liberated therefrom by alkali. An alternative manner of
separating the enantiomeric forms of the compounds of formula (I) involves liquid
chromatography using a chiral stationary phase. Said pure stereochemically isomeric forms may
also be derived from the corresponding pure stereochemically isomeric forms of the appropriate
starting materials, provided that the reaction occurs stereospecifically. Preferably if a specific
steroisomer is desired, said compound will be synthesized by stereospecific methods of
preparation. These methods will advantageously employ enantiomerically pure starting
materials.

[00022] The tautomeric forms of the compounds of formula (I) are meant to comprise those
compounds of formula (I) wherein e.g. an enol group is converted into a keto group (keto-enol
tautomerism). Tautomeric forms of the compounds of formula (I) or of intermediates of the present invention are intended to be embraced by the ambit of this invention.

[00023] The term "alkyl" as used herein denotes an unbranched or branched chain, saturated, monovalent hydrocarbon residue containing 1 to 20 carbon atoms. In one embodiment, the number of carbon atoms in the alkyl chain can be 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 carbon atoms. In another embodiment, the number of carbon atoms in the alkyl chain can be from 5 to 16 and referred to as "(C5 -Ci6)alkyl." The term "lower alkyl" denotes a straight or branched chain hydrocarbon residue containing 1 to 6 carbon atoms. "Ci-20 alkyl" as used herein refers to an alkyl composed of 1 to 20 carbons. Examples of alkyl groups include, but are not limited to, methyl, ethyl, propyl, /-propyl, «-butyl, /-butyl, t-buty1, penty1, isopentyl, neopentyl, hexyl, hepty1, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl and hexadecyl.

[00024] When the term "alkyl" is used as a suffix following another term, as in "phenylalkyl," or "hydroxyalkyl," this is intended to refer to an alkyl group, as defined above, being substituted with one to two substituents selected from the other specifically-named group. Thus, for example, "phenylalkyl" denotes the radical R'R", wherein R' is a phenyl radical, and R" is an alkylene radical as defined herein with the understanding that the attachment point of the phenylalkyl moiety will be on the alkylene radical. Examples of arylalkyl radicals include, but are not limited to, benzyl, phenylethyl, 3-phenylpropyl. The terms "arylalkyl" or "aralkyl" are interpreted similarly except R is an aryl radical. The terms "(het)arylalkyl" or "(het)aralkyl" are interpreted similarly except R is optionally an aryl or a heteroaryl radical.

[00025] The term "alkoxy" as used herein means an -O-alkyl group, wherein alkyl is as defined above such as methoxy, ethoxy, «-propyloxy, /-propyloxy, «-butyloxy, /-butyloxy, t-butyloxy, pentyloxy, hexyloxy, including their isomers. "Lower alkoxy" as used herein denotes an alkoxy group with a "lower alkyl" group as previously defined. "Ci-10 alkoxy" as used herein refers to an-O-alkyl wherein alkyl is Ci-10.
The term "halogen" means a fluorine, chlorine, bromine or iodine radical. In one embodiment, the halogen is fluorine.

A "patient" is a mammal, e.g., a human, mouse, rat, guinea pig, dog, cat, horse, cow, pig, or non-human primate, such as a monkey, chimpanzee, baboon or rhesus monkey, and the terms "patient" and "subject" are used interchangeably herein.

The term "carrier", as used in this disclosure, encompasses carriers, excipients, and diluents and means a material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a pharmaceutical agent from one organ, or portion of the body, to another organ, or portion of the body.

The term "treating", with regard to a subject, refers to improving at least one symptom of the subject's disorder. Treating can be curing, improving, or at least partially ameliorating the disorder.

The term "disorder" is used in this disclosure to mean, and is used interchangeably with, the terms disease, condition, or illness, unless otherwise indicated.

The term "administer", "administering", or "administration" as used in this disclosure refers to either directly administering a compound or pharmaceutically acceptable salt of the compound or a composition to a subject, or administering a prodrug derivative or analog of the compound or pharmaceutically acceptable salt of the compound or composition to the subject, which can form an equivalent amount of active compound within the subject's body.

The term "optionally substituted," as used in this disclosure, means a suitable substituent can replace a hydrogen bound to a carbon, nitrogen, or oxygen. When a substituent is oxo (i.e., =O) then 2 hydrogens on the atom are replaced by a single O. In one embodiment, an alkyl or lower alkyl group can substituted with, for example, -N₃, -C≡CH, phenyl or OH. It will be understood by those skilled in the art, with respect to any group containing one or more
substituents, that such groups are not intended to introduce any substitution or substitution patterns that are sterically impractical, synthetically non-feasible and/or inherently unstable. Furthermore, combinations of substituents and/or variables within any of the Formulae represented herein are permissible only if such combinations result in stable compounds or useful synthetic intermediates wherein stable implies a reasonable pharmacologically relevant half-life at physiological conditions.

**Dosage and Administration:**

[00033] The compounds of the present invention may be formulated in a wide variety of oral administration dosage forms and carriers. Oral administration can be in the form of tablets, coated tablets, dragees, hard and soft gelatin capsules, solutions, emulsions, syrups, or suspensions. Compounds of the present invention are efficacious when administered by other routes of administration including continuous (intravenous drip) topical parenteral, intramuscular, intravenous, subcutaneous, transdermal (which may include a penetration enhancement agent), buccal, nasal, inhalation and suppository administration, among other routes of administration. The preferred manner of administration is generally oral using a convenient daily dosing regimen which can be adjusted according to the degree of affliction and the patient's response to the active ingredient.

[00034] A compound or compounds of the present invention, as well as their pharmaceutically useable salts, together with one or more conventional excipients, carriers, or diluents, may be placed into the form of pharmaceutical compositions and unit dosages. The pharmaceutical compositions and unit dosage forms may be comprised of conventional ingredients in conventional proportions, with or without additional active compounds or principles, and the unit dosage forms may contain any suitable effective amount of the active ingredient commensurate with the intended daily dosage range to be employed. The pharmaceutical compositions may be employed as solids, such as tablets or filled capsules, semisolids, powders, sustained release formulations, or liquids such as solutions, suspensions, emulsions, elixirs, or filled capsules for oral use; or in the form of suppositories for rectal or vaginal administration; or in the form of
sterile injectable solutions for parenteral use. A typical preparation will contain from about 5% to about 95% active compound or compounds (w/w). The term "preparation" or "dosage form" is intended to include both solid and liquid formulations of the active compound and one skilled in the art will appreciate that an active ingredient can exist in different preparations depending on the target organ or tissue and on the desired dose and pharmacokinetic parameters.

[00035] The term "excipient" as used herein refers to a compound that is useful in preparing a pharmaceutical composition, generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes excipients that are acceptable for veterinary use as well as human pharmaceutical use. The compounds of this invention can be administered alone but will generally be administered in admixture with one or more suitable pharmaceutical excipients, diluents or carriers selected with regard to the intended route of administration and standard pharmaceutical practice.

[00036] "Pharmaceutically acceptable" means that which is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and neither biologically nor otherwise undesirable and includes that which is acceptable for veterinary as well as human pharmaceutical use.

[00037] A "pharmaceutically acceptable salt" form of an active ingredient may also initially confer a desirable pharmacokinetic property on the active ingredient which were absent in the non-salt form, and may even positively affect the pharmacodynamics of the active ingredient with respect to its therapeutic activity in the body. The phrase "pharmaceutically acceptable salt" of a compound means a salt that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound. Such salts include: (1) acid addition salts, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid, methanesulfonic
acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzensulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic acid, glucoheptonic acid, 3-phenylpropionic acid, trimethylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, and the like; or (2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like.

[00038] Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier may be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. In powders, the carrier generally is a finely divided solid which is a mixture with the finely divided active component. In tablets, the active component generally is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired. Suitable carriers include but are not limited to magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. Solid form preparations may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

[00039] Liquid formulations also are suitable for oral administration include liquid formulation including emulsions, syrups, elixirs, aqueous solutions, aqueous suspensions. These include solid form preparations which are intended to be converted to liquid form preparations shortly before use. Emulsions may be prepared in solutions, for example, in aqueous propylene glycol solutions or may contain emulsifying agents such as lecithin, sorbitan monooleate, or acacia. Aqueous solutions can be prepared by dissolving the active component in water and adding
suitable colorants, flavors, stabilizing, and thickening agents. Aqueous suspensions can be prepared by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well-known suspending agents.

[00040] The compounds of the present invention may be formulated for parenteral administration (e.g., by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, for example solutions in aqueous polyethylene glycol. Examples of oily or nonaqueous carriers, diluents, solvents or vehicles include propylene glycol, polyethylene glycol, vegetable oils (e.g., olive oil), and injectable organic esters (e.g., ethyl oleate), and may contain formulatory agents such as preserving, wetting, emulsifying or suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution for constitution before use with a suitable vehicle, e.g., sterile, pyrogen-free water.

[00041] The compounds of the present invention may be formulated for topical administration to the epidermis as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also containing one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents. Formulations suitable for topical administration in the mouth include lozenges comprising active agents in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.
[00042] The compounds of the present invention may be formulated for administration as suppositories. A low melting wax, such as a mixture of fatty acid glycerides or cocoa butter is first melted and the active component is dispersed homogeneously, for example, by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and to solidify.

[00043] The compounds of the present invention may be formulated for vaginal administration. Pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

[00044] The compounds of the present invention may be formulated for nasal administration. The solutions or suspensions are applied directly to the nasal cavity by conventional means, for example, with a dropper, pipette or spray. The formulations may be provided in a single or multidose form. In the latter case of a dropper or pipette, this may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray, this may be achieved for example by means of a metering atomizing spray pump.

[00045] The compounds of the present invention may be formulated for aerosol administration, particularly to the respiratory tract and including intranasal administration. The compound will generally have a small particle size for example of the order of five (5) microns or less. Such a particle size may be obtained by means known in the art, for example by micronization. The active ingredient is provided in a pressurized pack with a suitable propellant such as a chlorofluorocarbon (CFC), for example, dichlorodifluoromethane, trichlorofluoromethane, or dichlorotetrafluoroethane, or carbon dioxide or other suitable gas. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by a metered valve. Alternatively the active ingredients may be provided in a form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidine (PVP). The powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit
dose form for example in capsules or cartridges of e.g., gelatin or blister packs from which the powder may be administered by means of an inhaler.

[00046] When desired, formulations can be prepared with enteric coatings adapted for sustained or controlled release administration of the active ingredient. For example, the compounds of the present invention can be formulated in transdermal or subcutaneous drug delivery devices. These delivery systems are advantageous when sustained release of the compound is necessary and when patient compliance with a treatment regimen is crucial. Compounds in transdermal delivery systems are frequently attached to a skin-adhesive solid support. The compound of interest can also be combined with a penetration enhancer, e.g., Azone (1-dodecylaza-cycloheptan-2-one). Sustained release delivery systems are inserted subcutaneously into the subdermal layer by surgery or injection. The subdermal implants encapsulate the compound in a lipid soluble membrane, e.g., silicone rubber, or a biodegradable polymer, e.g., polylactic acid.

[00047] Suitable formulations along with pharmaceutical carriers, diluents and excipients are described in Remington: The Science and Practice of Pharmacy 1995, edited by E. W. Martin, Mack Publishing Company, 19th edition, Easton, Pennsylvania. A skilled formulation scientist may modify the formulations within the teachings of the specification to provide numerous formulations for a particular route of administration without rendering the compositions of the present invention unstable or compromising their therapeutic activity.

[00048] The modification of the present compounds to render them more soluble in water or other vehicle, for example, may be easily accomplished by minor modifications (salt formulation, esterification, etc.), which are well within the ordinary skill in the art. It is also well within the ordinary skill of the art to modify the route of administration and dosage regimen of a particular compound in order to manage the pharmacokinetics of the present compounds for maximum beneficial effect in patients.

[00049] The term "therapeutically effective amount" as used herein means an amount required to reduce symptoms of the disease in an individual. The dose will be adjusted to the individual
requirements in each particular case. That dosage can vary within wide limits depending upon numerous factors such as the severity of the disease to be treated, the age and general health condition of the patient, other medicaments with which the patient is being treated, the route and form of administration and the preferences and experience of the medical practitioner involved. For oral administration, a daily dosage of between about 0.01 and about 1000 mg/kg body weight per day should be appropriate in monotherapy and/or in combination therapy. A preferred daily dosage is between about 0.1 and about 500 mg/kg body weight, more preferred 0.1 and about 100 mg/kg body weight, and most preferred 1.0 and about 15 mg/kg body weight per day. Thus, for administration to a 70 kg person, the dosage range in one embodiment would be about 70 mg to .7 g per day. The daily dosage can be administered as a single dosage or in divided dosages, typically between 1 and 5 dosages per day. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect for the individual patient is reached. One of ordinary skill in treating diseases described herein will be able, without undue experimentation and in reliance on personal knowledge, experience and the disclosures of this application, to ascertain a therapeutically effective amount of the compounds of the present invention for a given disease and patient.

[00050] The pharmaceutical preparations are preferably in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

**General Schemes**

[00051] Compounds of the present invention can be prepared beginning with commercially available starting materials and utilizing general synthetic techniques and procedures known to those skilled in the art. Chemicals may be purchased from companies such as for example
SigmaAldrich, Argonaut Technologies, VWR and Lancaster. Chromatography supplies and equipment may be purchased from such companies as for example AnaLogix, Inc, Burlington, Wis.; Biotage AB, Charlottesville, Va.; Analytical Sales and Services, Inc., Pompton Plains, N.J.; Teledyne Isco, Lincoln, Nebr.; VWR International, Bridgeport, N.J.; and Waters Corporation, Milford, MA. Biotage, ISCO and Analogix columns are pre-packed silica gel columns used in standard chromatography.

[00052] Examples of synthetic pathways useful for making compounds of the present invention are set forth in the Examples below and generalized in Synthesis Method schemes 1-4 below.

**Synthesis Method 1**

![Synthesis Method 1 Diagram]

[00053] To a solution of 25 mL dichloromethane, 0.555 mmol of caffeic acid (0.1 g) or the corresponding derivative can be dissolved at room temperature under argon. Next, 3 drops from a Pasteur pipette of dimethylformamide can be added. Last, 400 µL of oxalyl chloride (4.66 mmol) can be added dropwise to the reaction. This reaction can be allowed to proceed for 3 hours. After completion, the solution can be rotary evaporated and the resulting precipitate can be dissolved in 5 mL of dichloromethane under argon. Next, 2.75 mmol of the corresponding alcohol can be added in one portion with 1 drop of triethylamine from a Pasteur pipette. The reaction can be capped and allowed to proceed at room temperature overnight. The next day, the sample can be rotary evaporated to 1 mL, purified using straight-phase silica chromatography, and verified by LC-MS. R_i can be H or OH; R_2 and R_3, independently of each other, can be H, OH, alkoxy, halogen, NH_2 or lower alkyl; R_4 can be H or OH; R_5 can be H, OH or halogen; R_6 can be (C_5-C_6)alkyl, unbranced or branched, optionally substituted with R_7; and R_7 can be -N_3, -C≡CH, phenyl or OH.
Synthesis Method 2

[00054] To a solution of 5 mL tetrahydrofuran, 0.555 mmol of caffeic acid (0.1 g) or the corresponding derivative can be dissolved at room temperature. Next, 1.1 mmol of the corresponding alcohol can be added in one portion. Last, 20 drops from a Pasteur pipette of sulfuric acid can be added. The reaction can be heated to reflux (80°C) and allowed to proceed overnight. The next day, the sample can be rotary evaporated to 1 mL, purified using straight-phase silica chromatography, and verified by LC-MS. R1 can be H or OH; R2 and R3, independently of each other, can be H, OH, alkoxy, halogen, NH2 or lower alkyl; R4 can be H or OH; R5 can be H, OH or halogen; R6 can be (C5-Ci5)alkyl, unbranced or branched, optionally substituted with R7; and R7 can be -N3, -C≡CH, phenyl or OH.

Synthesis Method 3

[00055] To a solution of 5 mL ethyl acetate, 0.555 mmol of caffeic acid (0.1 g) or the corresponding derivative can be dissolved at room temperature. Next, 0.6105 mmol (0.278 g) of N-[(5-Chloro-3-oxido-lH-benzotriazol-l-yl)-4-morpholinylmethylen]-N-methylmethylamminium hexafluorophosphate (HDMC) can be added in one portion. Last, 1.1 1
mmol of the corresponding amine can be added. The reaction can be capped and allowed to proceed at room temperature overnight. The next day, the reaction can be washed with 10 mL of 1 M hydrochloric acid three times and brine once. The sample can be rotary evaporated to 1 mL, purified using straight-phase silica chromatography, and verified by LC-MS. R_i can be H or OH; R_2 and R_3, independently of each other, can be H, OH, alkoxy, halogen, NH_2 or lower alkyl; R_4 can be H or OH; R_4 can be H, OH or halogen; R_6 can be (C_5-C_6)alkyl, unbranched or branched, optionally substituted with R_7; and R_7 can be -N_3, -C≡CH, phenyl or OH.

**Synthesis Method 4**

[00056] To a solution of 50 mL ethyl acetate, 5.55 mmol of caffeic acid (1.0 g) can be dissolved at room temperature. Next, 11.1 mmol (0.678 g) N,O-dimethylhydroxylamine can be added in one portion. Last, 6.105 mmol (2.78 g) of N-{[(5-Chloro-3-oxido-1 H-benzotriazol-1-yl)-N-morpholinylmethylene] N-methylmethanaminium hexafluorophosphate (HDMC) can be added in one portion. The reaction can be capped and allowed to proceed at room temperature overnight. The next day, the reaction can be washed with 10 mL of 1 M hydrochloric acid three times and brine once. The solution can be then rotary evaporated and the resulting precipitate can be resuspended in 1 mL dimethylformamide along with 2.5 equivalents of tert-butyldimethylsilylchloride and 5 equivalents of imidazole. The reaction can be capped and allowed to react at 0°C overnight. The next day, the reaction can be washed with saturated sodium bicarbonate three times and brine once. The sample can be purified using straight-phase silica chromatography, and verified by LC-MS. The resulting compound can be lyophilized overnight. The next day, the dried sample can be dissolved in 10 mL tetrahydrofuran and 2 equivalents of nonyl magnesium bromide can be added dropwise at 0°C. The mixture can be allowed to react for 4 hours then quenched with ammonium chloride. Next, 3 equivalents of
tetra-<i>-butylammonium</i> fluoride can be added and the reaction can be stirred at 0°C 30 minutes. The reaction can be purified using straight-phase silica chromatography, and verified by LC-MS. \( R_i \) can be H or OH; \( R_2 \) and \( R_3 \), independently of each other, can be H, OH, alkoxy, halogen, NH\(_2\) or lower alkyl; \( R_4 \) can be H or OH; \( R_5 \) can be H, OH or halogen; \( R_6 \) can be (C\(_5\)-C\(_6\))alkyl, unbranched or branched, optionally substituted with \( R_7 \); and \( R_7 \) can be -N\(_3\), -C≡CH, phenyl or OH.

**EXAMPLES**

[00057] The following examples further describe and demonstrate particular embodiments within the scope of the present invention. Techniques and formulations generally are found in *Remington's Pharmaceutical Sciences* (Mack Publishing Co., Easton, Pa.). The disclosure is further illustrated by the following examples, which are not to be construed as limiting this disclosure in scope or spirit to the specific procedures herein described. It is to be understood that the examples are provided to illustrate certain embodiments and that no limitation to the scope of the disclosure is intended thereby. It is to be further understood that resort may be had to various other embodiments, modifications, and equivalents thereof which may suggest themselves to those skilled in the art without departing from the spirit of the present disclosure and/or scope of the appended claims.

**Examples 1-52**

[00058] Using the procedures in Synthesis Methods 1 to 4 above, the compounds shown in the table below were prepared:

<table>
<thead>
<tr>
<th>Example No.</th>
<th>Name</th>
<th>Structure</th>
<th>Exact Mass</th>
<th>Mass to Charge (Ion Mode)</th>
<th>Synthesis Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3,4-dihydroxycinnamic acid n-octyl ester</td>
<td><img src="image" alt="Structure" /></td>
<td>292.17</td>
<td>291.20 (-)</td>
<td>1</td>
</tr>
</tbody>
</table>

- 20 -
<table>
<thead>
<tr>
<th></th>
<th>Formula</th>
<th>Molecular Weight</th>
<th>Retention Time</th>
<th>Enantiomer</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3,4-dihydroxycinnamic acid 1-methyl-nonyl ester (E61-1)</td>
<td>306.18</td>
<td>306.92 (+)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3,4-dihydroxycinnamic acid 2-methyl-nonyl ester (E61-2)</td>
<td>306.18</td>
<td>307.00 (+)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3,4-dihydroxycinnamic acid n-hexyl ester (E61-6)</td>
<td>264.14</td>
<td>265.08 (+)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3,4-dihydroxycinnamic acid 7-methyl-nonyl ester (E61-7)</td>
<td>306.18</td>
<td>307.08 (+)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3,4-dihydroxycinnamic acid n-nonyl ester (E61-9)</td>
<td>306.18</td>
<td>307.08 (+)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3,4-dihydroxycinnamic acid n-decyl ester (E61-10)</td>
<td>320.20</td>
<td>321.08 (+)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3,4-dihydroxycinnamic acid n-undecyl ester (E61-11)</td>
<td>334.21</td>
<td>335.08 (+)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>3,4-dihydroxycinnamic acid n-dodecyl ester (E61-12)</td>
<td>348.23</td>
<td>349.17 (+)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3,4-dihydroxycinnamic acid n-tridecyl ester (E61-13)</td>
<td>362.25</td>
<td>363.17 (+)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>3,4-dihydroxycinnamic acid n-tetradecyl ester (E61-14)</td>
<td>376.26</td>
<td>377.17 (+)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Chemical Structure</td>
<td>Molecular Formula</td>
<td>Molecular Weight</td>
<td>CAS Number</td>
<td>Score</td>
</tr>
<tr>
<td>-----</td>
<td>--------------------</td>
<td>-------------------</td>
<td>------------------</td>
<td>------------</td>
<td>-------</td>
</tr>
<tr>
<td>12</td>
<td>3,4-dihydroxycinnamic acid 1-methyl-tridecyl ester (E61-12-1)</td>
<td>C30H45O3</td>
<td>362.25</td>
<td>361.42</td>
<td>2 (-)</td>
</tr>
<tr>
<td>13</td>
<td>3,4-dihydroxycinnamic acid 1-methyl-tetradecyl ester (E61-13-1)</td>
<td>C32H49O3</td>
<td>376.26</td>
<td>375.50</td>
<td>2 (-)</td>
</tr>
<tr>
<td>14</td>
<td>3,4-dihydroxycinnamic acid n-octyl amide (E62)</td>
<td>C23H35NO2</td>
<td>291.18</td>
<td>290.24</td>
<td>3 (-)</td>
</tr>
<tr>
<td>15</td>
<td>3,4-dihydroxycinnamic acid n-decyl amide (E62-10)</td>
<td>C25H37NO2</td>
<td>319.21</td>
<td>318.26</td>
<td>3 (-)</td>
</tr>
<tr>
<td>16</td>
<td>3,4-dihydroxycinnamic acid n-undecyl amide (E62-11)</td>
<td>C27H41NO2</td>
<td>333.23</td>
<td>332.30</td>
<td>3 (-)</td>
</tr>
<tr>
<td>17</td>
<td>3,4-dihydroxycinnamic acid n-dodecyl amide (E62-12)</td>
<td>C29H43NO2</td>
<td>347.25</td>
<td>346.34</td>
<td>3 (-)</td>
</tr>
<tr>
<td>18</td>
<td>3,4-dihydroxycinnamic acid n-tridecyl amide (E62-13)</td>
<td>C31H45NO2</td>
<td>361.26</td>
<td>360.34</td>
<td>3 (-)</td>
</tr>
<tr>
<td>19</td>
<td>3,4-dihydroxycinnamic acid n-tetradecyl amide (E62-14)</td>
<td>C33H47NO2</td>
<td>375.28</td>
<td>374.34</td>
<td>3 (-)</td>
</tr>
<tr>
<td>20</td>
<td>3,4-dihydroxycinnamic acid n-hexadecyl amide (E62-16)</td>
<td>C35H49NO2</td>
<td>403.31</td>
<td>402.38</td>
<td>3 (-)</td>
</tr>
<tr>
<td>21</td>
<td>3,4-dihydroxycinnamic acid dodecan-12-ol amide (E62-13-OH)</td>
<td>C35H49NO3</td>
<td>363.24</td>
<td>362.34</td>
<td>3 (-)</td>
</tr>
<tr>
<td></td>
<td>Compound Description</td>
<td>Molecular Structure</td>
<td>Molecular Weight</td>
<td>MS/MS m/z</td>
<td>Retention Time</td>
</tr>
<tr>
<td>---</td>
<td>-----------------------------------------------------------</td>
<td>---------------------</td>
<td>------------------</td>
<td>-----------</td>
<td>---------------</td>
</tr>
<tr>
<td>22</td>
<td>3,4-dihydroxy-dihydrocinnamic acid n-octyl ester (E63)</td>
<td><img src="image1" alt="Molecular Structure" /></td>
<td>294.18</td>
<td>295.00 (+)</td>
<td>1</td>
</tr>
<tr>
<td>23</td>
<td>Cinnamic acid n-octyl ester (E61-CIN)</td>
<td><img src="image2" alt="Molecular Structure" /></td>
<td>260.18</td>
<td>261.08 (+)</td>
<td>1</td>
</tr>
<tr>
<td>24</td>
<td>3,4-dimethoxycinnamic acid n-octyl ester (E61-DM)</td>
<td><img src="image3" alt="Molecular Structure" /></td>
<td>320.20</td>
<td>321.08 (+)</td>
<td>1</td>
</tr>
<tr>
<td>25</td>
<td>3,4-difluorocinnamic acid n-octyl ester (E61-DF)</td>
<td><img src="image4" alt="Molecular Structure" /></td>
<td>296.16</td>
<td>297.08 (+)</td>
<td>1</td>
</tr>
<tr>
<td>26</td>
<td>3,4-dichlorocinnamic acid n-octyl ester (E61-DCl)</td>
<td><img src="image5" alt="Molecular Structure" /></td>
<td>328.10</td>
<td>329.00 (+)</td>
<td>1</td>
</tr>
<tr>
<td>27</td>
<td>3-methoxy-4-hydroxycinnamic acid n-tridecyl ester (E61-3M-13)</td>
<td><img src="image6" alt="Molecular Structure" /></td>
<td>376.26</td>
<td>377.08 (+)</td>
<td>1</td>
</tr>
<tr>
<td>28</td>
<td>3-hydroxy-4-methoxycinnamic acid n-tridecyl ester (E61-4M-13)</td>
<td><img src="image7" alt="Molecular Structure" /></td>
<td>376.26</td>
<td>375.50 (-)</td>
<td>1</td>
</tr>
<tr>
<td>29</td>
<td>2-hydroxycinnamic acid n-tridecyl ester (E61-2OH-13)</td>
<td><img src="image8" alt="Molecular Structure" /></td>
<td>346.25</td>
<td>345.42 (-)</td>
<td>1</td>
</tr>
<tr>
<td>30</td>
<td>3-hydroxycinnamic acid n-tridecyl ester (E61-3OH-13)</td>
<td><img src="image9" alt="Molecular Structure" /></td>
<td>346.25</td>
<td>345.42 (-)</td>
<td>1</td>
</tr>
<tr>
<td>31</td>
<td>4-hydroxycinnamic acid n-tridecyl ester (E61-4OH-13)</td>
<td><img src="image10" alt="Molecular Structure" /></td>
<td>346.25</td>
<td>347.08 (+)</td>
<td>1</td>
</tr>
</tbody>
</table>
Using the procedures in Schemes 1 to 4 above, the compounds shown in the table below can be prepared:

<table>
<thead>
<tr>
<th></th>
<th>Chemical Structure</th>
<th>Molecular Mass</th>
<th>Log P</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>3,4-dimethylcinnamic acid n-tridecyl ester (E61-DMe-13)</td>
<td>358.29</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td>33</td>
<td>2,4-dihydroxycinnamic acid n-tridecyl ester (E61-2,4OH-13)</td>
<td>362.25</td>
<td>361.33 (-)</td>
<td>1</td>
</tr>
<tr>
<td>34</td>
<td>3,4-dihydroxycinnamic acid n-octynyl ester (E61-Y)</td>
<td>288.14</td>
<td>289.08 (+)</td>
<td>2</td>
</tr>
<tr>
<td>35</td>
<td>3,4-dihydroxycinnamic acid n-nonynyl ester (E61-9-Y)</td>
<td>302.15</td>
<td>303.08 (+)</td>
<td>2</td>
</tr>
<tr>
<td>36</td>
<td>3,4-dihydroxycinnamic acid n-decynyl ester (E61-10-Y)</td>
<td>316.17</td>
<td>317.17 (+)</td>
<td>2</td>
</tr>
<tr>
<td>37</td>
<td>3,4-dihydroxycinnamic acid n-unynyl ester (E61-11-Y)</td>
<td>330.18</td>
<td>331.17 (+)</td>
<td>2</td>
</tr>
<tr>
<td>38</td>
<td>3,4-dihydroxycinnamic acid azidopentyl ester (E61-A)</td>
<td>291.12</td>
<td>290.17 (-)</td>
<td>2</td>
</tr>
<tr>
<td>39</td>
<td>2,3,4-trihydroxycinnamic acid n-octyl ester (E61-2,3,4OH)</td>
<td>308.16</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td>40</td>
<td>2,3,4-trihydroxycinnamic acid n-decyl amide (E62-2,3,4OH-10)</td>
<td>335.21</td>
<td>NA</td>
<td>3</td>
</tr>
<tr>
<td>Example No.</td>
<td>Name</td>
<td>Structure</td>
<td>Exact Mass</td>
<td>Synthesis Method</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------------------------</td>
<td>-----------</td>
<td>------------</td>
<td>------------------</td>
</tr>
<tr>
<td>41</td>
<td>3,4-dihydroxycinnamic acid n-pentadecyl amide (E62-15)*</td>
<td><img src="image1" alt="Structure" /></td>
<td>389.29</td>
<td>3</td>
</tr>
<tr>
<td>42</td>
<td>3-hydroxy-4-aminocinnamic acid n-decyl amide (E62-4N-10)*</td>
<td><img src="image2" alt="Structure" /></td>
<td>318.23</td>
<td>2</td>
</tr>
<tr>
<td>43</td>
<td>3-fluoro-4-hydroxycinnamic acid n-decyl amide (E62-3F-10)*</td>
<td><img src="image3" alt="Structure" /></td>
<td>321.21</td>
<td>2</td>
</tr>
<tr>
<td>44</td>
<td>3-chloro-4-hydroxycinnamic acid n-decyl amide (E62-3Cl-10)*</td>
<td><img src="image4" alt="Structure" /></td>
<td>337.18</td>
<td>2</td>
</tr>
<tr>
<td>45</td>
<td>3-bromo-4-hydroxycinnamic acid n-decyl amide (E62-3Br-10)*</td>
<td><img src="image5" alt="Structure" /></td>
<td>381.13</td>
<td>2</td>
</tr>
<tr>
<td>46</td>
<td>2,5-dihydroxycinnamic acid n-decyl amide (E62-2,5-OH-10)*</td>
<td><img src="image6" alt="Structure" /></td>
<td>319.21</td>
<td>2</td>
</tr>
<tr>
<td>47</td>
<td>2,3,4-trihydroxycinnamic acid n-dodecyl amide (E62-2,3,4-OH-12)*</td>
<td><img src="image7" alt="Structure" /></td>
<td>363.24</td>
<td>2</td>
</tr>
<tr>
<td>48</td>
<td>3,4,5-trihydroxycinnamic acid n-dodecyl amide (E62-2,3,4-OH-12)*</td>
<td><img src="image8" alt="Structure" /></td>
<td>363.24</td>
<td>2</td>
</tr>
<tr>
<td>49</td>
<td>2,4,5-trihydroxycinnamic acid n-dodecyl amide (E62-2,4,5-OH-12)*</td>
<td><img src="image9" alt="Structure" /></td>
<td>363.24</td>
<td>2</td>
</tr>
</tbody>
</table>
Example 50

2-fluoro-4,5-dihydroxycinnamic acid n-decyl amide (E62-2F-OH-10)*

Structure

Exact Mass 337.21

Synthesis Method 2

Example 51

3,4-dihydroxycinnamic acid phenyldecyl ester (E61-10P)*

Structure

Exact Mass 396.23

Synthesis Method 1

Example 52

3,4-dihydroxycinnamic acid nonyl ketone (E64)*

Structure

Exact Mass 290.19

Synthesis Method 4

Example 53

Synthesis of (E)-2-(2-fluoro-4,5-dihydroxyphenyl)tridec-2-en-4-one (E64FlMe)

Synthesis:

1. To a solution of undecan-2-one (3.06 g, 18 mmol) in THF (60 mL) at -78 °C was added LDA (2M solution in THF, 10 mL,
20 mmol) slowly. The mixture was stirred at -78 °C for 45 mins. A solution of 1-(2-fluoro-4,5-dimethoxyphenyl) ethanone (1.8 g, 9.1 mmol) in THF (5 mL) was added slowly. After stirred at -78 °C for 4 hrs, 100 mL of saturated aqueous NH₄Cl was added. This mixture was extracted with diethyl ether (100 mL) twice. The combined ether layer was washed with brine (60 mL), dried over Na₂SO₄ and concentrated under vacuum. The residue was purified by column chromatography (ISCO, 0—10% ethyl acetate/hexanes) to afford title compound 2.3 g (69% yield). LC/MS: Rf = 6.65 min, purity > 95%, (M+H)+ = 368.15. H NMR (300 MHz, CD₃Cl) δ ppm 7.15 (d, J=6.16 Hz, 1 H) 6.51 (d, J=12.90 Hz, 1 H) 3.82 (d, J=15.24 Hz, 6 H) 3.38 (d, J=17.00 Hz, 1 H) 2.75 (d, J=17.00 Hz, 1 H) 2.17 - 2.41 (m, 2 H) 1.34 - 1.53 (m, 5 H) 1.19 (br. s., 12 H) 0.83 (br. s., 3 H)

![Chemical Structure](image)

(E)-2-(2-fluoro-4,5-dimethoxyphenyl)tridec-2-en-4-one (2) The mixture of 1 (1.0 g, 2.72 mmol), TsOH (52 mg, 0.27 mmol) in toluene (10 mL) was heated at 60 °C for 5 hrs. After reaction was done, the mixture was concentrated. The residue was purified by column chromatography (ISCO) eluting with 0—10% ethyl acetate/hexanes to give the title compound (650 mg, 68% yield). LC/MS: Rf = 7.38 min, (M+H)+ = 351.15. H NMR (300 MHz, CD₃Cl) δ ppm 6.69 (d, J=5.57 Hz, 1 H) 6.57 (d, J=12.02 Hz, 1 H) 6.32 (br. s., 1 H) 3.74 - 3.88 (m, 6 H) 2.37 - 2.53 (m, 5 H) 1.57 (br. s., 2 H) 1.20 (br. s., 12 H) 0.81 (br. s., 3 H)

![Chemical Structure](image)

(E)-2-(2-fluoro-4,5-dihydroxyphenyl)tridec-2-en-4-one (E64FlMe) To a solution of in 2 (650 mg, 1.86 mmol) in CH₂Cl₂ (25 mL) was added 5.6 mL IM BBr₃ solution in CH₂Cl₂ at -78°C
and stayed at -78 °C for 1 hr. The mixture was then warmed up to -20 °C for 7 hrs. TLC indicated there was a lot S.M. left. The reaction mixture was kept in refrigerator 18 hrs. 40 mL H₂O and 50 mL ether were added, followed by 10 mL saturated aqueous NaHC0₃. The organic layer was separated, dried, concentrated. The residue was purified by column chromatography (ISCO). LC-MS showed the product was not pure enough, so the product was purified again using ISCO to give the title compound (402 mg, 67% yield). LC/MS: Rf = 6.29 min, (M+H)+ = 323.17. H NMR (300 MHz, CD₃Cl) δ ppm 6.79 (d, J=7.04 Hz, 1H) 6.66 (d, J=1.43 Hz, 1H) 6.36 (s, 1H) 2.35 - 2.56 (m, 5H) 1.50 - 1.68 (m, 2H) 1.26 (t, J=7.48 Hz, 12H) 0.79 - 0.93 (m, 3H).

**Example 54**

**Efficacy and SAR Data of E61 and Representative Derivatives**

PI resistant MM. 1S BzR cells were co-treated with E61 or vehicle (DMSO) and a dose range of Btz for 24 hrs. Compound E61 (Fig. 1B), showed strong synergy with Btz, decreasing the EC₅₀ from 54 nM to 9.4nM (Fig. 1A-B), which was comparable to that of the PI sensitive parental cell line. E61 also synergistically enhanced the cytotoxicity of next generation Pis, carfilzomib, ixazomib, and oprozomib (Fig. 1C). The PI enhancing effects that were observed in MM cells were absent in normal cell types, including human peripheral blood mononuclear cells (PBMCs) and lymphocytes (data not shown). This suggested that E61 has selective activity for MM cells and will have a wide therapeutic index. E61 also showed strong single agent activity, killing PI sensitive and resistant MM cells after 72 hours of treatment with EC₅₀ values of 0.9 µM and 4.5 µM, respectively (Fig. 4B; data not shown).

Redox Dependent Mechanism of Action of E61: PI resistant MM. 1S BzR cells were treated with E61 (5 µM) or vehicle (DMSO) for 2 hrs. E61 had significant effects on ROS levels in both untreated and Pi-treated cells, inducing the production of ROS as a single agent and cooperating with Btz to stimulate a synergistic increase in ROS production (Fig. 2A-B). Likewise, E61 increased the oxidation of protein thiols by itself and synergistically enhanced the oxidative damage of proteins by Btz (data not shown). E61 induced the expression of Nrf2 (data not shown), a master regulator of anti-oxidant gene expression that is stabilized in response to
oxidative stress, further implicating a role for redox in the mechanism of E61. Finally, the addition of the ROS scavenger, \(N\)-acetyl cysteine (NAC), to E61 and Btz treatments nearly completely reversed the effects of the combination on cell death in resistant cells (Fig. 2C), demonstrating a causal role of oxidative stress in the PI re-sensitizing mechanism of E61. Taken together, these results show that the redox pathway is a target of E61 and is primarily responsible for the PI re-sensitizing activity of the drug.

[00062] Anti-MM Activity of E61 In Vivo: Efficacy and tolerability of E61 in a mouse model of MM were investigated. Briefly, MM in mice was established by injecting PI resistant MM. 1S BzR cells into the lateral tail vein of NSG mice (The Jackson Laboratory). This method of injection disseminated cells widely throughout the body of the mouse, allowing the cells to invade the bone marrow and form osteolytic MM lesions that resemble the human pathology of MM. Median survival for mice in this model is approximately 50 days, with mice developing fore and hind limb paralysis, and 100% (N=18) of mice presenting with detectable levels of MM cells in their bone marrow. Mice were treated with daily injections of E61 (50 mg/kg, i.p.) at day 21 post injection. Survival analysis was conducted, and in parallel, mice from each group were randomly selected between days 45 and 50 for quantification of MM cells in the bone marrow of mice. MM cells were quantified by flow cytometry using antibodies for human CD138, a plasma cell specific cell surface marker, and human HLA-ABC antigens. E61 demonstrated significant anti-MM activity, improving animal survival from 8% for vehicle treated mice to 76% for E61-treated mice on day 55 of the study (day 34 of treatment; \(P= 0.0007\); Fig. 3A). Likewise, E61 significantly reduced the number of CD138 and human HLA positive cells in bone marrow of mice treated with E61 (Fig. 3B), demonstrating that the improvement in survival was indeed due to an anti-MM effect. The average number of CD138 positive MM cells in vehicle treated mice was 46.4 ± 4.6% versus 20.9 ± 6.7% in E61 treated mice (\(P=0.007\), N=6), and the average number of human HLA positive MM cells in vehicle treated mice was 72.3 ± 7.3% versus 29.6 ± 9.9% in E61 treated mice (\(P=0.004\), N=6). Two E61 treated mice appeared to be cured, as no MM cells were detected. Chronic, daily treatment with E61 for >6 weeks was overtly non-toxic,
as mice did not lose any body weight or show signs of discomfort or distress during the course of treatment (Fig. 3C).

**[00063]** E61 Structure Activity Relationships (SAR): SAR studies were conducted to identify the chemical groups of E61 that were responsible for the PI re-sensitizing activity of the molecule. Structurally, E61 is characterized by a catechol group with an α,β-unsaturated carbonyl group (i.e., caffeic acid) connected by an ester linkage to an 8-carbon alkyl chain. It was apparent from these SAR studies that the 8-carbon chain of E61 was one important element to the PI re-sensitizing activity of the drug. Evidence for this is that caffeic acid lacks activity, and reducing the alkyl chain of E61 to less than 7 carbons minimized the activity of the compound (data not shown). By contrast, increasing the length of the chain maintained or slightly improved the activity of E61. For example, E61-13 (E61 with alkyl chain extended to 13 carbons) showed the most potent single agent anti-MM activity and synergy with PIs (Fig. 4B). The hydroxyl groups on the catechol are also another important feature, as methoxylating, removing, or replacing them with halogens completely abrogates the PI re-sensitizing activity of the molecule.

**[00064]** A limitation of E61 is the ester linkage, which is a metabolic clearance risk due to the activity of serum esterase enzymes. In fact, E61 has a short half-life in human liver microsomes and in mouse blood plasma (Fig. 4A), suggesting a very short half-life *in vivo*. Despite this pharmacological limitation, E61 exhibited strong anti-MM activity in an aggressive MM mouse model.

**[00065]** The stability of E61 (an ester) and E62-10 (an amide) were evaluated *in vitro* by incubating the drugs in 50% mouse plasma for the indicated time points. The amount of remaining compound was quantified by LC-MS. E61-13 (E61 with a 13 carbon chain as opposed to an 8-carbon chain) was an efficacious derivative as a single agent and in combination with PIs (Fig. 4A). A second derivative (E62-10), where the alkyl chain has been lengthened to 10 carbons and the ester bond has been converted to an amide bond, showed comparable activity to E61 in cellular models (structures shown in Fig. 4B). A major advantage of E62-10 was that it
is nearly 50 times more stable than E61 in blood plasma (Fig. 4C; E61 $t_{1/2} = 3.3$ minutes, E62-10 $t_{1/2} = 155.9$ minutes). Therefore, while the two molecules have similar potencies in cell culture models, E62-10 is predicted to be much more potent in vivo. E61-13 will be further modified to produce a more stable derivative that is a ketone rather than an ester.

**Example 55**

**Analysis of (E)-2-(2-fluoro-4,5-dihydroxyphenyl)tridec-2-en-4-one (E64FlMe)**

Proteasome inhibitor re-sensitizing activity of E64FlMe was compared to E61. Proteasome inhibitor resistant MM.1S BzR cells were treated with the indicated drug or vehicle control (DMSO) and a dose range of bortezomib (Btz) for 24 hours. In the panel on the left, cell viability data are shown, indicating the relative responsiveness to the cytotoxic effects of Btz. A concentration of 2.5 DM was used for E61 and E64FlMe. In the panel on the right in Figure 5, EC50 values for Btz are shown after extrapolation from the dose response curves shown to the left. The vehicle treated control cells showed an EC50 of 33.2 nM compared to 13.4 nM for E61 (a 2.5-fold increase in Btz sensitivity) and 3.1 for E64FlMe (a 10.7-fold increase in Btz sensitivity). Thus, E64FlMe superior to E61 in terms of proteasome inhibitor sensitizing activity and anti-myeloma activity.

Figure 6 shows superior in vitro pharmacology of E64FlMe compared to E61 and E64. At left, the stability of the indicated compounds in human liver microsomes is shown. The intrinsic clearance rates were calculated at 97.6, 36.2, and 16.9 µl/min/mg for E61, E64, and E64FlMe, respectively, demonstrating that E64FlMe is metabolized significantly slower by hepatic enzymes than both E61 and E64. The middle panel shows data from catechol-O-methyltransferase (COMT) activity assays. The indicated compounds were incubated with recombinant COMT and the metabolism of the parent compound to the O-methylated metabolite was measured over time by LC-MS. The rates of COMT activity against E61, E64, and E64FlMe were 0.93, 0.82, and 0.56 %/min, respectively, demonstrating that modifications made to generate E64FlMe interfere metabolism by COMT. Chemical structures for E61, E64, and E64FlMe are shown in the right panel for reference. This set of data demonstrates that chemical
modifications made to generate compound E64FlMe significantly improve the metabolic
stability of the molecule.

[00068] Figure 7 shows superior pharmacokinetics of E64FlMe in mice compared to E61
and E64. Pharmacokinetic analysis was conducted in CD-1 mice using E61, E64, and E64FlMe
at a dose of 20 mg/kg (single dose, i.p.). Blood plasma concentrations (in nM) across the
indicated time points for each of the drugs are shown at the left (N=3 per time point). On the
right, the maximum plasma concentration achieved (in ng/ml; C\text{max}) and the area under the curve
(AUC) across the entire experiment are shown. It is evident from these experiments that the
chemical modifications made to generate lead E64FlMe have produced a molecule that is more
potent and superior in terms of pharmaceutical properties.

[00069] The invention is further described in the following numbered paragraphs:

1. A compound of formula (I):

\[
\begin{array}{c}
\text{R}_1 \quad \text{R}_2 \\
\text{R}_3 \quad \text{R}_4 \\
\text{R}_5 \quad \text{R}_6 \\
\text{R}_7 \quad \text{R}_8
\end{array}
\]

(\text{I})

wherein:

X is \(-\text{O}-\), -(NH)n-, -NH-SO2- or -CH-;

R\text{i} is H or OH;

R\text{2} and R\text{3}, independently of each other, are H, OH, alkoxy, halogen, \(\text{NH}_2\) or lower alkyl;

R\text{4} is H or OH;
R₅ is H, OH or halogen;

R₆ is (C₅-C₆)alkyl, unbranced or branched, optionally substituted with R₇;

R₇ is -N₃, -C≡CH, phenyl or OH;

R₈ is H or lower alkyl; and

n is 1 or 2,

or a pharmaceutically acceptable salt thereof.

2. The compound according to paragraph 1, wherein X is -O-.  
3. The compound according to paragraph 1, wherein X is -NH-.  
4. The compound according to paragraph 1, wherein X is -CH-.  
5. The compound according to paragraph 1, wherein R₁ is H.  
6. The compound according to paragraph 1, wherein R₂ and R₃ are both OH.  
7. The compound according to paragraph 1, wherein R₂ and R₃, independently of each other, are H, OH, methoxy, fluorine, chlorine, bromine, NH₂ or methyl.  
8. The compound according to paragraph 1, wherein R₄ is OH.  
9. The compound according to paragraph 1, wherein R₅ is H.  
10. The compound according to paragraph 1, wherein R₆ is halogen.  
11. The compound according to paragraph 1, wherein R₇ is fluorine.  
12. The compound according to paragraph 1, wherein R₆ is unsubstituted (C₅-C₆)alkyl, unbranced or branched.
13. The compound according to paragraph 1, wherein R₆ is unsubstituted (C₅-C₁₀)alkyl, unbranced or branched.

14. The compound according to paragraph 1, wherein R₆ is unsubstituted (C₁₀-C₁₆)alkyl, unbranced or branched.

15. The compound according to paragraph 1, wherein R₆ is (C₅-C₁₆)alkyl, unbranced or branched, substituted with R₇.

16. The compound according to paragraph 1, wherein R₇ is -N₃ or -C≡CH.

17. The compound according to paragraph 1, wherein R₇ is phenyl or OH.

18. The compound according to paragraph 1, wherein R₈ is methyl.

19. The compound according to paragraph 1, having the formula II:

\[
\begin{array}{c}
\text{R₁} \\
\text{R₂} \\
\text{R₃} \\
\text{R₄} \\
\text{R₅} \\
\text{R₆}
\end{array}
\]

wherein:

R₁ is H or OH;

R₂ and R₃, independently of each other, are H, OH, alkoxy, halogen, NH₂ or lower alkyl;

R₄ is H or OH;

R₆ is (C₅-C₁₆)alkyl, unbranced or branched, optionally substituted with R₇; and
R₇ is -N₃, -C≡CH, phenyl or OH,

or a pharmaceutically acceptable salt thereof.

20. The compound according to paragraph 1, wherein said compound is:

3,4-dihydroxycinnamic acid n-octyl ester;
3,4-dihydroxycinnamic acid 1-methyl-nonyl ester;
3,4-dihydroxycinnamic acid 2-methyl-nonyl ester;
3,4-dihydroxycinnamic acid n-hexyl ester;
3,4-dihydroxycinnamic acid 7-methyl-nonyl ester;
3,4-dihydroxycinnamic acid n-nonyl ester;
3,4-dihydroxycinnamic acid n-decyl ester;
3,4-dihydroxycinnamic acid n-undecyl ester;
3,4-dihydroxycinnamic acid n-dodecyl ester;
3,4-dihydroxycinnamic acid n-tridecyl ester;
3,4-dihydroxycinnamic acid n-tetradecyl ester;
3,4-dihydroxycinnamic acid 1-methyl-tridecyl ester;
3,4-dihydroxycinnamic acid 1-methyl-tetradecyl ester;
3,4-dihydroxycinnamic acid n-octyl amide;
3,4-dihydroxycinnamic acid n-decyl amide;
3,4-dihydroxycinnamic acid n-undecyl amide;
3,4-dihydroxycinnamic acid n-dodecyl amide;
3,4-dihydroxycinnamic acid n-tridecyl amide;
3,4-dihydroxycinnamic acid n-tetradecyl amide;
3,4-dihydroxycinnamic acid n-hexadecyl amide;
3,4-dihydroxycinnamic acid dodecan-12-ol amide;
3,4-dihydroxy-dihydrocinnamic acid n-octyl ester;
cinnamic acid n-octyl ester;
3,4-dimethoxycinnamic acid n-octyl ester;
3,4-difluorocinnamic acid n-octyl ester;
3,4-dichlorocinnamic acid n-octyl ester;
3-methoxy-4-hydroxy cinnamic acid n-tridecyl ester;
3-hydroxy-4-methoxy cinnamic acid n-tridecyl ester;
2-hydroxycinnamic acid n-tridecyl ester;
3-hydroxycinnamic acid n-tridecyl ester;
4-hydroxycinnamic acid n-tridecyl ester;
3,4-dimethylcinnamic acid n-tridecyl ester;
2,4-dihydroxycinnamic acid n-tridecyl ester;
3,4-dihydroxycinnamic acid n-octynyl ester;
3,4-dihydroxycinnamic acid n-nonynyl ester;
3,4-dihydroxycinnamic acid n-decynyl ester;
3,4-dihydroxycinnamic acid n-unynyl ester;
3,4-dihydroxycinnamic acid azidopentyl ester;
2,3,4-trihydroxycinnamic acid n-octyl ester;
3-hydroxy-4-aminocinnamic acid n-decyl amide;
3-fluoro-4-hydroxy cinnamic acid n-decyl amide;
3-chloro-4-hydroxy cinnamic acid n-decyl amide;
3-bromo-4-hydroxy cinnamic acid n-decyl amide;
2,5-dihydroxycinnamic acid n-decyl amide;
2,3,4-trihydroxycinnamic acid n-dodecyl amide;
3,4,5-trihydroxycinnamic acid n-dodecyl amide;
2,4,5-trihydroxycinnamic acid n-dodecyl amide;
2-fluoro-4,5-dihydroxycinnamic acid n-decyl amide;
3,4-dihydroxycinnamic acid phenyldecyl ester;
3,4-dihydroxycinnamic acid phenyldecyl ester;
3,4-dihydroxycinnamic acid nonyl ketone; or
(E)-2-(2-fluoro-4,5-dihydroxyphenyl)tridec-2-en-4-one,
or a pharmaceutically acceptable salt thereof.

21. A pharmaceutical composition, comprising a therapeutically effective amount of a compound according to paragraph 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

22. A method for the treatment of cancer, comprising the step of administering to a patient in need thereof a therapeutically effective amount of a compound according to paragraph 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

23. The method according to paragraph 22, wherein said cancer is multiple myeloma.

24. A method for reducing resistance to proteasome inhibitors, comprising the step of administering to a patient in need thereof a therapeutically effective amount of a compound according to paragraph 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

25. A method for reducing resistance to proteasome inhibitors during treatment of multiple myeloma, comprising the step of administering to a patient in need thereof a therapeutically effective amount of a compound according to paragraph 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

* * *

It is to be understood that the invention is not limited to the particular embodiments of the invention described above, as variations of the particular embodiments may be made and still fall within the scope of the appended claims.
CLAIMS:

1. A compound of formula (I):

   \[
   \begin{array}{c}
   \text{R}_1 \quad \text{R}_2 \\
   \text{R}_3 \quad \text{R}_4 \\
   \text{R}_5 \quad \text{R}_6 \\
   \text{X} \quad \text{R}_7 \\
   \end{array}
   \]

   (I),

wherein:

   \( X \) is \(-\text{O}-\), -(NH)n-, \(-\text{NH-SO}_2\)- or \(-\text{CH}-\);

   \( \text{R}_1 \) is H or OH;

   \( \text{R}_2 \) and \( \text{R}_3 \), independently of each other, are H, OH, alkoxy, halogen, \( \text{NH}_2 \) or lower alkyl;

   \( \text{R}_4 \) is H or OH;

   \( \text{R}_5 \) is H, OH or halogen;

   \( \text{R}_6 \) is \( (\text{C}_5-\text{C}_6) \)alkyl, unbranced or branched, optionally substituted with \( \text{R}_7 \);

   \( \text{R}_7 \) is \(-\text{N}_3\), \(-\text{C}≡\text{CH}\), phenyl or OH;

   \( \text{R}_8 \) is H or lower alkyl; and

   \( n \) is 1 or 2,

   or a pharmaceutically acceptable salt thereof.
2. The compound according to claim 1, wherein X is -0-.

3. The compound according to claim 1, wherein X is -NH-.

4. The compound according to claim 1, wherein X is -CH-.

5. The compound according to claim 1, wherein R_i is H.

6. The compound according to claim 1, wherein R_2 and R_3 are both OH.

7. The compound according to claim 1, wherein R_2 and R_3, independently of each other, are H, OH, methoxy, fluorine, chlorine, bromine, NH_2 or methyl.

8. The compound according to claim 1, wherein R_4 is OH.

9. The compound according to claim 1, wherein R_5 is H.

10. The compound according to claim 1, wherein R_5 is halogen.

11. The compound according to claim 1, wherein R_5 is fluorine.

12. The compound according to claim 1, wherein R_i is unsubstituted (C_5-C_{16})alkyl, unbranched or branched.

13. The compound according to claim 1, wherein R_i is unsubstituted (C_5-C_{10})alkyl, unbranched or branched.

14. The compound according to claim 1, wherein R_i is unsubstituted (C_10-C_{16})alkyl, unbranched or branched.

15. The compound according to claim 1, wherein R_i is (C_5-C_{16})alkyl, unbranched or branched, substituted with R_7.

16. The compound according to claim 1, wherein R_7 is -N_3 or -C≡CH.
17. The compound according to claim 1, wherein \( R_7 \) is phenyl or OH.

18. The compound according to claim 1, wherein \( R_8 \) is methyl.

19. The compound according to claim 1, having the formula II:

\[
\begin{align*}
R_1 & \quad \text{or} \quad \text{OH}; \\
R_2 \text{ and } R_3, \text{ independently of each other, are } & \text{H, OH, alkoxy, halogen, NH}_2 \text{ or lower alkyl; } \\
R_4 & \quad \text{H or OH; } \\
R_6 & \text{is (C}_5\text{-Cl}_6\text{)alkyl, unbranched or branched, optionally substituted with } R_7; \text{ and } \\
R_7 & \text{is } -N_3, -C=\text{CH, phenyl or OH,} \\
& \text{or a pharmaceutically acceptable salt thereof.}
\end{align*}
\]

20. The compound according to claim 1, wherein said compound is:

- 3,4-dihydroxycinnamic acid n-octyl ester;
- 3,4-dihydroxycinnamic acid 1-methyl-nonyl ester;
3,4-dihydroxycinnamic acid 2-methyl-nonyl ester;
3,4-dihydroxycinnamic acid n-hexyl ester;
3,4-dihydroxycinnamic acid 7-methyl-nonyl ester;
3,4-dihydroxycinnamic acid n-nonyl ester;
3,4-dihydroxycinnamic acid n-decyl ester;
3,4-dihydroxycinnamic acid n-undecyl ester;
3,4-dihydroxycinnamic acid n-dodecyl ester;
3,4-dihydroxycinnamic acid n-tridecyl ester;
3,4-dihydroxycinnamic acid n-tetradecyl ester;
3,4-dihydroxycinnamic acid 1-methyl-tridecyl ester;
3,4-dihydroxycinnamic acid 1-methyl-tetradecyl ester;
3,4-dihydroxycinnamic acid n-octyl amide;
3,4-dihydroxycinnamic acid n-decyl amide;
3,4-dihydroxycinnamic acid n-undecyl amide;
3,4-dihydroxycinnamic acid n-dodecyl amide;
3,4-dihydroxycinnamic acid n-tridecyl amide;
3,4-dihydroxycinnamic acid n-tetradecyl amide;
3,4-dihydroxycinnamic acid n-hexadecyl amide;
3,4-dihydroxycinnamic acid dodecan-12-ol amide;
3,4-dihydroxy-dihydrocinnamic acid n-octyl ester;
cinnamic acid n-octyl ester;
3,4-dimethoxycinnamic acid n-octyl ester;
3,4-difluorocinnamic acid n-octyl ester;
3,4-dichlorocinnamic acid n-octyl ester;
3-methoxy-4-hydroxy cinnamic acid n-tridecyl ester;
3-hydroxy-4-methoxy cinnamic acid n-tridecyl ester;
2-hydroxycinnamic acid n-tridecyl ester;
3-hydroxycinnamic acid n-tridecyl ester;
4-hydroxycinnamic acid n-tridecyl ester;
3,4-dimethylcinnamic acid n-tridecyl ester;
2,4-dihydroxycinnamic acid n-tridecyl ester;
3,4-dihydroxycinnamic acid n-octynyl ester;
3,4-dihydroxycinnamic acid n-nonynyl ester;
3,4-dihydroxycinnamic acid n-decynyl ester;
3,4-dihydroxycinnamic acid n-unynyl ester;
3,4-dihydroxycinnamic acid azidopentyl ester;
2,3,4-trihydroxycinnamic acid n-octyl ester;
3,4-dihydroxycinnamic acid n-pentadecyl amide;
3-hydroxy-4-aminocinnamic acid n-decyl amide;
3-fluoro-4-hydroxycinnamic acid n-decyl amide;
3-chloro-4-hydroxycinnamic acid n-decyl amide;
3-bromo-4-hydroxycinnamic acid n-decyl amide;
2,5-dihydroxycinnamic acid n-decyl amide;
2,3,4-trihydroxycinnamic acid n-dodecyl amide;
3,4,5-trihydroxycinnamic acid n-dodecyl amide;
2,4,5-trihydroxycinnamic acid n-dodecyl amide;
2-fluoro-4,5-dihydroxycinnamic acid n-decyl amide;
3,4-dihydroxycinnamic acid phenyldecyl ester;
3,4-dihydroxycinnamic acid nonyl ketone; or
(E)-2-(2-fluoro-4,5-dihydroxyphenyl)tridec-2-en-4-one,
or a pharmaceutically acceptable salt thereof.

21. A pharmaceutical composition, comprising a therapeutically effective amount of a
compound according to claim 1, or a pharmaceutically acceptable salt thereof, and a
pharmaceutically acceptable carrier.
22. A method for the treatment of cancer, comprising the step of administering to a patient in need thereof a therapeutically effective amount of a compound according to claim 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

23. The method according to claim 22, wherein said cancer is multiple myeloma.

24. A method for reducing resistance to proteasome inhibitors, comprising the step of administering to a patient in need thereof a therapeutically effective amount of a compound according to claim 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

25. A method for reducing resistance to proteasome inhibitors during treatment of multiple myeloma, comprising the step of administering to a patient in need thereof a therapeutically effective amount of a compound according to claim 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.
FIGURE 1

A

- DMSO
- E61 5μM

Cell Viability (%)

120
100
80
60
40
20
0
1 10 100
Btz [nM]

B

EC₅₀ Btz, nM

32
24
16
8
0
0 1 2 3 4 5
[E61], μM

C

MM.1S BzR

$EC_{50}$ (nM)

1000
100
10
1

E61: - + - + - + - +

- Btz
- Crflz
- Ixazomib
- Oprozomib
FIGURE 2

A

- $H_2$DCFDA
- + $H_2$DCFDA

P < 0.0001

ROS (RFU)

DMSO  DMSO  E61  E61

B

ROS Induction ($\Delta$ RFU)

DMSO  E61

Time (sec)

-250  0  250  500  750

C

- NAC
- + NAC

Cell Viability (%)

DMSO  DMSO  E61  E61  E61  E61 + Btz

P = 0.005

SUBSTITUTE SHEET (RULE 26)
FIGURE 5
**FIGURE 6**

![Graph showing % Remaining over time for different samples.](image)

![Bar graph showing COMT Activity (µmol/min).](image)

![Chemical structures of E61, E64, and E64F1Me.](image)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC  -  A61K 31/495, 31/1 92, 31/215; C07C 69/73 (2017.01)
CPC  -  A61K 31/495, 31/192, 31/215; C07C 69/73

B. FIELDS SEARCHED

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>YO 2014/182744 A1 (THE JOHNS HOPKINS UNIVERSITY) 13 November 2014; abstract; paragraphs [007], [011]; claims 1, 10, 12, 15</td>
<td>23-25</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier application or patent but published on or after the international filing date
  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed
  "Q" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  "R" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  "S" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  "T" document member of the same patent family

Date of the actual completion of the international search 05 May 2017 (05.05.2017)

Date of mailing of the international search report 16 AUG 2017

Name and mailing address of the ISA/
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer Shane Thomas
PCT Helpdesk: 571-272-4300
PCT OSP: 971-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)
This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

This International Searching Authority found multiple inventions in this international application, as follows:

- "-Please See Extra Sheet Below.-"-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: Claims 1-25 (in-part)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

- ☒ No protest accompanied the payment of additional search fees.
**INTERNATIONAL SEARCH REPORT**

**International application No.**
PCT/US 17/22665

- Continued from Box III: Observations where unity of invention is lacking-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I, Claims 1-25 (in-part); compound of Formula (I), wherein X is -O-; R1 is H; R2 and R3 are H; R4 is H; R5 is H; R6 is (C5-C16) alkyl, unbranched or branched, optionally substituted with R7; R7 is -N3; and R6 is H (first exemplary compound structure), and methods associated therewith.

The compound, compositions and methods will be searched to the extent the compound encompasses a compound of Formula (I), wherein X is -O-; R1 is H; R2 and R3 are H; R4 is H; R5 is H; R6 is (C5-C16)alkyl; and R8 is H (first exemplary compound structure). Applicant is invited to elect additional compound(s), with fully specified structure (e.g. no optional or variable atoms or substituents) for each, to be searched. Additional compound(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 2 (in-part), 5 (in-part), 7 (in-part), 9 (in-part), 12 (in-part), 13 (in-part), 14 (in-part), 21 (in-part), 22 (in-part), 23 (in-part), 24 (in-part), and 25 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass a compound of Formula (I), wherein X is -O-; R1 is H; R2 and R3 are H; R4 is H; R5 is H; R6 is (C5-C16)alkyl; and R8 is H (compound structure). Applicants must specify the claims that encompass any additionally elected compound structure(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "-" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a compound of Formula (I), wherein X is -O-; R1 is H; R2 and R3 are H; R4 is H; R5 is H; R6 is (C5-C16)alkyl; and R8 is H (first exemplary elected compound structure).

Groups I share the technical features including: a compound of Formula (I), X is -O-; R1 is H; R2 and R3 are H; R4 is H; R5 is H; R6 is (C5-C16)alkyl; and R8 is H, for use in the treatment of cancer, comprising the step of administering to a patient in need thereof a therapeutically effective amount of a compound, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

However, these shared technical features are previously disclosed by US 5,216,024 A to Markaverich, et al. (hereinafter 'Markaverich').

Markaverich discloses a compound of Formula (I) (second shown compound; column 3, lines 30-35), X is -O- (second shown compound (X is -O-); column 3, lines 30-35); R1 is H (second shown compound (R1 is H); column 3, lines 30-35); R2 and R3 are H (second shown compound wherein R2 and R3 are H; column 3, lines 30-35; column 4, line 20); R4 is H (second shown compound (R4 is H); column 3, lines 30-35); R5 is H (second shown compound (R5 is H); column 3, lines 30-35); R6 is (C5-C16)alkyl, unbranched or branched (second shown compound wherein R1 (R6) is C6 alkyl; column 3, lines 30-35; column 4, lines 18-19); and R8 is H (second shown compound (R8 is H); column 3, lines 30-35); for use in the treatment of cancer (method of treating cancer; column 3, lines 5-6), comprising the step of administering to a patient in need thereof a therapeutically effective amount of a compound (method comprises administering a therapeutic dose of a compound of the second shown compound; column 3, lines 18-35), and a pharmaceutically acceptable carrier (carrier; column 6, lines 39-44). Markaverich does not provide a single concise embodiment with each of the selected moieties, from the list of possible moieties. However, provided that Markaverich discloses the chosen substituents (Markaverich; column 3, lines 5-35; column 4, lines 1-20), it would have been obvious to one of ordinary skill in the art, at the time of the invention, to have modified the compound of Markaverich, by narrowing the range of substituents so to as select the chosen substituents for the second recited compound, for enhancing the compound's efficacy as caffeic acid derivative useful in the treatment of cancer.

Since none of the special technical features of the Groups I inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Markaverich reference, unity of invention is lacking.

Form PCT/ISA/2 10 (extra sheet) (January 2015)