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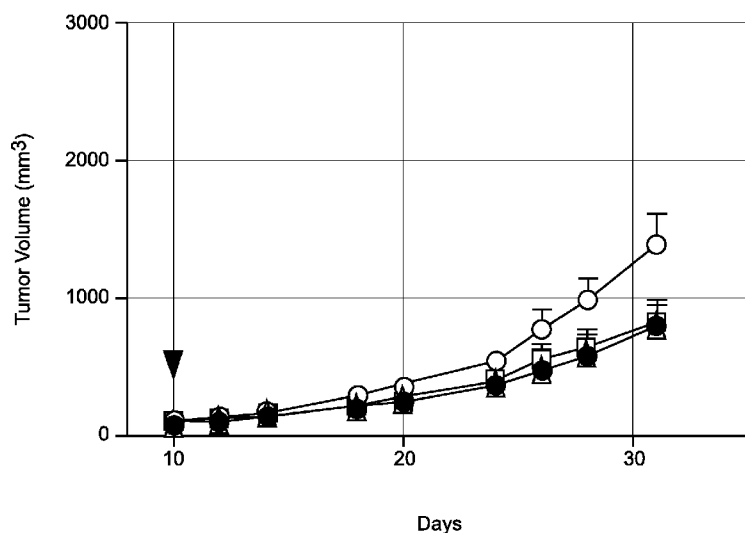
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(54) Title: LIPOSOMAL COMPOSITIONS OF EPOXYKETONE-BASED PROTEASOME INHIBITORS

**FIG. 7**

(57) Abstract: Liposomal compositions comprising peptide epoxyketone compounds are described, as well as methods of making and using such liposomal compositions. These liposomal compositions provide prolonged drug exposure (relative to non-liposomal compositions comprising peptide epoxyketone compounds) without significantly affecting biodistribution of the drug. Tolerability of peptide epoxyketone compounds was also enhanced when formulated in the liposomal compositions.



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LIPOSOMAL COMPOSITIONS OF EPOXYKETONE-BASED PROTEASOME INHIBITORS

Cross-Reference to Related Applications

[0001] This application claims the benefit of U.S. Provisional Application Ser. No. 61/673,017, filed 18 July 2012, now pending, and U.S. Provisional Application Ser. No. 61/794,603, filed 15 March 2013, now pending, both of which applications are herein incorporated by reference in their entireties.

Technical Field

[0002] The present invention relates to compositions for the treatment of cancer, as well as methods of preparing such compositions. Aspects of the present invention include, but are not limited to, methods for formulating stable liposomal compositions comprising peptide epoxyketone compounds, liposomal compositions comprising peptide epoxyketone compounds, and methods of using such liposomal compositions.

Background of the Invention

[0003] In eukaryotes, protein degradation is predominately mediated through the ubiquitin pathway in which proteins targeted for destruction are ligated to the 76 amino acid polypeptide ubiquitin. Once targeted, ubiquitinated proteins then serve as substrates for the 26S proteasome, a multicatalytic protease, which cleaves proteins into short peptides through the action of its three major proteolytic activities. While having a general function in intracellular protein turnover, proteasome-mediated degradation also plays a key role in many processes such as major histocompatibility complex (MHC) class I presentation, apoptosis, cell growth regulation, NF- κ B activation, antigen processing, and transduction of pro-inflammatory signals.

[0004] The 20S proteasome is a 700 kDa cylindrical-shaped multicatalytic protease complex comprised of 28 subunits organized into four rings. In yeast and other eukaryotes, 7 different α subunits form the outer rings and 7 different β subunits comprise the inner rings. The α subunits serve as binding sites for the 19S (PA700) and 11S (PA28) regulatory complexes, as well as a physical barrier for the inner proteolytic chamber formed by the two β subunit rings. Thus, *in vivo*, the proteasome is believed to exist as a 26S particle ("the 26S proteasome"). *In vivo* experiments have shown that inhibition of the 20S form of the proteasome can be readily correlated to inhibition of 26S proteasome. Cleavage of amino-terminal prosequences of β subunits during particle formation exposes amino-terminal threonine residues, which serve as the catalytic nucleophiles.

[0005] The subunits responsible for catalytic activity in proteasomes thus possess an amino terminal nucleophilic residue, and these subunits belong to the family of N-terminal nucleophile (Ntn) hydrolases (where the nucleophilic N-terminal residue is, for example, Cys, Ser, Thr, or other nucleophilic moieties). This family includes, for example, penicillin G acylase (PGA), penicillin V acylase (PVA), glutamine PRPP amidotransferase (GAT), and bacterial glycosylasparaginase. In addition to the ubiquitously expressed β subunits, higher vertebrates also possess three interferon- γ -inducible β subunits (LMP7, LMP2 and MECL1), which replace their normal counterparts, $\beta 5$, $\beta 1$ and $\beta 2$ respectively, thus altering the catalytic activities of the proteasome.

[0006] Through the use of different peptide substrates, three major proteolytic activities have been defined for the eukaryote 20S proteasome: chymotrypsin-like activity (CT-L), which cleaves after large hydrophobic residues; trypsin-like activity (T-L), which cleaves after basic residues; and caspase-like (C-L), which cleaves after acidic residues. The major proteasome proteolytic activities appear to be contributed by different catalytic sites, because inhibitors, point mutations in β subunits and the exchange of interferon- γ -inducing β subunits alter these activities to various degrees.

[0007] There are several examples of small molecules that have been used to inhibit proteasome activity and have been shown to be effective against cancer, particularly multiple myeloma. However, these compounds generally lack the specificity, stability, or potency necessary to explore and exploit the roles of the proteasome at the cellular and molecular level, and thus maximize their anti-cancer activity.

Summary of the Invention

[0008] The present invention generally relates to liposomal compositions for increasing the therapeutic activity of proteasome inhibitors, particularly by enhancing their pharmacokinetic properties. Aspects of the present invention include, liposomal compositions comprising a peptide epoxyketone compound, methods of making such compositions, and methods of using such compositions.

[0009] In one aspect, the present invention relates to pharmaceutical liposomal compositions. In some embodiments, the pharmaceutical liposomal compositions comprise liposome entrapped peptide epoxyketone compound, wherein (i) liposomes of the liposomal composition comprise one or more lipids (e.g., L- α -phosphatidylcholine, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, and/or sphingomyelin), and in the liposomes the weight

ratio of peptide epoxyketone compound:lipid is between about 0.01:1 and about 1:1. In a preferred embodiment, the weight ratio of peptide epoxyketone compound:lipid is between about 0.05:1 to about 0.5:1. Typically, the liposomes have an average size of between about 0.05 microns to about 0.5 microns, preferably an average size of between about 0.05 microns to about 0.2 microns.

[0010] Embodiments of the pharmaceutical liposomal compositions present invention include, but are not limited to, the lipids of the liposomes comprising a phospholipid, a cholesterol, a hydrophilic polymer-derivatized lipid, and/or combinations thereof.

[0011] In some embodiments of this aspect of the present invention, the liposomal composition comprises liposomes comprising the peptide epoxyketone compound and a solubilizing agent (e.g., a compound).

[0012] The pharmaceutical liposomal composition of the present invention can also include one or more excipients.

[0013] In other aspects, the present invention relates to methods of making the pharmaceutical liposomal compositions described herein. One method of making a pharmaceutical liposomal composition comprises preparing a dried film comprising a peptide epoxyketone compound and at least one lipid, and rehydrating the dried film with an aqueous solution to form a liposomal composition comprising liposomes and an aqueous solution. Another method of making a pharmaceutical liposomal composition comprises preparing a dried film comprising one or more lipids and rehydrating the dried film with an aqueous solution comprising a peptide epoxyketone compound to form a liposomal composition comprising liposomes dispersed in aqueous solution. Typically the aqueous solution comprises a peptide epoxyketone compound and a solubilizing agent. Yet another method of making a pharmaceutical liposomal composition comprises preparing a lipid solution comprising one or more lipids and a solvent and injecting the lipid solution into an aqueous solution comprising a peptide epoxyketone compound. Typically the aqueous solution comprises a peptide epoxyketone compound and a solubilizing agent.

[0014] In preferred embodiments, the methods of making pharmaceutical liposomal compositions further comprises sizing the liposomes to have an average size of between about 0.05 microns to about 0.5 microns (preferably an average size of between about 0.05 microns to about 0.2 microns).

[0015] In some embodiments, excess peptide epoxyketone compounds are removed from the non-encapsulated aqueous solution.

[0016] In some embodiments, the method further comprises adding one or more excipients, including, but not limited to, a pH adjusting agent (e.g., a buffer) and/or an agent to maintain isotonicity, to the aqueous solution in which the liposomes are dispersed.

[0017] In another aspect, the present invention relates to pharmaceutical liposomal compositions made by the methods of the invention.

[0018] In a further aspect, the present invention relates to methods of treating a disease or condition in a subject in need of treatment, comprising administering a therapeutically effective amount of a pharmaceutical liposomal composition comprising liposomes comprising a peptide epoxyketone compound. In some embodiments the methods of treating further comprise simultaneous, sequential, or separate administration of a therapeutically effective amount of another therapeutic agent, for example, a chemotherapeutic agent, a cytokine, a steroid, an immunotherapeutic agent, or combinations thereof.

[0019] In another aspect the present invention relates to dry pharmaceutical compositions comprising, one or more amphipathic lipids (e.g., a phospholipid), and a peptide epoxyketone compound.

[0020] These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

Brief Description of the Figures

[0021] FIG. 1 presents the characterization of exemplary liposomal carfilzomib compositions (A-G) used to generate the data presented in the Examples and Tables herein.

[0022] FIG. 2A presents data related to pharmacodynamic responses in BALB/C mice to different compositions of carfilzomib. In the figure, the vertical axis is the percent (%) enzymatic activity relative to vehicle, wherein the enzymatic activity corresponds to proteasome CT-L activity in whole blood (primarily erythrocytes). Three groups of data are presented on the horizontal axis as follows. The first group, represented in the figure as cross-hatched bars, presents data for an injectable composition of carfilzomib (CFZ) formulated in 10% sulfobutylether beta cyclodextrin (SBE- β -CD, also referred to herein as SBE-CD), and 10 mM Citrate, pH 3.5 (non-liposomal): the first bar presents control data for the placebo vehicle without carfilzomib, the second bar presents data for CFZ SBE-CD at 1 hour, the third bar presents data for CFZ SBE-CD at 8 hours, and the fourth bar presents data for CFZ SBE-CD at 24 hours. The second group, represented in the figure as diagonal-lined bars, presents data for a liposomal composition of carfilzomib: the first bar presents control data for the liposomal vehicle without carfilzomib, the second bar presents data for the

carfilzomib liposomal composition at 1 hour, the third bar presents data for the carfilzomib liposomal composition at 8 hours, and the fourth bar presents data for the carfilzomib liposomal composition at 24 hours. The third group, represented in the figure as white, outlined bars, presents data for a pegylated liposomal composition of carfilzomib: the first bar presents control data for the pegylated liposomal vehicle without carfilzomib, the second bar presents data for the carfilzomib pegylated liposomal composition at 1 hour, the third bar presents data for the carfilzomib pegylated liposomal composition at 8 hours, and the fourth bar presents data for the carfilzomib pegylated liposomal composition at 24 hours.

[0023] FIG. 2B presents data related to pharmacodynamic responses in BALB/C mice to different compositions of carfilzomib. In the figure, the vertical axis is the percent (%) enzymatic activity relative to vehicle, wherein the enzymatic activity corresponds to proteasome CT-L activity in adrenal tissue. Three groups of data are presented on the horizontal axis as follows. The first group, represented in the figure as cross-hatched bars, presents data for injectable CFZ SBE-CD (non-liposomal): the first bar presents control data for the vehicle without carfilzomib, the second bar presents data for CFZ SBE-CD at 1 hour, the third bar presents data for CFZ SBE-CD at 8 hours, and the fourth bar presents data for CFZ SBE-CD at 24 hours. The second group, represented in the figure as diagonal-lined bars, presents data for a liposomal composition of carfilzomib: the first bar presents control data for the liposomal vehicle without carfilzomib, the second bar presents data for the carfilzomib liposomal composition at 1 hour, the third bar presents data for the carfilzomib liposomal composition at 8 hours, and the fourth bar presents data for the carfilzomib liposomal composition at 24 hours. The third group, represented in the figure as white, outlined bars, presents data for a pegylated liposomal composition of carfilzomib: the first bar presents control data for the pegylated liposomal vehicle without carfilzomib, the second bar presents data for the carfilzomib pegylated liposomal composition at 1 hour, the third bar presents data for the carfilzomib pegylated liposomal composition at 8 hours, and the fourth bar presents data for the carfilzomib pegylated liposomal composition at 24 hours.

[0024] FIG. 2C presents data related to pharmacodynamic responses in BALB/C mice to different compositions of carfilzomib. In the figure, the vertical axis is the percent (%) enzymatic activity relative to vehicle, wherein the enzymatic activity corresponds to proteasome CT-L activity in liver tissue. Three groups of data are presented on the horizontal axis as follows. The first group, represented in the figure as cross-hatched bars, presents data for injectable CFZ SBE-CD (non-liposomal): the first bar presents control data for the vehicle without carfilzomib, the second bar presents data for the carfilzomib SBE-CD

composition at 1 hour, the third bar presents data for the carfilzomib composition at 8 hours, and the fourth bar presents data for the carfilzomib composition at 24 hours. The second group, represented in the figure as diagonal-lined bars, presents data for a liposomal composition of carfilzomib: the first bar presents control data for the liposomal vehicle without carfilzomib, the second bar presents data for the carfilzomib liposomal composition at 1 hour, the third bar presents data for the carfilzomib liposomal composition at 8 hours, and the fourth bar presents data for the carfilzomib liposomal composition at 24 hours. The third group, represented in the figure as white, outlined bars, presents data for a pegylated liposomal composition of carfilzomib: the first bar presents control data for the pegylated liposomal vehicle without carfilzomib, the second bar presents data for the carfilzomib pegylated liposomal composition at 1 hour, the third bar presents data for the carfilzomib pegylated liposomal composition at 8 hours, and the fourth bar presents data for the carfilzomib pegylated liposomal composition at 24 hours.

[0025] FIG. 2D presents data related to pharmacodynamic responses in BALB/C mice to different compositions of carfilzomib. In the figure, the vertical axis is the percent (%) enzymatic activity relative to vehicle, wherein the enzymatic activity corresponds to proteasome CT-L activity in heart tissue. Three groups of data are presented on the horizontal axis as follows. The first group, represented in the figure as cross-hatched bars, presents data for injectable CFZ SBE-CD (non-liposomal): the first bar presents control data for the vehicle without carfilzomib, the second bar presents data for CFZ SBE-CD at 1 hour, the third bar presents data for CFZ SBE-CD at 8 hours, and the fourth bar presents data for CFZ SBE-CD at 24 hours. The second group, represented in the figure as diagonal-lined bars, presents data for a liposomal composition of carfilzomib: the first bar presents control data for the liposomal vehicle without carfilzomib, the second bar presents data for the carfilzomib liposomal composition at 1 hour, the third bar presents data for the carfilzomib liposomal composition at 8 hours, and the fourth bar presents data for the carfilzomib liposomal composition at 24 hours. The third group, represented in the figure as white, outlined bars, presents data for a pegylated liposomal composition of carfilzomib: the first bar presents control data for the pegylated liposomal vehicle without carfilzomib, the second bar presents data for the carfilzomib pegylated liposomal composition at 1 hour, the third bar presents data for the carfilzomib pegylated liposomal composition at 8 hours, and the fourth bar presents data for the carfilzomib pegylated liposomal composition at 24 hours.

[0026] FIG. 3A presents data related to pharmacodynamic responses in BALB/C mice to different compositions of carfilzomib. In the figure, the vertical axis (CT-L activity)

is the percent (%) enzymatic activity relative to a corresponding vehicle without carfilzomib (CFZ), wherein the enzymatic activity corresponds to proteasome CT-L activity in whole blood (primarily erythrocytes). The horizontal axis is the time in hours (Hour). Four groups of data are presented. The first group (open squares containing an X) presents data for an injectable composition of carfilzomib (CFZ) formulated in 10% sulfobutylether beta cyclodextrin (SBE-CD), and 10 mM Citrate, pH 3.5, (non-liposomal) administered at 5 mg/kg with data points at 0, 1, 4, 6, 8, and 24 hours. The second group (open circles containing an X) presents data for an injectable composition of carfilzomib (CFZ) formulated in 10% sulfobutylether beta cyclodextrin (SBE-CD), and 10 mM Citrate, pH 3.5, (non-liposomal) administered at 10 mg/kg with data points at 0, 1, 8, and 24 hours. The third group (solid squares) presents data for a pegylated liposomal composition of carfilzomib wherein the aqueous core of the pegylated liposomes comprises carfilzomib and SBE-CD (ap-L11) administered at 5 mg/kg with data points at 0, 1, 4, 6, and 24 hours. The fourth group (solid circles) presents data for a pegylated liposomal composition of carfilzomib wherein the aqueous core of the pegylated liposomes comprises carfilzomib and SBE-CD (ap-L11) administered at 15 mg/kg with data points at 0, 1, 4, 6, and 24 hours.

[0027] FIG. 3B presents data related to pharmacodynamic responses in BALB/C mice to different compositions of carfilzomib. In the figure, the vertical axis (CT-L activity) is the percent (%) enzymatic activity relative to a corresponding vehicle without carfilzomib (CFZ), wherein the enzymatic activity corresponds to proteasome CT-L activity in heart tissue. The horizontal axis is the time in hours (Hour). Four groups of data are presented. The first group (open squares containing an X) presents data for an injectable composition of carfilzomib (CFZ) formulated in 10% sulfobutylether beta cyclodextrin (SBE-CD), and 10 mM Citrate, pH 3.5, (non-liposomal) administered at 5 mg/kg with data points at 0, 1, 4, 6, 8, and 24 hours. The second group (open circles containing an X) presents data for an injectable composition of carfilzomib (CFZ) formulated in 10% sulfobutylether beta cyclodextrin (SBE-CD), and 10 mM Citrate, pH 3.5, (non-liposomal) administered at 10 mg/kg with data points at 0, 1, 8, and 24 hours. The third group (solid squares) presents data for a pegylated liposomal composition of carfilzomib wherein the aqueous core of the pegylated liposomes comprises carfilzomib and SBE-CD (ap-L11) administered at 5 mg/kg with data points at 0, 1, 4, 6, and 24 hours. The fourth group (solid circles) presents data for a pegylated liposomal composition of carfilzomib wherein the aqueous core of the pegylated liposomes comprises carfilzomib and SBE-CD (ap-L11) administered at 15 mg/kg with data points at 0, 1, 4, 6, and 24 hours.

[0028] FIG. 3C presents data related to pharmacodynamic responses in BALB/C mice to different compositions of carfilzomib. In the figure, the vertical axis (CT-L activity) is the percent (%) enzymatic activity relative to a corresponding vehicle without carfilzomib (CFZ), wherein the enzymatic activity corresponds to proteasome CT-L activity in liver tissue. The horizontal axis is the time in hours (Hour). Four groups of data are presented. The first group (open squares containing an X) presents data for an injectable composition of carfilzomib (CFZ) formulated in 10% sulfobutylether beta cyclodextrin (SBE-CD), and 10 mM Citrate, pH 3.5, (non-liposomal) administered at 5 mg/kg with data points at 0, 1, 4, 6, 8, and 24 hours. The second group (open circles containing an X) presents data for an injectable composition of carfilzomib (CFZ) formulated in 10% sulfobutylether beta cyclodextrin (SBE-CD), and 10 mM Citrate, pH 3.5, (non-liposomal) administered at 10 mg/kg with data points at 0, 1, 8, and 24 hours. The third group (solid squares) presents data for a pegylated liposomal composition of carfilzomib wherein the aqueous core of the pegylated liposomes comprises carfilzomib and SBE-CD (ap-L11) administered at 5 mg/kg with data points at 0, 1, 4, 6, and 24 hours. The fourth group (solid circles) presents data for a pegylated liposomal composition of carfilzomib wherein the aqueous core of the pegylated liposomes comprises carfilzomib and SBE-CD (ap-L11) administered at 15 mg/kg with data points at 0, 1, 4, 6, and 24 hours.

[0029] FIG. 3D presents data related to pharmacodynamic responses in BALB/C mice to different compositions of carfilzomib. In the figure, the vertical axis (CT-L activity) is the percent (%) enzymatic activity relative to a corresponding vehicle without carfilzomib (CFZ), wherein the enzymatic activity corresponds to proteasome CT-L activity in adrenal tissue. The horizontal axis is the time in hours (Hour). Four groups of data are presented. The first group (open squares containing an X) presents data for an injectable composition of carfilzomib (CFZ) formulated in 10% sulfobutylether beta cyclodextrin (SBE-CD), and 10 mM Citrate, pH 3.5, (non-liposomal) administered at 5 mg/kg with data points at 0, 1, 4, 6, 8, and 24 hours. The second group (open circles containing an X) presents data for an injectable composition of carfilzomib (CFZ) formulated in 10% sulfobutylether beta cyclodextrin (SBE-CD), and 10 mM Citrate, pH 3.5, (non-liposomal) administered at 10 mg/kg with data points at 0, 1, 8, and 24 hours. The third group (solid squares) presents data for a pegylated liposomal composition of carfilzomib wherein the aqueous core of the pegylated liposomes comprises carfilzomib and SBE-CD (ap-L11) administered at 5 mg/kg with data points at 0, 1, 4, 6, and 24 hours. The fourth group (solid circles) presents data for a pegylated liposomal composition of carfilzomib wherein the aqueous core of the pegylated

liposomes comprises carfilzomib and SBE-CD (ap-L11) administered at 15 mg/kg with data points at 0, 1, 4, 6, and 24 hours.

[0030] FIG. 4 presents data related to the circulation half-life in BALB/C mice of different compositions of carfilzomib. In the figure, the vertical axis is the concentration of carfilzomib in $\mu\text{mol/L}$ (Concentration ($\mu\text{mol/L}$)), and the horizontal axis is the time in minutes (Time (min)). The line with open squares containing an X corresponds to administration of 5 mg/kg of an injectable carfilzomib SBE-CD composition (non-liposomal). The line with solid squares corresponds to administration of 5 mg/kg of apL11, a pegylated liposomal composition of carfilzomib wherein the aqueous core of the pegylated liposomes comprises carfilzomib and SBE-CD. The line with solid circles corresponds to administration of 15 mg/kg of apL11, a pegylated liposomal composition of carfilzomib wherein the aqueous core of the pegylated liposomes comprises carfilzomib and SBE-CD.

[0031] FIG. 5A presents data related to pharmacodynamic responses in BALB/C mice to different compositions of carfilzomib. In the figure, the vertical axis (CT-L Activity) is the percent (%) enzymatic activity relative to a corresponding vehicle without carfilzomib (CFZ), wherein the enzymatic activity corresponds to proteasome CT-L activity in whole blood (primarily erythrocytes). The horizontal axis is the time in hours (Hour). Two groups of data are presented. The first group (open circles) presents data for an injectable composition of carfilzomib (CFZ) formulated in 10% sulfobutylether beta cyclodextrin (SBE-CD), and 10 mM Citrate, pH 3.5, (non-liposomal) administered at 10 mg/kg with data points at 0, 1, 8, and 24 hours. The second group (solid squares) presents data for a liposomal composition of carfilzomib comprising liposomes comprising entrapped carfilzomib (pL-6) administered at 15 mg/kg with data points at 0, 1, 4, 6, and 24 hours.

[0032] FIG. 5B presents data related to pharmacodynamic responses in BALB/C mice to different compositions of carfilzomib. In the figure, the vertical axis (CT-L Activity) is the percent (%) enzymatic activity relative to a corresponding vehicle without carfilzomib (CFZ), wherein the enzymatic activity corresponds to proteasome CT-L activity in heart tissue. The horizontal axis is the time in hours (Hour). Two groups of data are presented. The first group (open circles) presents data for an injectable composition of carfilzomib (CFZ) formulated in 10% sulfobutylether beta cyclodextrin (SBE-CD), and 10 mM Citrate, pH 3.5, (non-liposomal) administered at 10 mg/kg with data points at 0, 1, 8, and 24 hours. The second group (solid squares) presents data for a liposomal composition of carfilzomib comprising liposomes comprising entrapped carfilzomib (pL-6) administered at 15 mg/kg with data points at 0, 1, 4, 6, and 24 hours.

[0033] FIG. 5C presents data related to pharmacodynamic responses in BALB/C mice to different compositions of carfilzomib. In the figure, the vertical axis (CT-L Activity) is the percent (%) enzymatic activity relative to a corresponding vehicle without carfilzomib (CFZ), wherein the enzymatic activity corresponds to proteasome CT-L activity in liver tissue. The horizontal axis is the time in hours (Hour). Two groups of data are presented. The first group (open circles) presents data for an injectable composition of carfilzomib (CFZ) formulated in 10% sulfobutylether beta cyclodextrin (SBE-CD), and 10 mM Citrate, pH 3.5, (non-liposomal) administered at 10 mg/kg with data points at 0, 1, 8, and 24 hours. The second group (solid squares) presents data for a liposomal composition of carfilzomib comprising liposomes comprising entrapped carfilzomib (pL-6) administered at 15 mg/kg with data points at 0, 1, 4, 6, and 24 hours.

[0034] FIG. 5D presents data related to pharmacodynamic responses in BALB/C mice to different compositions of carfilzomib. In the figure, the vertical axis (CT-L Activity) is the percent (%) enzymatic activity relative to a corresponding vehicle without carfilzomib (CFZ), wherein the enzymatic activity corresponds to proteasome CT-L activity in adrenal tissue. The horizontal axis is the time in hours (Hour). Two groups of data are presented. The first group (open circles) presents data for an injectable composition of carfilzomib (CFZ) formulated in 10% sulfobutylether beta cyclodextrin (SBE-CD), and 10 mM Citrate, pH 3.5, (non-liposomal) administered at 10 mg/kg with data points at 0, 1, 8, and 24 hours. The second group (solid squares) presents data for a liposomal composition of carfilzomib comprising liposomes comprising entrapped carfilzomib (pL-6) administered at 15 mg/kg with data points at 0, 1, 4, 6, and 24 hours.

[0035] FIG. 6 presents data related to the circulation half-life in BALB/C mice of different compositions of carfilzomib. In the figure, the vertical axis is the concentration of carfilzomib in $\mu\text{mol/L}$ (Concentration ($\mu\text{mol/L}$)), and the horizontal axis is the time post dose in minutes (Time Post Dose (min)). The line with open circles corresponds to administration of 5 mg/kg of an injectable carfilzomib SBE-CD composition (non-liposomal). The line with solid squares corresponds to administration of 15 mg/kg of a liposomal composition of carfilzomib comprising liposomes comprising entrapped carfilzomib (pL-6).

[0036] FIG. 7 presents data related to the dosing frequency of different compositions of carfilzomib. In the figure, the vertical axis is tumor volume in mm^3 (Tumor Volume (mm^3)), and the horizontal axis is days post-tumor challenge (Days). The line (top line in the figure at 30 days) with open circles correspond to once-weekly administration of vehicle (liposomes comprising 12.5 mg/mL EPC, 3.1 mg/mL Cholesterol, 1.2 mg/mL mPEG-DSPE

with no carfilzomib); the line with open squares corresponds to 5 mg/kg of an injectable carfilzomib SBE-CD composition (non-liposomal) administered on days 1 and 2 of each week; the line with solid circles corresponds to administration of pL6 = 2 mg/mL CFZ, 12.5 mg/mL Sphingomylin, 3.2 mg/mL cholesterol, 1.3 mg/mL mPEG-DSPE providing a dose of 10 mg/kg of carfilzomib administered on days 1 and 2 of each week. The line with open triangles corresponds to administration of pL6 = 2 mg/mL CFZ, 12.5 mg/mL Sphingomylin, 3.2 mg/mL cholesterol, 1.3 mg/mL mPEG-DSPE providing a dose of 15 mg/kg of carfilzomib administered once weekly. The solid arrow indicates the start of the dosing period.

Detailed Description of the Invention

[0037] All patents, publications, and patent applications cited in this specification are herein incorporated by reference as if each individual patent, publication, or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

1.0.0 Definitions

[0038] It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a lipid” includes one or more lipids, or mixtures of lipids, reference to “a hydrophilic polymer” includes one or more hydrophilic polymers, or mixtures of hydrophilic polymers, reference to “a drug” includes one or more drugs, and the like.

[0039] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although other methods and materials similar, or equivalent, to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

[0040] In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out below.

[0041] The term “enzyme” as used herein refers to any partially or wholly proteinaceous molecule that carries out a chemical reaction in a catalytic manner. Such enzymes can be native enzymes, fusion enzymes, proenzymes, apoenzymes, denatured enzymes, farnesylated enzymes, ubiquitinated enzymes, fatty acylated enzymes,

gerangeranylated enzymes, GPI-linked enzymes, lipid-linked enzymes, prenylated enzymes, naturally-occurring or artificially generated mutant enzymes, enzymes with side chain or backbone modifications, enzymes having leader sequences, and enzymes complexed with non-proteinaceous material, such as proteoglycans and proteoliposomes. Enzymes can be made by any means, including natural expression, promoted expression, cloning, various solution-based and solid-based peptide syntheses, and similar methods known to those skilled in the art.

[0042] The term “C_{x-y} alkyl” as used herein refers to substituted or unsubstituted saturated hydrocarbon groups, including straight-chain alkyl and branched-chain alkyl groups that contain from x to y carbons in the chain, including haloalkyl groups such as trifluoromethyl and 2,2,2-trifluoroethyl, etc. C₀ alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal. The terms “C_{2-y} alkenyl” and “C_{2-y} alkynyl” refer to substituted or unsubstituted unsaturated aliphatic groups analogous in length and possible substitution to the alkyls, but that contain at least one double or triple bond respectively.

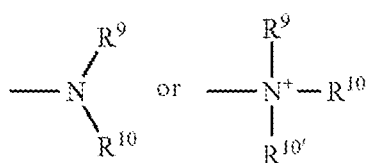
[0043] The term “alkoxy” as used herein refers to an alkyl group having an oxygen attached thereto. Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy, and the like.

[0044] The term “ether” as used herein refers to two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxy.

[0045] The term “C₁₋₆ alkoxyalkyl” as used herein refers to a C₁₋₆ alkyl group substituted with an alkoxy group, thereby forming an ether.

[0046] The term “C₁₋₆ aralkyl” as used herein refers to a C₁₋₆ alkyl group substituted with an aryl group.

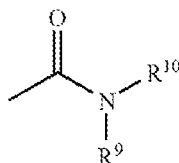
[0047] The terms “amine” and “amino” as used herein are art-recognized and refer to both unsubstituted and substituted amines and salts thereof, *e.g.*, a moiety that can be represented by the general formulae:



[0048] wherein R⁹, R¹⁰ and R^{10'} each independently represent a hydrogen, an alkyl, an alkenyl, --(CH₂)_m--R⁸, or R⁹ and R¹⁰ taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R⁸ represents an aryl, a cycloalkyl, a cycloalkenyl, a heterocyclyl or a polycyclyl; and m is zero or an

integer from 1 to 8. In preferred embodiments, only one of R^9 or R^{10} can be a carbonyl, *e.g.*, R^9 , R^{10} , and the nitrogen together do not form an imide. In even more preferred embodiments, R^9 and R^{10} (and optionally $R^{10'}$) each independently represent a hydrogen, an alkyl, an alkenyl, or $-(CH_2)_m-R^8$. In certain embodiments, the amino group is basic, meaning the protonated form has a $pK_a \geq 7.00$.

[0049] The terms “amide” and “amido” are art-recognized as referring to an amino-substituted carbonyl and including a moiety that can be represented by the general formula:

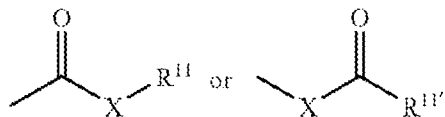


[0050] wherein R^9 , R^{10} are as defined above. Preferred embodiments of the amide does not include imides that can be unstable.

[0051] The term “aryl” as used herein refers to 5- membered, 6- membered, and 7- membered substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon. The term “aryl” also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings, wherein at least one of the rings is aromatic, *e.g.*, the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Aryl groups include benzene, naphthalene, phenanthrene, phenol, aniline, and the like.

[0052] The terms “carbocycle” and “carbocyclyl” as used herein refer to a non-aromatic substituted or unsubstituted ring in which each atom of the ring is carbon. The terms “carbocycle” and “carbocyclyl” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is carbocyclic, *e.g.*, the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls.

[0053] The term “carbonyl” as used herein is art-recognized and refers to moieties as can be represented by the general formula:



[0054] wherein X is a bond or represents an oxygen or a sulfur, and R^{11} represents a hydrogen, an alkyl, an alkenyl, $-(CH_2)_m-R^8$, or a pharmaceutically acceptable salt; $R^{11'}$ represents a hydrogen, an alkyl, an alkenyl, or $-(CH_2)_m-R^8$, where m and R^8 are as defined below. Where X is an oxygen and R^{11} or $R^{11'}$ is not hydrogen, the formula represents an

“ester.” Where X is an oxygen and R¹¹ is a hydrogen, the formula represents a “carboxylic acid.”

[0055] The term “C₁₋₆ heteroaralkyl” as used herein refers to a C₁₋₆ alkyl group substituted with a heteroaryl group.

[0056] The term “heteroaryl” as used herein refers to substituted or unsubstituted aromatic 5-membered to 7-membered ring structures, more preferably 5-membered to 6-membered rings, whose ring structures include one to four heteroatoms. The term “heteroaryl” also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings, wherein at least one of the rings is heteroaromatic, *e.g.*, the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine, pyrimidine, and the like.

[0057] The term “heteroatom” as used herein refers to an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, phosphorus, and sulfur.

[0058] The terms “heterocyclyl” and “heterocyclic group” as used herein refer to substituted or unsubstituted non-aromatic 3-membered to 10-membered ring structures, more preferably 3-membered to 7-membered rings, whose ring structures include one to four heteroatoms. The terms “heterocyclyl” or “heterocyclic group” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings, wherein at least one of the rings is heterocyclic, *e.g.*, the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heterocyclyl groups include, for example, piperidine, piperazine, pyrrolidine, morpholine, lactones, lactams, and the like.

[0059] The term “C₁₋₆hydroxyalkyl” as used herein refers to a C₁₋₆alkyl group substituted with a hydroxy group.

[0060] The term “thioether” as used herein refers to an alkyl group having a sulfur moiety attached thereto. In preferred embodiments, the “thioether” is represented by --S-alkyl. Representative thioether groups include methylthio, ethylthio, and the like.

[0061] The term “substituted” as used herein refers to moieties having substituents replacing a hydrogen on one or more carbons of the backbone. The terms “substitution” or “substituted with” include the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results

in a stable compound, *e.g.*, which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term “substituted” is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein that satisfy the valences of the heteroatoms. Substituents can include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxy carbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxy, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate.

[0062] The term “inhibitor” as used herein refers to a compound that blocks or reduces an activity of an enzyme (*e.g.*, inhibition of proteolytic cleavage of standard fluorogenic peptide substrates such as suc-LLVY-AMC, Box-LLR-AMC and Z-LLE-AMC, inhibition of various catalytic activities of the 20S proteasome). An inhibitor can act with competitive, uncompetitive, or noncompetitive inhibition. An inhibitor can bind reversibly or irreversibly, and therefore the term includes compounds that are suicide substrates of an enzyme. An inhibitor can modify one or more sites on or near the active site of the enzyme, or it can cause a conformational change elsewhere on the enzyme.

[0063] The term “peptide” as used herein refers not only to standard amide linkage with standard α -substituents, but also to commonly used peptidomimetics, other modified linkages, non-naturally occurring side chains, and side chain modifications, for example, as described in U.S. Patent No. 7,417,042.

[0064] The terms “polycyclyl” and “polycyclic” as used herein refer to two or more rings (*e.g.*, cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, *e.g.*, the rings are “fused rings.” Each of the rings of the polycycle can be substituted or unsubstituted.

[0065] The term “prodrug” as used herein refers to compounds that, under physiological conditions, are converted into therapeutically active agents. A common

method for making a prodrug is to include selected moieties that are hydrolyzed under physiological conditions to reveal the desired molecule. In other embodiments, the prodrug is converted by an enzymatic activity of the host animal.

[0066] The term “preventing” as used herein is art-recognized, and when used in relation to a condition, such as a local recurrence (*e.g.*, pain), a disease such as cancer, a syndrome complex such as heart failure or any other medical condition, is well understood in the art, and includes administration of a composition that reduces the frequency of, or delays the onset of, symptoms of a medical condition in a subject relative to a subject who does not receive the composition. Thus, prevention of cancer includes, for example, reducing the number of detectable cancerous growths in a population of subjects receiving a prophylactic treatment relative to an untreated control population, and/or delaying the appearance of detectable cancerous growths in a treated population versus an untreated control population, *e.g.*, by a statistically and/or clinically significant amount. Prevention of an infection includes, for example, reducing the number of diagnoses of the infection in a treated population versus an untreated control population and/or delaying the onset of symptoms of the infection in a treated population versus an untreated control population. Prevention of pain includes, for example, reducing the magnitude of, or alternatively delaying, pain sensations experienced by subjects in a treated population versus an untreated control population.

[0067] The term “prophylactic or therapeutic” treatment, as used herein, is art-recognized and refers to administration to the subject of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (*e.g.*, disease or other unwanted state of the subject) then the treatment is prophylactic, (*i.e.*, it protects the subject against developing the unwanted condition), whereas if it is administered after manifestation of the unwanted condition, the treatment is therapeutic, (*i.e.*, it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof).

[0068] The term “proteasome” as used herein refers to immuno- and constitutive proteasomes.

[0069] The term “therapeutically effective amount” as used herein refers to an amount of the compound(s) in a preparation that, when administered as part of a desired dosage regimen (to a mammal, preferably a human) alleviates a symptom, ameliorates a condition, or slows the onset of disease conditions according to clinically acceptable standards for the disorder or condition to be treated or the cosmetic purpose, *e.g.*, at a reasonable benefit/risk ratio applicable to any medical treatment.

[0070] The terms “treating” and “treatment” as used herein refer to reversing, reducing, or arresting the symptoms, clinical signs, and underlying pathology of a condition in manner to improve or stabilize a subject’s condition.

[0071] The term “amphipathic lipids” as used herein refers to molecules that are mostly lipid-like (hydrophobic) in structure, but at one end have a region that is polar, charged, or a combination of polar and charged (hydrophilic). The hydrophilic region is referred to as the head group, and the lipid portion is known as the tail group(s). Examples of amphipathic lipids include phospholipids, glycolipids, and sphingolipids.

[0072] The terms “hydrophilic polymer” and “water-soluble polymer” as used herein refer to polymers, for example, polyethylene glycol (PEG) or other polyethoxylated polymers, used to shield liposomes and thereby enhance liposomal circulatory half-life. “Hydrophilic polymer” encompasses free hydrophilic polymers associated non-covalently with the liposomes and hydrophilic polymers that are conjugated or covalently linked to a component of the liposome (*e.g.*, PEG-modified lipids). Additional exemplary hydrophilic polymers include, but are not limited to, polyvinyl alcohol, polylactic acid, polyglycolic acid, polyacrylamide, polyvinylpyrrolidone, polyglycerol, polyaxozlines, etc.

[0073] The term “free sterol” as used herein refers to a sterol that is not covalently bound to another compound. “Free cholesterol” refers to cholesterol that is not covalently bound as a moiety in a sterol-modified amphiphilic lipid compound.

[0074] The terms “sterol” and “steroid alcohols” as used herein refer to the subgroup of steroids having a free hydroxyl or a derivative thereof. Exemplary sterols include, but are not limited to, the class cholesterol and derivatives thereof, the class phytosterols and derivatives thereof, and the class fungal sterols and derivatives thereof. Sterols can be natural or synthetic.

[0075] The term “sterol-modified amphiphilic lipid” as used herein refers to amphiphilic lipid compounds having a hydrophilic head group, and two or more hydrophobic tails of which at least one is sterol. “Sterol-modified amphiphilic phospholipids” refers to a sterol-modified amphiphilic lipid comprising a phosphate-containing moiety, such as phosphocholine or phosphoglycerol.

[0076] The term “therapeutic agent” as used herein refers to an agent used in testing, development, or application as a therapeutic, including drugs and pharmaceutical agents.

[0077] The term “drug” as used herein refers to any chemical compound (*e.g.*, a peptide epoxyketone compound) used in the diagnosis, treatment, or prevention of disease or other abnormal condition.

[0078] The term “prodrug” as used herein refers to compounds that, under physiological conditions, are converted into therapeutically active agents. A common method for making a prodrug is to include selected moieties that are hydrolyzed under physiological conditions to reveal the desired molecule. In other embodiments, the prodrug is converted by an enzymatic activity of the subject.

[0079] The terms “therapeutically acceptable” and “pharmaceutically acceptable” as used herein refer to a material that is not biologically or otherwise undesirable, *i.e.*, the material can be administered to a subject together with an active ingredient without causing undesirable biological effects or interacting adversely with any other component of the composition.

[0080] The term “emulsion” as used herein refers to a mixture of two immiscible (unblendable) substances.

[0081] The term “bilayer” as used herein refers to a structure composed of amphiphilic lipid molecules (often phospholipids) arranged in two molecular layers, with the hydrophobic tails on the interior and the polar head groups on the exterior surfaces.

[0082] The term “monolayer” as used herein refers to a single molecular layer of amphipathic molecules with the head groups aligned on one side, and hydrophobic groups on the opposite side.

[0083] The term “liposome” as used herein refers to a vesicle comprising a lipid bilayer, for example, a closed vesicle formed when phospholipids or their derivatives are dispersed in water. The liposomes of the present invention typically comprise one or more phospholipids, and may also contain mixed lipid chains with surfactant properties (*e.g.*, egg phosphatidylethanolamine). Liposome can employ surface ligands to target binding to unhealthy tissue (*e.g.*, tumors or neoplastic cells). Liposomes typically have an aqueous core.

[0084] The term “entrapped” as used herein refers to the non-covalent association of peptide epoxyketone compounds with a liposome bilayer and/or the liposome’s interior aqueous volume (also called the liposome's aqueous core).

[0085] The terms “liposomal composition” and “liposome-containing composition” are used interchangeably herein and refer to liposome formulations or mixtures comprising lipids and peptide epoxyketone compounds, and such liposome mixtures or formulations can further comprise additional excipients. A liposomal composition typically comprises an aqueous solution comprising the liposomes. Encapsulated aqueous solution is aqueous solution in the aqueous core of the liposomes. Non-encapsulated aqueous solution is aqueous solution in which the liposomes are dispersed.

[0086] The term “excipient” as used herein typically refers to any pharmacologically inactive substance used for in the formulation or administration of the liposomal compositions of the present invention, for example, phospholipid, buffer, a carrier or vehicle (such as diluents), and so on. Examples of excipients useful in the practice of the present invention are described herein.

[0087] The term "pH adjusting agent" as used herein refers to any agent used to modify the pH of an aqueous solution. pH is adjusted by using acidifying (e.g., acids) and alkalizing agents (e.g., salts of acids or bases). Acidifying agents are used in a formulation to lower the pH and alkalizing agents are used to increase the pH. pH adjusting agents include buffering systems (e.g., combinations of acids and bases). Pharmaceutical compositions of the present invention can contain one or more of these agents to achieve a desirable pH either for preparation (i.e., in bulk solution) of the composition or upon reconstitution for therapeutic administration.

[0088] The term "solublizing agent" as used herein refers to an agent, typically a compound, pH adjusting agent, or cosolvent, that increases the solubility of a peptide epoxyketone compound in an aqueous solution.

[0089] The term “physiological conditions” as used herein refers to conditions compatible with living cells, *e.g.*, predominantly aqueous conditions of a temperature, pH, salinity, etc.

[0090] The terms “therapeutic composition,” “pharmaceutical composition,” “therapeutic preparation,” and “pharmaceutical preparation” are used interchangeably herein and encompass liposomal compositions of the present invention suitable for application or administration to a subject, typically a human. In general such compositions are safe, sterile or aseptic, and preferably free of contaminants that are capable of eliciting undesirable responses in the subject (*i.e.*, the compound(s) comprising the composition are pharmaceutically acceptable). Compositions can be formulated for application or administration to a subject in need thereof by a number of different routes of administration including oral (*i.e.*, administered by mouth or alimentary canal) or parenteral (*e.g.*, buccal, rectal, transdermal, transmucosal, subcutaneous, intravenous, intraperitoneal, intradermal, intratracheal, intrathecal, pulmonary, and the like).

[0091] The term "aseptic conditions " as used herein typically refers to manufacturing or processing conditions wherein the manufactured product is free from contamination with pathogens.

[0092] The term “subject” as used herein refers to any member of the subphylum *chordata*, including, without limitation, humans and other primates, including non-human primates such as rhesus macaque, chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese; and the like. The term does not denote a particular age. Thus, adult, young, and newborn individuals are intended to be covered.

2.0.0 General Overview of the Invention

[0093] Before describing the present invention in detail, it is to be understood that this invention is not limited to particular types of liposomes, particular sources of drugs, particular lipids, particular polymers, and the like, as use of such particulars can be selected in view of the teachings of the present specification. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

[0094] Peptide epoxyketone compounds are proteasome inhibitors useful for the treatment of a wide variety of diseases and conditions. Many peptide epoxyketone compounds (*e.g.*, carfilzomib) have poor water solubility (*e.g.*, carfilzomib is essentially insoluble in water). At present, carfilzomib for injection is prepared by dissolving carfilzomib drug substance in sulfobutylether beta cyclodextrin (SBE- β -CD) with citric acid using a slurry method to create a bulk solution that is then lyophilized to yield a lyophilized carfilzomib suitable for reconstitution and injection.

[0095] However, intravenous administration of the carfilzomib SBE- β -CD composition results in a short half-life due to rapid metabolism. Clearance of carfilzomib is largely extrahepatic, and carfilzomib is predominantly eliminated by peptidase cleavage and epoxide hydrolysis. Therefore, multiple weekly injections are used for treatment regimens. In addition, the use of the SBE- β -CD composition can limit dose increases of carfilzomib, which can impact its best profile activities.

[0096] Liposomes are spherical vesicles, typically comprising phospholipids, that have an internal aqueous volume that is enclosed by one or more concentric lipid bilayers with the polar head groups oriented towards the interior and exterior aqueous phases. Phospholipids are biocompatible and biodegradable as they are naturally occurring in the body and are a major constituent of cell membranes. Liposomes can act as drug carriers by

entrapping drugs in the aqueous core and/or within the lipid bilayers. Liposomes range in size and can exist as unilamellar or multilamellar vesicles.

[0097] The present application describes development of a variety of liposomal compositions incorporating sparingly soluble hydrophobic epoxyketone-based proteasome inhibitors. In some aspects, entrapment of peptide epoxyketone compounds is described. In other aspects, incorporation of peptide epoxyketone compounds into the interior aqueous core of liposomes is described. The liposomal compositions comprising peptide epoxyketone compounds described herein maximize the therapeutic window of peptide epoxyketone compounds by improving tolerability, efficacy and *in vivo* half-life.

[0098] The pharmaceutical liposomal compositions of the present invention are either sterile or aseptic and methods of making the pharmaceutical liposomal compositions are typically carried out under sterile or aseptic conditions. Terminal sterilization of the pharmaceutical liposomal compositions of the present invention can also be employed.

[0099] In a first aspect, the present invention relates to pharmaceutical liposomal compositions. In some embodiments, the pharmaceutical liposomal compositions comprise liposome entrapped peptide epoxyketone compound, wherein (i) liposomes of the liposomal composition comprise one or more lipids (e.g., L- α -phosphatidylcholine, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, and/or sphingomyelin), and in the liposomes the weight ratio of peptide epoxyketone compound:lipid is between about 0.01:1 and about 1:1. In a preferred embodiment, the weight ratio of peptide epoxyketone compound:lipid is between about 0.05:1 to about 0.5:1. Typically, the liposomes have an average size of between about 0.05 microns to about 0.5 microns, preferably an average size of between about 0.05 microns to about 0.2 microns. Pharmaceutical liposomal compositions of the present invention typically comprise an aqueous solution in which liposomes are dispersed.

[00100] Embodiments of the pharmaceutical liposomal compositions present invention include, but are not limited to, the following: wherein the lipids of the liposomes comprise between about 20 to about 100 weight percent phospholipids; wherein the lipids of the liposomes further comprise between about 10 and about 50 weight percent cholesterol; wherein the lipids of the liposomes comprise between about 1 and about 20 weight percent of a hydrophilic polymer-derivatized lipid; and combinations thereof.

[00101] In some embodiments, the lipid of the hydrophilic polymer-derivatized lipid is cholesterol or a phospholipid.

[00102] In some embodiments, the hydrophilic polymer of a hydrophilic polymer-derivatized lipid is a polyethylene glycol.

[00103] In a preferred embodiment, the hydrophilic polymer-derivatized lipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (mPEG2000DSPE).

[00104] The lipids of the liposomes, in some embodiments, further comprise α -tocopherol, for example, at about 0.001 to about 5 weight percent.

[00105] The aqueous solution in which the liposomes are dispersed can also comprise one or more excipients, including, but not limited to, a pH adjusting agent (e.g., a buffer) and/or an agent to maintain isotonicity.

[00106] In other embodiments of this first aspect of the present invention, the liposomal composition comprises liposomes comprising the peptide epoxyketone compound and a solubilizing agent (e.g., a compound) in an internal aqueous core of the liposomes. In some embodiments, the solubilizing agent is a compound (e.g., a cyclodextrin), and the liposomes of the liposomal composition comprise the peptide epoxyketone compound complexed with the compounds (e.g., a cyclodextrin) in the internal aqueous core of the liposomes. A preferred solubilizing agent that is a compound is a cyclodextrin, for example, a sulfobutylether-beta-cyclodextrin or a hydroxypropyl-beta-cyclodextrin.

[00107] The pharmaceutical liposomal composition of the present invention can also include liposomal compositions wherein the aqueous solution is adjusted to between about pH 3.5 and about pH 7.0. Preferably, the aqueous solution is adjusted to a human physiological pH.

[00108] Examples of peptide epoxyketone compounds for use in liposomal compositions of the present invention include, but are not limited to, compound I. Preferred peptide epoxyketone compounds for use in liposomal compositions include compound II, compound III, compound IV, and, most preferably carfilzomib (compound V).

[00109] Preferred embodiments of pharmaceutical liposomal compositions comprising peptide epoxyketone compounds include, but are not limited to, the following: peptide epoxyketone compound – EPC – mPEG2000DSPE – cholesterol; and peptide epoxyketone compound – sphingomyelin – mPEG2000DSPE – cholesterol.

[00110] In a second aspect the present invention relates to dry pharmaceutical compositions formed by drying the pharmaceutical liposomal compositions described herein. One embodiment of this second aspect of the present invention is a dry pharmaceutical composition comprising one or more lipids, the lipids comprising at least one phospholipid

selected from the group consisting of L- α -phosphatidylcholine, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, and sphingomyelin, wherein the weight ratio of peptide epoxyketone compound:lipid is between about 0.01:1 and about 1:1. In some embodiments, dry pharmaceutical compositions further comprise additional excipients, for example cryoprotectant agents (e.g., glycerol, dimethylamine, dimethylsulfoxide), glass transition modifying agents (e.g. sugars, polyols, polymers, amino acids), combinations thereof, and/or other stabilizing excipients.

[00111] In a third aspect, the present invention relates to a method of making a pharmaceutical liposomal composition, the method comprising: preparing a dried film comprising a peptide epoxyketone compound and at least one lipid (e.g., a phospholipid such as L- α -phosphatidylcholine, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, and/or sphingomyelin), wherein the weight ratio of peptide epoxyketone compound:lipid is between about 0.01:1 and about 1:1 (preferably the weight ratio of peptide epoxyketone compound:lipid is between about 0.05:1 to about 0.5:1); and rehydrating the dried film with an aqueous solution to form a liposomal composition comprising liposomes and an aqueous solution.

[00112] In preferred embodiments, the method further comprises sizing the liposomes to have an average size of between about 0.05 microns to about 0.5 microns (preferably an average size of between about 0.05 microns to about 0.2 microns).

[00113] Embodiments of the methods of this aspect of the present invention for making pharmaceutical liposomal compositions include, but are not limited to, the following: wherein the lipids of the dried film comprise between about 20 to about 100 weight percent phospholipids; wherein the lipids of the dried film further comprise between about 10 and about 50 weight percent cholesterol; wherein the lipids of the dried film further comprise between about 1 and about 20 weight percent of a hydrophilic polymer-derivatized lipid; and combinations thereof.

[00114] In some embodiments, the lipid of the hydrophilic polymer-derivatized lipid is cholesterol or a phospholipid.

[00115] In some embodiments, the hydrophilic polymer of a hydrophilic polymer-derivatized lipid is a polyethylene glycol.

[00116] In a preferred embodiment, the hydrophilic polymer-derivatized lipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000].

[00117] The lipids of the dried film, in some embodiments, further comprise α -tocopherol, for example, at about 0.001 to about 5 weight percent.

[00118] In some embodiments, the method further comprises adding one or more excipients, including, but not limited to, a pH adjusting agent (e.g., a buffer) and/or an agent to maintain isotonicity, to the aqueous solution in which the liposomes are dispersed.

[00119] In some embodiments, the method further comprises adjusting the pH of the aqueous solution to between about pH 3.5 and about pH 7.0, preferably adjusting the pH of the aqueous solution to a human physiological pH.

[00120] Examples of peptide epoxyketone compounds for use in the method of making liposomal compositions of the present invention include, but are not limited to, compound I. Preferred peptide epoxyketone compounds for use in liposomal compositions include compound II, compound III, compound IV, and, most preferably carfilzomib (compound V).

[00121] In a fourth aspect, the present invention relates to pharmaceutical liposomal compositions made by the methods of the third aspect of the invention.

[00122] In a fifth aspect, the present invention relates to a method of making a pharmaceutical liposomal composition comprising preparing a dried film comprising one or more lipids and rehydrating the dried film with an aqueous solution comprising a peptide epoxyketone compound. Typically the method comprises preparing a dried film comprising at least one phospholipid (e.g., L- α -phosphatidylcholine, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, and/or sphingomyelin; and rehydrating the dried film with an aqueous solution comprising a peptide epoxyketone compound and one or more solubilizing agent to form a liposomal composition comprising liposomes dispersed in the aqueous solution.

[00123] In some embodiments of this fifth aspect of the present invention, one or more solubilizing agent is, for example, a compound, a pH adjusting agent (e.g., an organic or non-organic acid), a cosolvent (e.g., ethanol or dimethylsulfoxide), or a combination thereof. Cyclodextrin (preferably a sulfobutylether-betacyclodextrin or a hydroxypropyl-betacyclodextrin) is an example of a compound used as a solubilizing agent. In some embodiments, the pH adjusting agent is used to bring the pH of the aqueous solution to between about pH 0.5 and pH 3.0, preferably to a pH of about pH 1.0. In some embodiments, after formation of the liposomes the pH of the liposomal composition is adjusted to between about pH 3.5 and about pH 7.0, preferably to about a human physiological pH. In some embodiments, for example for pH adjustment and/or

removal of a cosolvent, the method further comprises processing the liposomal composition using dialysis, desalting, buffer exchange, and/or gel filtration.

[00124] The weight ratio of peptide epoxyketone compound:lipid in the liposomes of the liposomal composition can be, for example, between about 0.01:1 and about 1:1.

[00125] The method can further comprise sizing the liposomes to have an average size of between about 0.05 microns to about 0.5 microns, an average size of between about 0.05 microns to about 0.2 microns.

[00126] Embodiments of the methods of this aspect of the present invention for making pharmaceutical liposomal compositions include, but are not limited to, the following: wherein the lipids of the dried film comprise between about 20 to about 100 weight percent phospholipids; wherein the lipids of the dried film further comprise between about 10 and about 50 weight percent cholesterol; wherein the lipids of the dried film further comprise between about 1 and about 20 weight percent of a hydrophilic polymer-derivatized lipid; and combinations thereof.

[00127] In some embodiments, the lipid of the hydrophilic polymer-derivatized lipid is cholesterol or a phospholipid.

[00128] In some embodiments, the hydrophilic polymer of a hydrophilic polymer-derivatized lipid is a polyethylene glycol.

[00129] In a preferred embodiment, the hydrophilic polymer-derivatized lipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000].

[00130] The lipids of the dried film, in some embodiments, further comprise α -tocopherol, for example, at about 0.001 to about 5 weight percent.

[00131] In some embodiments, the method further comprises, after forming the liposomal composition, removing peptide epoxyketone compound from the aqueous solution in which the liposomes are dispersed (that is, non-encapsulated aqueous solution) using, for example, dialysis, ultracentrifugation, gel filtration, or a combination thereof.

[00132] In some embodiments, the method further comprises adding one or more excipients, including, but not limited to, a pH adjusting agent (e.g., a buffer) and/or an agent to maintain isotonicity, to the aqueous solution in which the liposomes are dispersed.

[00133] In some embodiments, the method further comprises adjusting the pH of the aqueous solution to between about pH 3.5 and about pH 7.0, preferably adjusting the pH of the aqueous solution to a human physiological pH.

[00134] Examples of peptide epoxyketone compounds for use in the method of making liposomal compositions of the present invention include, but are not limited to, compound I.

Preferred peptide epoxyketone compounds for use in liposomal compositions include compound II, compound III, compound IV, and, most preferably carfilzomib (compound V).

[00135] In a sixth aspect, the present invention relates to pharmaceutical liposomal compositions made by the methods of the fifth aspect of the invention.

[00136] In a seventh aspect, the present invention relates to a method of making a pharmaceutical liposomal composition comprising preparing a lipid solution comprising one or more lipids and a solvent and injecting the lipid solution into an aqueous solution comprising a peptide epoxyketone compound. Typically the method comprises preparing a lipid solution comprising a solvent and at least one phospholipid (e.g., L- α -phosphatidylcholine, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, and/or sphingomyelin); and injecting the lipid solution into an aqueous solution comprising a peptide epoxyketone compound and one or more solubilizing agent to form a liposomal composition comprising liposomes dispersed in the aqueous solution.

[00137] In some embodiments the solvent is an organic solvent, for example an alcohol (e.g., ethanol).

[00138] In some embodiments of this seventh aspect of the present invention, the one or more solubilizing agent is, for example, a compound, a pH adjusting agent (e.g., an organic or non-organic acid), a cosolvent (e.g., ethanol or dimethylsulfoxide), or a combination thereof. Cyclodextrin (preferably a sulfobutylether-betacyclodextrin or a hydroxypropyl-betacyclodextrin) is an example of a compound used as a solubilizing agent. In some embodiments, the pH adjusting agent is used to bring the pH of the aqueous solution to between about pH 0.5 and pH 3.0, preferably to a pH of about pH 1.0.

[00139] In some embodiments, for example for pH adjustment and/or removal of a solvent and/or a cosolvent, the method further comprises processing the liposomal composition using dialysis, desalting, buffer exchange, and/or gel filtration.

[00140] The weight ratio of peptide epoxyketone compound:lipid in the liposomes of the liposomal composition can be, for example, between about 0.01:1 and about 1:1.

[00141] The method can further comprise sizing the liposomes to have an average size of between about 0.05 microns to about 0.5 microns, an average size of between about 0.05 microns to about 0.2 microns.

[00142] Embodiments of the methods of this aspect of the present invention for making pharmaceutical liposomal compositions include, but are not limited to, the following: wherein the lipids of the liposomes of the liposomal composition comprise between about 20

to about 100 weight percent phospholipids; wherein the lipids of the liposomes of the liposomal composition further comprise between about 10 and about 50 weight percent cholesterol; wherein the lipids of the liposomes of the liposomal composition further comprise between about 1 and about 20 weight percent of a hydrophilic polymer-derivatized lipid; and combinations thereof.

[00143] In some embodiments, the lipid of the hydrophilic polymer-derivatized lipid is cholesterol or a phospholipid.

[00144] In some embodiments, the hydrophilic polymer of a hydrophilic polymer-derivatized lipid is a polyethylene glycol.

[00145] In a preferred embodiment, the hydrophilic polymer-derivatized lipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000].

[00146] The lipids of the liposomes of the liposomal composition, in some embodiments, further comprise α -tocopherol, for example, at about 0.001 to about 5 weight percent.

[00147] In some embodiments, the method further comprises, after forming the liposomal composition, removing peptide epoxyketone compound from the aqueous solution in which the liposomes are dispersed (that is, non-encapsulated aqueous solution) using, for example, dialysis, ultracentrifugation, gel filtration, or a combination thereof.

[00148] In some embodiments, the method further comprises adding one or more excipients, including, but not limited to, a pH adjusting agent (e.g., a buffer) and/or an agent to maintain isotonicity, to the aqueous solution in which the liposomes are dispersed.

[00149] In some embodiments, the method further comprises adjusting the pH of the aqueous solution to between about pH 3.5 and about pH 7.0, preferably adjusting the pH of the aqueous solution to a human physiological pH.

[00150] Examples of peptide epoxyketone compounds for use in the method of making liposomal compositions of the present invention include, but are not limited to, compound I. Preferred peptide epoxyketone compounds for use in liposomal compositions include compound II, compound III, compound IV, and, most preferably carfilzomib (compound V).

[00151] In an eighth aspect, the present invention relates to pharmaceutical liposomal compositions made by the methods of the seventh aspect of the invention.

[00152] In a ninth aspect, the present invention relates to methods of treating a disease or condition in a subject in need of treatment, comprising administering a therapeutically effective amount of a pharmaceutical liposomal composition, as described herein, comprising liposomes comprising a peptide epoxyketone compound. In some embodiments the methods

of treating further comprise simultaneous, sequential, or separate administration of a therapeutically effective amount of another therapeutic agent, for example, a chemotherapeutic agent, a cytokine, a steroid, an immunotherapeutic agent, or combinations thereof. Examples of diseases or conditions that are treated using the liposomal compositions of the present invention comprising peptide epoxyketone compounds include, but are not limited to, multiple myeloma, solid tumors, infections, and autoimmune diseases.

[00153] In another aspect, the present invention includes dry pharmaceutical compositions comprising one or more lipids and a peptide epoxyketone compound. Such dry pharmaceutical compositions can be rehydrated for use in the methods of the present invention. In one embodiment, a dry pharmaceutical composition comprises, one or more amphipathic lipids (*e.g.*, lipids comprising at least one phospholipid), and (ii) a peptide epoxyketone compound. Dry pharmaceutical compositions can be made using any of the liposomal compositions of the present invention described herein (*e.g.*, compositions further comprising a solubilizing agent), as well as liposomal compositions comprising peptide epoxyketone compounds made by any of the methods described herein. In some embodiments, dry pharmaceutical compositions further comprise additional excipients, for example cryoprotectant agents (*e.g.*, glycerol, dimethylamine, dimethylsulfoxide), glass transition modifying agents (*e.g.* sugars, polyols, polymers, amino acids), and/or other stabilizing excipients.

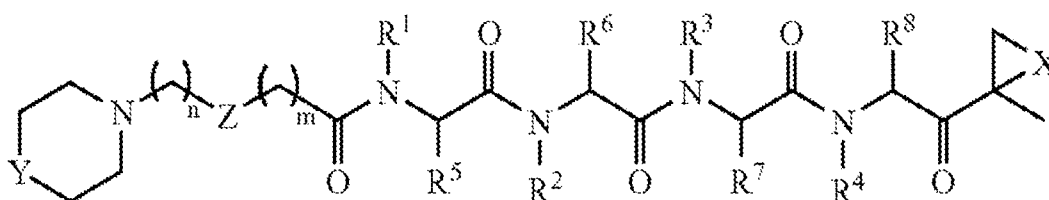
3.0.0 Pharmaceutical Compositions

[00154] The present invention relates to liposomal compositions of peptide epoxyketones compounds (*e.g.*, carfilzomib) and prodrugs thereof, and methods of making and using such compositions.

3.1.0 Peptide Epoxyketone Compounds

[00155] Examples of peptide epoxyketone compounds useful in the practice of the present invention are described in U.S. Patent No. 7,417,042, and include, but are not limited to, a peptide epoxyketone compound having the structure of formula I:

I

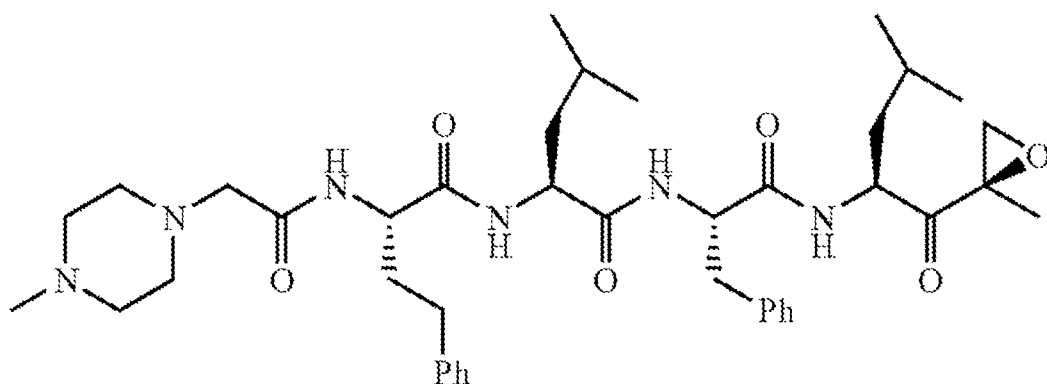


[00156] wherein X is O, NH, or N-alkyl; Y is NH, N-alkyl, O, or C(R⁹)₂; Z is O or C(R⁹)₂; R¹, R², R³, and R⁴ are all hydrogen; each R⁵, R⁶, R⁷, R⁸, and R⁹ is independently selected from hydrogen, C₁₋₆alkyl, C₁₋₆hydroxyalkyl, C₁₋₆alkoxyalkyl, aryl, and C₁₋₆aralkyl, each of which is optionally substituted with a group selected from alkyl, amide, amine, carboxylic acid or a pharmaceutically acceptable salt thereof, carboxyl ester, thiol, and thioether; m is an integer from 0 to 2; and n is an integer from 0 to 2. Terms used to describe these compounds are further set forth in the "Definitions" section.

[00157] Examples of specific peptide epoxyketone compounds useful in the practice of the present invention include the following compounds having formulas II, III, and IV ("Ph" in the following compounds represents a phenyl group):

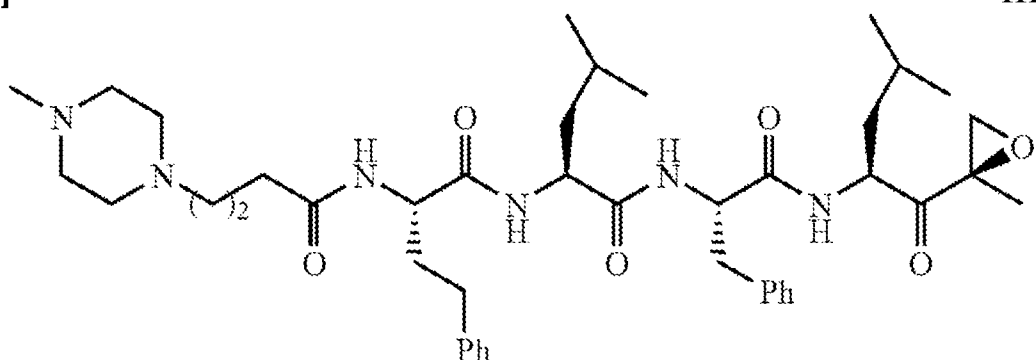
[00158]

II

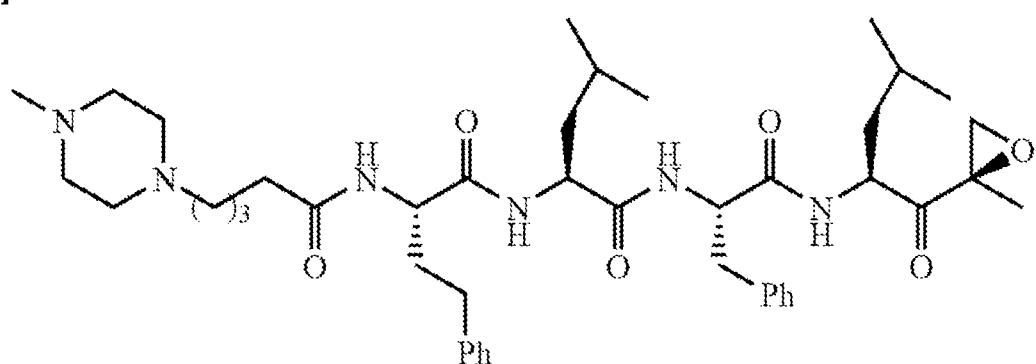


[00159]

III

**[00160]**

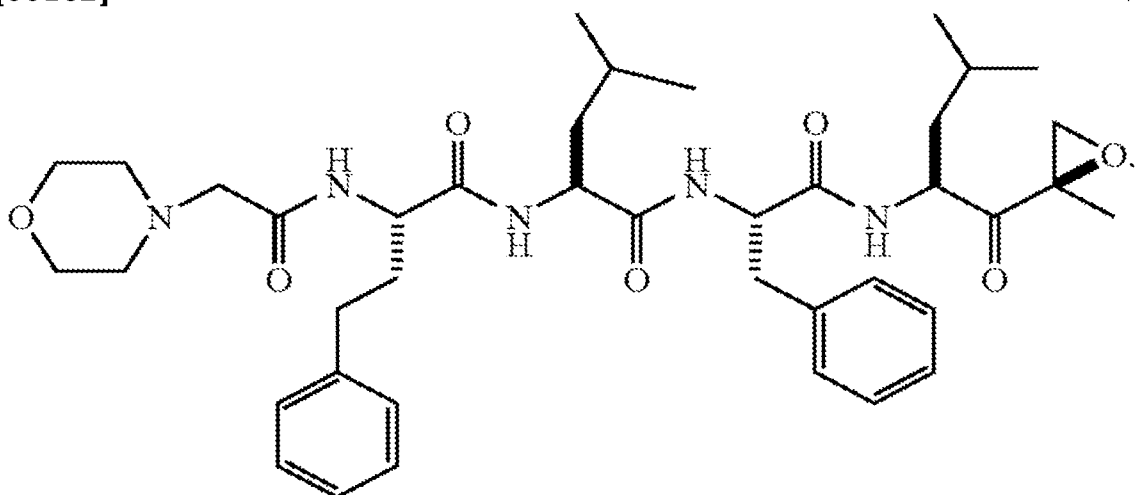
IV



[00161] In a preferred embodiment of the present invention, the peptide epoxyketone compound is carfilzomib having formula V:

[00162]

V



[00163] In the liposomal compositions of the present invention, the weight ratio of peptide epoxyketone compound:total lipid in the liposomes is between about 0.01:1 and about 1:1, preferably between about 0.025:1 to about 0.5:1, and more preferably between about 0.05:1 to about 0.25:1.

3.2.0 Liposome Components

[00164] Types of lipids used in the practice of the present invention include, but are not limited to phospholipids, sterols, and modifications and derivatives thereof. Additional amphipathic lipids can also be used in the practice of the present invention.

[00165] Preferred vesicle forming amphipathic lipids for use in the practice of the present invention include phospholipids and derivatives thereof. Phospholipids fall generally into three classes, neutral, cationic, and anionic. Examples of phospholipids useful in the practice of the present invention include, but are not limited to, the following:

phosphatidylcholine; L- α -phosphatidylcholine (egg phosphatidylcholine (EPC), or hydrogenated soy phosphatidylcholine (HSPC)); 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC); 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC); phosphatidylserine (PS); phosphatidylinositol (PI); phosphatidylglycerol (PG); phosphatidylethanolamine (PE); dioleoyl phosphatidylglycerol (DOPG); dioleoyl phosphatidylcholine (DOPC); dioleoyl phosphatidylserine (DOPS); 1,2-dioleoyl-*sn*-3-phosphoethanolamine (DOPE); diacylphosphatidylcholine; diacylphosphatidic acid; N-dodecanoyl phosphatidylethanolamine; N-succinyl phosphatidylethanolamine; N-glutaryl phosphatidylethanolamine; lysylphosphatidylglycerol; sphingolipids (*e.g.*, sphingomyelin); and mixtures thereof.

[00166] Further lipids useful in the practice of the present invention include, for example, N,N-dioleoyl-N,N-dimethylammonium chloride (DODAC); N-(2,3-dioleoyloxy)propyl-N,N,N-triethylammonium chloride (DOTMA); N,N-distearoyl-N,N-dimethylammonium bromide (DDAB); N-(2,3-dioleoyloxy)propyl-N,N,N-trimethylammonium chloride (DOTAP); N-(1-(2,3-dioleoyloxy)propyl)-N-2-(sperminecarboxamido)ethyl-N,N-dimethylammonium trifluoroacetate (DOSPA); dioctadecylamidoglycylcarboxyspermine (DOGS); N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE); stearylamine; dicetyl phosphate; β -oleoyl- γ -palmitoyl; and mixtures thereof.

[00167] Preferred lipids for use in the practice of the present invention include, but are not limited to: L- α -phosphatidylcholine (*e.g.*, egg phosphatidylcholine (EPC), or hydrogenated soy phosphatidylcholine (HSPC)); 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC); and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). In some embodiments, the lipids of the liposomes comprise between about 20 to about 100 weight percent phospholipid, preferably between about 30 and about 90 weight percent phospholipid.

[00168] A variety of sterols and derivatives thereof (*e.g.*, cholesterol) can be used to stabilize liposomes. Sterol-modified amphiphilic lipids are known in the art (see, *e.g.*, U.S. Patent Application Publication No. 2011/0177156). Sterols for use in the practice of the present invention, such as cholesterol, also can be derivatized with a variety of hydrophilic polymers (PEG-cholesterol derivatives; see, *e.g.*, U.S. Patent No. 6,270,806). In an embodiment of the present invention, sterols or derivatives thereof can be added to the liposomal composition to stabilize the lipid bilayer. Preferred sterols for use in the practice of the present invention are cholesterol and its derivatives (*e.g.*, cholesterol hemisuccinate), for example, the lipids of the liposomes of the liposomal compositions of the present invention can comprise between about 10 and about 50 weight percent cholesterol, preferably between about 15 and about 40 weight percent cholesterol.

[00169] In other embodiments, cholesterol is chemically modified with a ligand designed to be recognized by a particular organ or cell type such as a long chain fatty acid, an amino acid, an oligosaccharide, a hormone, an amino acid derivative, a protein, glycoprotein, modified protein, or the like. The resultant liposome is suitable for being targeted to a specific organ or cell type (see, *e.g.*, U.S. Patent No. 4,544,545).

[00170] Additional examples of liposomal compositions including targeting factors that can be used, in view of the teachings of the present specification, include U.S. Patent Nos. 5,049,390; 5,780,052; 5,786,214; 5,830,686; 6,056,973; 6,110,666; 6,177,059; 6,245,427; 6,316,024; 6,524,613; 6,530,944; 6,749,863; 6,803,360; 6,960,560; 7,060,291; 7,101,985; and U.S. Patent Application Nos. 2002/0198164; 2003/0027779; 2003/0220284; 2003/0224037; 2003/0228285; 2003/143742; and 2004/0022842.

[00171] Steric stabilization refers to the colloidal stability conferred on the liposome by a variety of hydrophilic polymers or hydrophilic glycolipids, for example, polyethylene glycol and the ganglioside GM1. Liposomes can contain PEG-PE, GM1, or another such glycolipid or polymer that demonstrates a relatively long half-life in the general circulation. Hydrophilic polymers such as PEG and other polyethoxylated polymers can be used to shield liposomes to enhance the circulatory half-life of the liposome. Such hydrophilic polymers can be associated non-covalently with the liposomes or conjugated or covalently linked to a particular component of the liposome (*e.g.*, PEG-modified lipids; 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt) (mPEG-DSPE)). Additional exemplary hydrophilic polymers include, but are not limited to, polyvinyl alcohol, polylactic acid, polyglycolic acid, polyvinylpyrrolidone, polyacrylamide, polyglycerol, polyaxozlines, and mixtures thereof.

[00172] In some embodiments of the liposomal compositions described herein, the lipids of the liposomes can comprise between about 0.1 and about 30 weight percent, preferably between about 1 and about 20 weight percent of a hydrophilic polymer-derivatized lipid. Preferred hydrophilic polymers for use in the practice of the present invention are polyethylene glycols (*e.g.*, phospholipids conjugated to monomethoxy polyethylene glycol, for example, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (mPEG-DSPE)).

[00173] Additional examples of liposomal compositions that can be used, in view of the teachings of the present specification, include: U.S. Patent Nos. 4,789,633; 4,925,661; 4,983,397; 5,013,556; 5,534,241; 5,593,622; 5,648,478; 5,676,971; 5,756,069; 5,834,012; 5,846,458; 5,891,468; 5,945,122; 6,056,973; 6,057,299; 6,077,834; 6,126,966; 6,153,596; 6,287,593; 6,316,024; 6,387,397; 6,476,068; 6,586,559; 6,627,218; 6,723,338; 6,897,196; 6,936,272; 6,960,560; 7,122,202; 7,311,924; 7,361,640; and 7,901,708; and U.S. Patent Application Publication Nos. 2003/0072794; 2003/0082228; 2003/0166601; 2003/0203865; 2003/0215490; 2003/0224037; 2004/0022842; 2004/0234588; and 2005/0136064.

[00174] The liposomal compositions typically comprise liposome entrapped peptide epoxyketone compounds and an aqueous carrier.

[00175] Typical excipients useful in the practice of the present invention include, but are not limited to, the following: carrier or vehicle (*e.g.*, water or buffered aqueous solutions); pH adjusting agents; antioxidants (*e.g.*, α -tocopherol, methionine, ascorbic acid, sodium thiosulfate, ethylenediaminetetraacetic acid, citric acid, cysteins, thioglycerol, thioglycolic acid, thiosorbitol, butylated hydroxanisole, butylated hydroxytoluene, and propyl gallate, and mixtures thereof); agents to maintain isotonicity (*e.g.*, sodium chloride, sugars, polyols (sugar alcohols), boric acid, sodium tartrate, propylene glycol, and mixtures thereof); one or more sugars (*e.g.*, trehalose, maltose, sucrose, lactose, mannose, dextrose, fructose, etc.) or sugar alcohol (*e.g.*, sorbitol, maltitol, lactitol, mannitol, glycerol, etc.), alcohol (*e.g.*, ethanol, t-butanol, etc.); and preservatives (alcohols, benzoic acid, salicylic acid, phenol and its derivatives (*e.g.*, cresol, p-cresol, m-cresol and o-cresol), cetrimide, BHA (butylated hydroxytoluene), BHA (butylated hydroxyanisole); and mixtures thereof).

[00176] pH adjusting agents useful in the practice of the present invention include, but are not limited to hydrochloric acid, sodium hydroxide, citric acid, phthalic acid, acetic acid, ascorbic acid, phosphate, glutamate, sodium or potassium succinate, tartrate, histidine, sodium or potassium phosphate, Tris (tris (hydroxymethyl)aminomethane), and diethanolamine. Buffers comprising both acids and bases/salts can also be used.

[00177] In a preferred embodiment of the present invention, the liposomes can be rehydrated using buffered aqueous solutions (*e.g.*, phosphate buffer saline (PBS)), 0.9% Saline, 5% Dextrose, 10% Sucrose, or water for injection (WFI) as the rehydration medium. In some embodiments, the pH of the aqueous phase of the liposomal compositions is adjusted, for example, to approximately human physiological pH (*i.e.*, between about pH 6.5 and about pH 7.5). Excipients typically present in the aqueous phase include, but are not limited to, buffer systems, agents to maintain isotonicity, sugars, sugar alcohols, and/or preservatives.

[00178] Exemplary embodiments of liposomal compositions of peptide epoxyketones include, but are not limited to, the following: carfilzomib – EPC; carfilzomib – EPC – cholesterol; carfilzomib – DSPC; carfilzomib – DSPC – cholesterol; carfilzomib – DPPC; carfilzomib – DPPC – cholesterol; carfilzomib – sphingomyelin; carfilzomib – sphingomyelin – cholesterol.

[00179] Further examples comprise PEGylated liposomal compositions of peptide epoxyketones that include, but are not limited to, the following: carfilzomib – EPC – mPEG2000DSPE; carfilzomib – EPC – mPEG2000DSPE – cholesterol; carfilzomib – DSPC – mPEG2000DSPE; carfilzomib – DSPC – mPEG2000DSPE – cholesterol; carfilzomib – sphingomyelin – mPEG2000DSPE; and carfilzomib – sphingomyelin – mPEG2000DSPE – cholesterol.

[00180] Preferred embodiments of liposomal compositions of peptide epoxyketones include, but are not limited to, the following: carfilzomib – EPC – mPEG2000DSPE – cholesterol; and carfilzomib – sphingomyelin – mPEG2000DSPE – cholesterol.

[00181] Examples of specific embodiments of liposomal compositions are set forth in Examples 1, 7, 10, and 11.

4.0.0 Preparing Liposomal Compositions

[00182] Liposomes can be prepared by a variety of techniques (*e.g.*, Szoka, F., Jr., et al., “Comparative Properties and Methods of Preparation of Lipid Vesicles (Liposomes),” *Annual Review of Biophysics and Bioengineering*, June 1980, 9:467-508; U.S. Patent No. 4,235,871) including reverse phase evaporation methods. The reverse phase evaporation vesicles initially have typical average sizes between about 2-4 microns.

[00183] In some embodiments, liposomes are formed by simple lipid-film hydration techniques (see, *e.g.*, Examples 1 and 2). In this procedure, a mixture of liposome-forming lipids of the type described herein and peptide epoxyketone compounds are dissolved in a suitable organic solvent and evaporated in a vessel to form a thin film, which is then covered

by an aqueous medium. The lipid film hydrates to form vesicles typically with sizes between about 0.1 to 10 microns.

[00184] Other embodiments of the present invention include, a method of passively encapsulating a hydrophobic, water-insoluble, peptide expoxyketone compound into the internal aqueous core of the liposome. Such encapsulation in the aqueous core can be facilitated using one or more solubilizing agent. Solubilizing agents increase the solubility of a peptide expoxyketone compound in an aqueous solution. Solubilizing agents include, for example, compounds to facilitate solubilization (e.g., cyclodextrin), pH adjusting agents, cosolvents, and combinations thereof. Advantages of encapsulating peptide expoxyketone compounds in the interior aqueous core of liposomes include greater protection from chemical and biological degradation, slower diffusion, greater tissue distribution and extended drug release profiles.

[00185] Cyclodextrins are an example of compounds to facilitate solubilization of peptide expoxyketone compounds in aqueous solution. Cyclodextrins can be charged or neutral, native (cyclodextrins α , β , γ , δ , ϵ), branched or polymerized. In certain aspects, cyclodextrins can be chemically modified, for example, by substitution of one or more hydroxypropyls by groups such as alkyls, aryls, arylalkyls, glycosidics, or by etherification, esterification with alcohols or aliphatic acids. From these groups, particular preference is given to those from hydroxypropyl, methyl m, sulfobutylether groups (*see, e.g.,* Stella V.J., et al., *Toxicol. Pathol.* 36(1):30-42 (2008)). In certain aspects, cyclodextrins comprise six, seven, or eight glucopyranose units.

[00186] Cyclodextrins include α -cyclodextrin, β -cyclodextrin, and γ -cyclodextrin. Suitable α -cyclodextrins include but are not limited to hydroxypropyl- α -cyclodextrin and hydroxyethyl- α -cyclodextrin. Suitable β -cyclodextrins include but are not limited to hydroxypropyl- β -cyclodextrin (e.g., such as 2-hydroxypropyl cyclodextrin), carboxymethyl- β -cyclodextrin, dihydroxypropyl- β -cyclodextrin, hydroxyethyl- β -cyclodextrin, 2,6-di-O-methyl- β -cyclodextrin, methyl- β -cyclodextrin, randomly methylated cyclodextrin, and sulfated- β -cyclodextrin. Suitable γ -cyclodextrins include hydroxypropyl γ -cyclodextrin, dihydroxypropyl- γ -cyclodextrin, hydroxyethyl γ -cyclodextrin, and sulfated- γ -cyclodextrin.

[00187] Preferred cyclodextrins for use in the practice of the present invention include β -cyclodextrins (such as sulfobutyl ether- β -cyclodextrins (abbreviated as SBE- β -CD or SBE-CD; CAPTISOL® (Ligand Pharmaceuticals, Inc., La Jolla, CA)); or hydroxypropyl-betacyclodextrin (HP- β -CD; Janssen, Titusville NJ; see also Gould S, et al., *Food Chem.*

Toxicol.43(10):1451-9 (2005)); see also U.S. Patent Nos. 4,920,214; 5,385,891; 5,718,905; and 6,046,177).

[00188] Peptide expoxyketone compounds are typically hydrophobic and have low solubility in water. Peptide expoxyketone compounds have increased aqueous solubility in acidic solutions. Accordingly, lowering the pH of the aqueous solution in which a peptide expoxyketone compound is being dissolved can enhance aqueous solubilization. For example, the pH of the aqueous solution can be lowered using a pH adjusting agent to a pH of less than ~pH 2 using an acid, for example, hydrochloric acid. Examples of pH adjusting agents are listed above. Preferred pH adjusting agents for solubilization of peptide expoxyketone compounds include, but are not limited to, hydrochloric acid, citric acid, methanesulfonic acid, sulfuric acid, tartaric acid, acetic acid, and/or maleic acid. A preferred pH for solubilization is typically ~pH 1.

[00189] Further, solubility of peptide expoxyketone compounds in aqueous solutions can be increased by the use of cosolvent solubilization. Examples of cosolvents as solubilizing agents include, but are not limited to, dimethylsulfoxide, methylpyrrolidone, dimethylimidazolidinone, tetrahydrofuran, *N,N*-dimethylacetamide, propylene glycol, benzyl alcohol, polyethylene glycol, ethanol, and combinations thereof.

[00190] As noted above, solubility of peptide expoxyketone compounds in aqueous solutions can be increased by use of solubilizing agents, including, but not limited to, compounds, pH adjusting agents, cosolvents, and combinations thereof.

[00191] In some embodiments, liposomes are formed by a thin film hydration method followed by rehydration using an aqueous solution comprising a peptide expoxyketone compound and solubilizing agent. In such a method, a lipid film is formed wherein the lipid film comprises, for example, any one or combination of phospholipids, including but not limited to the following: L- α -phosphatidylcholine (egg phosphatidylcholine, EPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), and sphingomyelin (SPH); phospholipids conjugated to monomethoxy polyethylene glycol (PEG); and cholesterol. The lipids are typically dissolved in an organic solvent (e.g., Methanol:Chloroform) followed by solvent removal to form a lipid film.

[00192] The peptide expoxyketone compound is solubilized in an aqueous solution comprising, for example, about 1% to about 60% (w/w), preferably about 5% to about 40% of a solubilizing agent (e.g., sulfobutylether-betacyclodextrin or hydroxypropyl-betacyclodextrin). The aqueous solution can also include, for example, a pH adjusting agent

(e.g., citrate buffer, ~pH 3, or Glycine-HCl; ~pH 2) and/or a cosolvent for solubilization of the peptide epoxyketone compound. The aqueous drug solution is used to rehydrate the lipid film. Upon rehydration, self-assembling vesicles form concentric lipid bilayers encapsulating an internal aqueous volume (i.e., aqueous core) of the aqueous solution comprising the peptide epoxyketone compound. The unencapsulated free drug can be removed, for example, by centrifugation and the liposomal composition washed, for example, with phosphate buffer saline. Example 7 describes making liposomal compositions following this method.

[00193] In other embodiments, liposomes are formed by a lipid solution injection method wherein a lipid solution is injected into an aqueous solution comprising a peptide epoxyketone compound. This method typically comprises solubilizing a peptide epoxyketone compound (e.g., in different solid states, such as crystalline or amorphous), using pH control, with or without, cosolvent solubilization in an aqueous solution. The lipids are dissolved in a solvent, for example, ethanol, followed by injection into the aqueous solution comprising the peptide epoxyketone compound while stirring. Liposome vesicles are formed upon injection into the aqueous solution trapping small amounts of aqueous solution in the internal aqueous compartment(s) of the vesicles. Example 10 describes making liposomal compositions following this method.

[00194] In some embodiments, for example for pH adjustment and/or removal of solvent and/or a cosolvent, the methods of the invention further comprise processing the liposomal composition using dialysis, desalting, buffer exchange, and/or gel filtration.

[00195] A liposomal composition of the present invention generally contains a non-homogenous mixture of lipids, peptide epoxyketone compound, and aqueous solution, wherein the liposomes are of substantially homogenous size, with an average size of less than about 1 micron, preferably between about 0.01 to about 1.0 microns, and more preferably between about 0.05 and about 0.5 microns. In some embodiments, liposomes of the liposomal compositions of the present invention have average diameters of less than about 0.2 microns. Sizing serves to eliminate larger liposomes and to produce a defined size range having optimal pharmacokinetic properties.

[00196] One effective sizing method for vesicles involves extruding an aqueous suspension of the liposomes through a series of polycarbonate membranes having a selected uniform pore size in the range of 0.03 to 0.2 micron, typically 0.05, 0.08, 0.1, or 0.2 microns. The pore size of the membrane corresponds roughly to the largest sizes of liposomes produced by extrusion through that membrane, particularly where the preparation is extruded

two or more times through the same membrane. The liposomes can be extruded through successively smaller-pore membranes, to achieve a gradual reduction in liposome size. This method of liposome sizing is used in preparing homogeneous-size vesicle compositions. A more recent method involves extrusion through an asymmetric ceramic filter (see, *e.g.*, U.S. Patent No. 4,737,323). Homogenization methods are also useful for down-sizing liposomes to sizes of 0.1 micron or less.

[00197] Sonication of a liposome suspension either by bath or probe sonication can be used to produce progressive size reduction down to small unilamellar vesicles (SUVs) less than about 0.05 microns in size. Homogenization is another method that relies on shearing energy to fragment large liposomes into smaller ones. In a typical homogenization procedure, vesicles are recirculated through a standard emulsion homogenizer until selected liposome sizes, typically between about 0.1 and 0.5 microns, are observed. In both methods, the particle size distribution can be monitored by conventional laser-beam particle size discrimination. A further sizing method includes use of a microfluidizer.

[00198] Centrifugation and molecular sieve chromatography are other methods available for producing a liposome suspension with particle sizes below a selected threshold less than 1 micron. These two methods both involve preferential removal of larger liposomes, rather than conversion of large particles to smaller ones.

[00199] Examples of preparation, rehydration, and characterization of liposomal compositions of the present invention are presented in Example 1, Example 2, Example 3, Example 7, Example 10, and Example 11 herein.

[00200] In one aspect, the present invention includes methods for the preparation of the liposomal compositions described herein. In one embodiment, a method of making a liposomal composition comprises mixing (typically dissolving) lipid and peptide epoxyketone compound in a suitable solvent, evaporating the solvent to produce a dried film, rehydrating the dried film (which in this embodiment comprises lipid and peptide epoxyketone compound) to form liposomes, and sizing the liposomes. In another embodiment, a method of making a liposomal composition comprises a thin film hydration method which produces a dried film comprising liposomal components followed by rehydration using an aqueous solution comprising a peptide epoxyketone compound as well as a solubilizing agent, a pH adjusting agent, and/or a cosolvent. In yet another embodiment, a method of making a liposomal composition comprises dissolving lipid(s) in solvent(s) and injecting the resulting lipid solution into an aqueous solution comprising a peptide

epoxyketone compound as well as a solubilizing agent, a pH adjusting agent, and/or a cosolvent.

[00201] The present invention also includes liposomal compositions comprising peptide epoxyketone compounds made by the methods described herein.

[00202] Dry pharmaceutical compositions comprising one or more lipids and a peptide epoxyketone compound can be formed from the liposomal compositions described herein, for example, by lyophilization, desiccation, freeze-drying, spray-drying, or similar method. In some embodiments, dry pharmaceutical compositions further comprise additional excipients, for example cryoprotectant agents (e.g., glycerol, dimethylamine, dimethylsulfoxide), glass transition modifying agents (e.g. sugars, polyols, polymers, amino acids), and/or other stabilizing excipients. Such dry pharmaceutical compositions can be rehydrated for use in the methods of the present invention. The rehydration media used for reconstitution of such dry pharmaceutical compositions can include excipients including, but not limited to, a pH adjusting agent, an antioxidant, an agent to maintain isotonicity, a sugar, a sugar alcohol, an alcohol, and/or a preservative.

5.0.0 Uses of the Liposomal Compositions of the Present Invention

[00203] In one aspect of the present invention, the liposomal compositions comprising peptide epoxyketone compounds are useful for the treatment of cancer. Compounds of the invention also can be used to inhibit NF- κ B activation, and stabilize p53 levels in cell culture.

[00204] In one embodiment of the present invention, the liposomal compositions can be used for anti-inflammatory therapeutic intervention in treating conditions associated with chronic inflammation, including, but not limited to COPD, psoriasis, bronchitis, emphysema, and cystic fibrosis.

[00205] In another embodiment of the present invention, the liposomal compositions can be used to treat neurodegenerative diseases and conditions, including, but not limited to: stroke; ischemic damage to the nervous system; neural trauma (e.g., percussive brain damage, spinal cord injury, and traumatic damage to the nervous system); multiple sclerosis and other immune-mediated neuropathies (e.g., Guillain-Barre syndrome and its variants, acute motor axonal neuropathy, acute inflammatory demyelinating polyneuropathy, and Fisher Syndrome); HIV/AIDS dementia complex; axonopathy; diabetic neuropathy; Parkinson's disease; Huntington's disease; multiple sclerosis; bacterial, parasitic, fungal, and viral meningitis; encephalitis; vascular dementia; multi-infarct dementia; Lewy body dementia; frontal lobe dementia such as Pick's disease; subcortical dementias (such as Huntington or

progressive supranuclear palsy); focal cortical atrophy syndromes (such as primary aphasia); metabolic-toxic dementias (such as chronic hypothyroidism or B12 deficiency); and dementias caused by infections (such as syphilis or chronic meningitis).

[00206] In yet another embodiment of the present invention, the liposomal compositions can be used as a treatment for Alzheimer's disease, comprising administering to a subject an effective amount of peptide epoxyketone-containing liposomal compositions disclosed herein. In such cases, the liposomal compositions reduce the rate of β -AP processing, reduce the rate of β -AP plaque formation, reduce the rate of β -AP generation, and reduce the clinical signs of Alzheimer's disease.

[00207] Other embodiments of the present invention relate to methods for treating cachexia and muscle-wasting diseases, cancer, chronic infectious diseases, fever, muscle disuse (atrophy) and denervation, nerve injury, fasting, renal failure associated with acidosis, diabetes, and hepatic failure. Embodiments of the invention encompass methods for: reducing the rate of muscle protein degradation in a cell; reducing the rate of intracellular protein degradation; reducing the rate of degradation of p53 protein in a cell; and inhibiting the growth of p53-related cancers.

[00208] Certain embodiments of the present invention relate to a method for treating hyperproliferative conditions such as diabetic retinopathy, macular degeneration, diabetic nephropathy, glomerulosclerosis, IgA nephropathy, cirrhosis, biliary atresia, congestive heart failure, scleroderma, radiation-induced fibrosis, and lung fibrosis (idiopathic pulmonary fibrosis, collagen vascular disease, sarcoidosis, interstitial lung diseases, and extrinsic lung disorders). The treatment of burn victims often is hampered by fibrosis; thus, an additional embodiment of the invention is the topical or systemic administration of the peptide epoxyketone-containing liposomal composition for burn treatment. Wound closure following surgery often is associated with disfiguring scars, which can be prevented by inhibition of fibrosis. Thus, in certain embodiments, the invention relates to a method for prevention or reduction of scarring.

[00209] Certain embodiments of the present invention relate to a method of treating ischemia and reperfusion injury, which are associated with hypoxia, a deficiency of oxygen reaching the tissues of the body. Examples of such injuries or conditions include, but are not limited to, acute coronary syndrome (vulnerable plaques), arterial occlusive disease (cardiac, cerebral, peripheral arterial and vascular occlusions), atherosclerosis (coronary sclerosis, coronary artery disease), infarctions, heart failure, pancreatitis, myocardial hypertrophy, stenosis, and restenosis.

[00210] Two further embodiments of the present invention are a method for inhibiting or reducing HIV infection in a subject, and a method for decreasing the level of viral gene expression.

[00211] In certain embodiments, compounds of the present invention can be used for the inhibition of TNF α to prevent and/or treat septic shock.

[00212] An additional embodiment of the present invention is a method for inhibiting antigen presentation in a cell. In such method, the liposomal composition is used to treat immune-related conditions such as allergy, asthma, organ/tissue rejection (graft-versus-host disease), and auto-immune diseases, including, but not limited to, lupus, rheumatoid arthritis, psoriasis, multiple sclerosis, and inflammatory bowel diseases (such as ulcerative colitis and Crohn's disease). Thus, a further embodiment is a method for suppressing the immune system of a subject (*e.g.*, inhibiting transplant rejection, allergies, auto-immune diseases, and asthma), including administering to the subject an effective amount of a compound described herein.

[00213] One embodiment of the invention is a method for inhibiting I κ B- α degradation, including contacting the cell with the liposomal composition. A further embodiment is a method for reducing the cellular content of NF- κ B in a cell, muscle, organ, or subject, including contacting the cell, muscle, organ, or subject with the liposomal composition.

[00214] A further embodiment of the invention is a method for treating a proliferative disease in a subject (*e.g.*, cancer, psoriasis, or restenosis), including administering to the subject an effective amount of the liposomal composition. The invention also encompasses a method for treating cyclin-related inflammation in a subject.

[00215] Another embodiment of the present invention is a method for treating p53-related apoptosis.

[00216] In a certain embodiments, the invention's liposomal compositions are useful for the treatment of a parasitic infection, such as infections in humans caused by a protozoan parasite selected from *Plasmodium* spp. (including *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*, which cause malaria), *Trypanosoma* spp. (including *T. cruzi*, which causes Chagas' disease, and *T. brucei* which causes African sleeping sickness), *Leishmania* spp. (including *L. amazonensis*, *L. donovani*, *L. infantum*, *L. mexicana*, etc.), *Pneumocystis carinii* (a protozoan known to cause pneumonia in AIDS and other immunosuppressed patients), *Toxoplasma gondii*, *Entamoeba histolytica*, *Entamoeba invadens*, and *Giardia lamblia*. In certain embodiments, the disclosed compounds are useful for the treatment of parasitic infections in

animals and livestock caused by a protozoan parasite selected from *Plasmodium hermani*, *Cryptosporidium* sps., *Echinococcus granulosus*, *Eimeria tenella*, *Sarcocystis neurona*, and *Neurospora crassa*.

[00217] In one embodiment of the present invention, the liposomal compositions can be useful in the treatment and/or prevention of diseases associated with bone loss, such as osteoporosis.

[00218] Actual dosage levels of peptide epoxyketone compounds in pharmaceutical compositions of this invention can be varied so as to obtain an amount of the peptide epoxyketone compound that is effective to achieve the desired therapeutic response for a particular subject, composition, and mode of administration, without being toxic to the subject.

[00219] The concentration of peptide epoxyketone compound in a pharmaceutically acceptable mixture will vary depending on several factors, including dosage of the compound to be administered, pharmacokinetic characteristics of the compound(s) employed, and route of administration. In general, the liposomal compositions of this invention can be provided in an aqueous solution for parenteral administration. Typical dose ranges are from about 0.01 to about 50 mg/kg of body weight per day of peptide epoxyketone compound, and can be administered in single or divided doses. Each divided dose may contain the same or different compounds of the invention. The dosage will be an effective amount depending on several factors including the overall health of a patient, and the composition and route of administration of the selected peptide epoxyketone compound(s).

[00220] Another aspect of the present invention provides a combination treatment wherein one or more other therapeutic agents are administered with the peptide epoxyketone-containing liposomal composition. Such combination treatment can be achieved by simultaneous, sequential, or separate dosing of the individual components of the treatment.

[00221] In certain embodiments of the present invention, a peptide epoxyketone-containing liposomal composition described herein is used as part of a combination treatment that includes one or more other proteasome inhibitor(s).

[00222] In other embodiments, a liposomal composition of the invention is part of a combination treatment that includes a chemotherapeutic. Suitable chemotherapeutics may include natural products such as vinca alkaloids (*i.e.*, vinblastine, vincristine, and vinorelbine), paclitaxel, epidipodophyllotoxins (*i.e.*, etoposide, teniposide), antibiotics (dactinomycin (actinomycin D), daunorubicin, doxorubicin, and idarubicin), anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin), and mitomycin, enzymes (L-

asparaginase, which systemically metabolizes L-asparagine and deprives cells that do not have the capacity to synthesize their own asparagine); antiplatelet agents; antiproliferative/antimitotic alkylating agents such as nitrogen mustards (mechlorethamine, cyclophosphamide, and analogs, melphalan, chlorambucil), ethylenimines and methylmelamines (hexamethylmelamine and thiotepa), alkyl sulfonates (busulfan), nitrosoureas (carmustine (BCNU) and analogs, streptozocin), trazenes--dacarbazine (DTIC); antiproliferative/antimitotic antimetabolites such as folic acid analogs (methotrexate), pyrimidine analogs (fluorouracil, floxuridine, and cytarabine), purine analogs and related inhibitors (mercaptopurine, thioguanine, pentostatin, and 2-chlorodeoxyadenosine); aromatase inhibitors (anastrozole, exemestane, and letrozole); and platinum coordination complexes (cisplatin, carboplatin), procarbazine, hydroxyurea, mitotane, aminoglutethimide; histone deacetylase (HDAC) inhibitors (trichostatin, sodium butyrate, apicidan, suberoyl anilide hydroamic acid); hormones (*i.e.*, estrogen) and hormone agonists such as leutinizing hormone releasing hormone (LHRH) agonists (goserelin, leuprolide, and triptorelin). Other chemotherapeutic agents may include lenalidomide, mechlorethamine, camptothecin, ifosfamide, tamoxifen, raloxifene, gemcitabine, navelbine, or any analog or derivative variant of the foregoing.

[00223] In some embodiments, the present invention relates to a method of treating cancer (*e.g.*, multiple myeloma or solid tumor) in a subject in need of treatment. The method typically comprises administering a therapeutically effective amount of a pharmaceutical liposomal composition of the present invention (*e.g.*, comprising carfilzomib), and may further comprise simultaneous, sequential, or separate administration of a therapeutically effective amount of a chemotherapeutic agent.

[00224] In certain embodiments of the present invention, a liposomal composition described herein is used in a combination treatment that includes a cytokine. Cytokines include, but are not limited to, Interferon- γ , Interferon- α , and Interferon- β ; Interleukins 1-8, 10, and 12; Granulocyte Monocyte Colony-Stimulating Factor (GM-CSF); TNF- α and TNF- β ; and TGF- β .

[00225] Embodiments of the present invention include combination treatments incorporating a liposomal composition described herein and a steroid. Suitable steroids may include, but are not limited to, 21-acetoxypregnenolone, alclometasone, algestone, amcinonide, beclomethasone, betamethasone, budesonide, chloroprednisone, clobetasol, clocortolone, cloprednol, corticosterone, cortisone, cortivazol, deflazacort, desonide, desoximetasone, dexamethasone, diflorasone, diflucortolone, difuprednate, enoxolone,

fluazacort, flucoronide, flumethasone, flunisolide, fluocinolone acetonide, fluocinonide, fluocortin butyl, fluocortolone, fluorometholone, fluperolone acetate, fluprednidene acetate, fluprednisolone, flurandrenolide, fluticasone propionate, formocortal, halcinonide, halobetasol propionate, halometasone, hydrocortisone, loteprednol etabonate, mazipredone, medrysone, meprednisone, methylprednisolone, mometasone furoate, paramethasone, prednicarbate, prednisolone, prednisolone 25-diethylaminoacetate, prednisolone sodium phosphate, prednisone, prednival, prednylidene, rimexolone, tixocortol, triamcinolone, triamcinolone acetonide, triamcinolone benetonide, triamcinolone hexacetonide, and salts and/or derivatives thereof.

[00226] In certain embodiments of the present invention, a liposomal composition described herein is part of a combination treatment that includes an immunotherapeutic agent. Suitable immunotherapeutic agents may include, but are not limited to, MDR modulators (verapamil, valspodar, biricodar, tariquidar, laniquidar), cyclosporine, thalidomide, and monoclonal antibodies. The monoclonal antibodies can be either naked or conjugated such as rituximab, tositumomab, alemtuzumab, epratuzumab, ibritumomab tiuxetan, gemtuzumab ozogamicin, bevacizumab, cetuximab, erlotinib, and trastuzumab.

[00227] Experiments performed in support of the present invention demonstrated that liposomal compositions of the present invention provided increased maximum tolerated dose (MTD) relative to a non-liposomal composition comprising peptide epoxyketone compound. For example, in mice, a first liposomal composition resulted in a 2.5-fold increase in the MTD, and a second liposomal composition resulted in a 50% increase. In rats, both liposomal compositions resulted in increases in tolerability (Example 4). Biodistribution, as measured by proteasome inhibition in blood and tissues, was similar across the various compositions (Example 5, Example 11). Further, the liposomal peptide epoxyketone compound compositions of the present invention provided about 3 to 5 and 7-fold increased exposure (AUC) in mice and rats, respectively, compared to a non-liposomal composition comprising peptide epoxyketone compound. This increased exposure was the result of a decrease in plasma clearance (Example 6, Example 11).

[00228] Further, liposomal compositions comprising a peptide epoxyketone compound entrapped in the liposomes' aqueous core demonstrated enhanced tolerability by increasing the maximum tolerated dose (MTD) of carfilzomib in mice by 50%, from 10 mg/kg to 15 mg/kg as compared to the injectable, non-liposomal, SBE-CD formulation. These results indicate that liposomal compositions comprising a peptide epoxyketone compound entrapped in the liposomes' aqueous core release carfilzomib over a longer period of time with a lower

maximum plasma concentration (C_{max}) relative to the injectable, non-liposomal, SBE-CD formulation.

[00229] The liposomal compositions comprising liposomes having a peptide epoxyketone compound entrapped in their aqueous core also resulted in delayed proteasome recovery at 24 hours in some mouse tissues whereas the current drug product (i.e., injectable, non-liposomal, CFZ SBE-CD) resulted in recovery from proteasome inhibition by 24 hours post-dose (Example 8, FIG. 3A, FIG. 3B, FIG. 3C, FIG. 3D). These results support that the liposomal compositions provide long-term exposure of peptide epoxyketone compounds.

[00230] In addition, when delivered in the non-liposomal CFZ SBE-CD formulation, the plasma concentration of carfilzomib declined rapidly and was not detectable after 1 hour post-dose (Example 9, FIG. 4). When carfilzomib was delivered as liposomal compositions comprising a peptide epoxyketone compound entrapped in the liposomes' aqueous core, systemic exposure was extended with detectable total drug (both encapsulated and released) for up to 24 hours post-dose (Example 9, FIG. 4). These data demonstrate that the liposomal compositions comprising a peptide epoxyketone compound entrapped in the liposomes' aqueous core resulted in significantly greater exposure and longer circulation relative to an injectable, non-liposomal, peptide epoxyketone compound formulation.

[00231] Also, liposomal compositions of the present invention maintain efficacy at a reduced dosing frequency relative to a non-liposomal formulation (Example 11).

[00232] Accordingly, the data in the Examples demonstrate that liposomal compositions of the present invention resulted in prolonged exposure without affecting biodistribution. Tolerability of the peptide epoxyketone compound was also enhanced in animals, likely due to reduced exposure to high concentrations of free drug. The liposomal compositions comprising peptide epoxyketone compounds of the present invention provide the following improvements relative to the current injectable, non-liposomal, CFZ SBE-CD formulation: improve the pharmacodynamic profile of peptide epoxyketone compounds by delaying proteasome recovery; improve the pharmacokinetic profile by decreasing clearance and extending plasma half-life; improve the safety profile of peptide epoxyketone compounds (i.e. tolerability); and allow reduced dosing frequency.

Experimental

[00233] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to practice the present invention, and are not intended to limit the scope of what the inventors regard as the invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts,

concentrations, percent changes, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, temperature is in degrees Centigrade and pressure is at or near atmospheric.

[00234] The compositions used to practice the methods of the present invention meet the specifications for content and purity required of pharmaceutical products.

1.0 Materials and Methods

[00235] Particle size reduction of liposomal suspension

[00236] Particle size reduction or deagglomeration of the rehydrated liposomal suspension can be carried out either by sonication (20 to 60 minutes) or by high-pressure homogenization/microfluidizer (up to 30,000 psi).

[00237] Content determination by HPLC assay

[00238] The liposomal suspension can be mixed with an organic solvent, for example, methanol, to dissolve lipids and free the carfilzomib. The solution can be filtered through a 0.2µm PTFE filter prior to HPLC analysis.

[00239] Carfilzomib content can be determined by a gradient HPLC assay according to the method in Table 1 using sodium perchlorate buffer, 0.1M, pH 3.1 and acetonitrile (50:50 v/v).

[00240] **Table 1**

Column: Phenomenex Gemini™ C18, 150 x 4.6 mm, 5 µm particle size
Column Temperature: 30 ± 2 °C
Autosampler Temperature: 5 ± 3 °C
Detection Wavelength: 220 nm
Flow Rate: 1.5 mL/min
Injection Volume: 10 µL
Total Run Time: 17 min

[00241] Liposome morphology

[00242] The vesicle size, shape and surface morphology of the liposomal composition can be determined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

[00243] Liposome/carfilzomib solid state

[00244] Polarized light microscopy, differential scanning calorimetry (DSC), X-ray diffraction (XRD), and freeze fracture electron microscopy can be used to elucidate the phase behavior of the vesicles.

[00245] Liposome size and distribution

[00246] Polarized light microscopy, dynamic light scattering, and TEM can be used to determine the size and size distribution range of the liposomes of the liposomal composition.

[00247] Determination of Free Drug

[00248] Because carfilzomib has extremely low aqueous solubility, the amount of entrapped drug versus free drug can be qualitatively determined based on polarized light microscope. Free drug precipitates in the aqueous medium, due to its extremely low aqueous solubility, and the precipitated material can be seen using a polarized light microscope.

[00249] Sulfobutyl ether- β -cyclodextrins

[00250] Sulfobutyl ether- β -cyclodextrins (SBE-CD), for example, CAPTISOL®, can be synthetically produced and/or are commercially available, for example from Ligand Pharmaceuticals, Inc., La Jolla, CA.

2.0 Examples

Example 1

Preparation of Molecularly Dispersed Carfilzomib in Thin Lipid Film

[00251] To make liposomal carfilzomib, the following materials in the indicated ratios were added to a suitably sized round bottom flask: drug to total lipid weight ratio of 1:19 to 1:2.33. Total lipids typically comprise the lipids EPC, HSPC, DSPC, DPPC, DSPE, and/or sphingomyelin (SPH) alone, or with cholesterol. If cholesterol is added, the lipid to cholesterol weight ratio (lipid:cholesterol) is from 0.9:0.1 to 0.5:0.5. An appropriate volume of phosphate buffer saline was used to rehydrate the lipid film to give a target carfilzomib concentration of 1 and 2 mg/mL, respectively.

[00252] To make PEGylated liposomal carfilzomib, the following materials in the indicated ratios were added to a suitably sized round bottom flask: drug to total lipid weight ratio of 1:19 to 1:2.33. Total lipids typically comprise the lipids EPC, HSPC, DSPC, DPPC, DSPE, and/or sphingomyelin with PEG-modified lipids (e.g., PEG-modified phospholipids) in weight ratio (lipids:PEG-modified lipids) of from 0.9:0.1 to 0.75:0.25, or when cholesterol is added the lipid to PEG-modified lipid to cholesterol weight ratio (lipids:PEG-modified lipids:cholesterol) of from 0.83:0.083:0.083 to 0.57:0.14:0.29. An appropriate volume of

phosphate buffer saline was used to rehydrate the lipid film to give a target carfilzomib concentration of 2 mg/mL.

[00253] To dissolve the lipids and carfilzomib, an appropriate volume of organic solvent (*e.g.*, chloroform:MeOH (60:40 or 50:50 v/v)), enough to achieve between 10-20 mg/mL dissolved lipid, was added to the flask. The flask was attached to a rotary evaporator spinning at 50-100 rpm and immersed in a water bath set above the highest gel-liquid crystal phase transition (T_c) temperature of the lipids used. Although the T_c for egg phosphatidylcholine is below room temperature (-15°C to -7°C), the temperature bath used for EPC was approximately 45°C to 50°C . For DSPC, DPPC, and mPEG-DSPE, the water bath temperature should be set greater than 55°C , 41°C , and 50°C , respectively.

[00254] The flask was allowed to rotate in the water bath for approximately 1 minute to equilibrate. A slow vacuum was pulled, to as low as < 10 Torr, to obtain a thin dry film on the walls of the flask without precipitation. To remove any residual solvent, the flask was subjected to high vacuum at room temperature for a few hours or overnight.

Table 2 presents nominal concentrations of the components of exemplary liposomal carfilzomib compositions (liposomal carfilzomib compositions, L-CFZ; pegylated liposomal carfilzomib compositions, pL-CFZ), as well as control compositions (*i.e.*, “empty” liposomes).

[00255] **Table 2**

Composition Designation	Composition Name	CFZ (mg/mL)	EPC (mg/mL)	mPEG-DSPE (mg/mL)	Cholesterol (mg/mL)
A	Empty Liposomes	0	12.5	0	0
B	1 mg/mL Liposomal CFZ	1	12.5	0	0
C	2 mg/mL Liposomal CFZ	2	12.5	0	0
D	Empty PEG-Liposomes	0	12.5	1.3	0
E	2 mg/mL PEG-Liposomal CFZ	2	12.5	1.3	0
F	Empty PEG-Liposomes w/ Cholesterol	0	12.5	1.3	3.1
G	2 mg/mL PEG-Liposomal CFZ w/ Cholesterol	2	12.5	1.3	3.1

[00256] Table 3 presents example ranges of components to be used in liposomal carfilzomib compositions of the present invention, as well as control compositions (*i.e.*,

“empty” liposomes). In the table, where EPC is listed, additional lipids can also be added for example, HSPC, DSPC, DPPC, DSPE, and/or sphingomyelin.

[00257]

Table 3

Composition Type	Weight ratio
Empty Liposomes = Empty L	CFZ to EPC w/w ratio 0:1
Liposomal CFZ = L-CFZ	CFZ to EPC w/w ratio from 1:19 to 1:2.33
Empty Liposomes w/ Cholesterol = Empty L Chol	CFZ to Total Lipid ¹ w/w ratio 0:1 where EPC to Cholesterol w/w ratio is from 0.9:0.1 to 0.5:0.5
Liposomal CFZ w/ Cholesterol = L-CFZ Chol	CFZ to Total Lipid ¹ w/w ratio from 1:19 to 1:2.33 where EPC to Cholesterol w/w ratio is from 0.9:0.1 to 0.5:0.5
Empty PEGylated Liposomes = Empty pL	CFZ to Total Lipid ² w/w ratio 0:1 where EPC to mPEG w/w ratio is from 0.9:0.1 to 0.75:0.25
PEGylated Liposomal CFZ = pL-CFZ	CFZ to Total Lipid ² w/w ratio from 1:19 to 1:2.33 where EPC to mPEG w/w ratio is from 0.9:0.1 to 0.75:0.25
Empty PEGylated Liposomes w/ Cholesterol = Empty pL Chol	CFZ to Total Lipid ³ w/w ratio 0:1 where EPC to mPEG to Cholesterol w/w ratio is from 0.83:0.083:0.083 to 0.57:0.14:0.29
PEGylated Liposomal CFZ w Cholesterol = pL-CFZ Chol	CFZ to Total Lipid ³ w/w ratio 1:19 to 1:2.33 where EPC to mPEG to Cholesterol w/w ratio is from 0.83:0.083:0.083 to 0.57:0.14:0.29

1 - Total Lipid = EPC + Cholesterol; 2 - Total Lipid = EPC + mPEG;

3 - Total Lipid = EPC + mPEG + Cholesterol

Example 2

Lipid Hydration

[00258] The thin-filmed, round-bottom flask was immersed in a water bath set above the highest gel-liquid crystal phase transition. When EPC was used, rehydration occurred at room temperature. For DSPC, DPPC, and mPEG-DSPE, the water bath temperature should be set greater than 55°C, 41°C, and 50°C, respectively. An appropriate volume of phosphate buffered saline, pH 7.2, or water for injection was added to the lipid film to achieve the desired target carfilzomib concentration or dose. The flask was mildly agitated or shaken with intermittent vortexing, as needed, and sonicated in water bath at the appropriate T_c temperature for 1 to 2 minutes to facilitate complete hydration from flask walls.

[00259] After the film was dispersed, the mixture was transferred to a vial and sonicated for an additional 20 to 40 minutes in a water bath above the T_c to size the liposomes. When EPC was used, the temperature of the water bath in the sonicator was kept

near room temperature. Upon hydration the lipid suspension appeared as a slightly hazy or milky solution.

[00260] FIG. 1 sets forth the composition of exemplary liposomal carfilzomib compositions of the present invention, as well as control compositions (*i.e.*, “empty” liposomes) used in the studies described below.

Example 3

Characterization of Liposomes

[00261] Particle size reduction and/or deagglomeration of the rehydrated liposomal suspension was carried out by sonication (20 to 60 minutes).

[00262] The CFZ content of exemplary liposomal carfilzomib compositions was determined by HPLC as described above. The liposomal compositions were each diluted in methanol to dissolve lipids and carfilzomib. The solution was filtered through a 0.2 μ m PTFE filter prior to HPLC analysis. The percent difference between the theoretical and experimental liposomal drug concentrations for the prepared lots (FIG. 1) were typically 2% or less (except for one of the first lots which had a 12% difference). The results of the HPLC analysis are presented in FIG. 1.

[00263] Based on polarized light microscope it was qualitatively determined that nearly all of the drug was entrapped in the liposomes as any free drug would precipitate in the aqueous medium due to its extremely low aqueous solubility of < 1 μ g/mL and any precipitated material could be easily observed under the polarized light microscope.

Example 4

Tolerability of Liposomal Carfilzomib

[00264] The tolerability of carfilzomib incorporated in either liposomal (L-CFZ) or pegylated liposomal (pL-CFZ) carfilzomib compositions was evaluated in both mice and rats above the maximum tolerated dose (MTD) achieved using an injectable composition of carfilzomib formulated in 10% sulfobutylether beta cyclodextrin (SBE- β -CD, also referred to as CFZ SBE-CD), 10 mM Citrate, pH 3.5 (see, *e.g.*, U.S. Patent Publication Nos. 2011/0236428).

[00265] Liposomal compositions prepared in Example 2 and characterized in Example 3 were rehydrated with an appropriate volume of aqueous medium to achieve a target carfilzomib concentration in the range of approximately 1 to 2 mg/ml (*see* composition data

in FIG. 1). Toxicity for both L-CFZ and pL-CFZ compositions were tested in mice (Table 4). Toxicity for L-CFZ was tested in rats (Table 5).

[00266] Female BALB/c mice (7-8 week old; 5/cohort) were dosed intravenously as follows: 15 mg/kg CFZ SBE-CD (7.5 mL/kg); 10 mg/kg L-CFZ (5 mL/kg); 10 mg/kg pL-CFZ (5 mL/kg); 15 mg/kg L-CFZ (7.5 mL/kg); 15 mg/kg pL-CFZ (7.5 mL/kg); 20 mg/kg L-CFZ (10 mL/kg); 20 mg/kg pL-CFZ (10 mL/kg); 25 mg/kg L-CFZ (12.5 mL/kg); 25 mg/kg pL-CFZ (12.5 mL/kg); 30 mg/kg L-CFZ (15 mL/kg); 35 mg/kg L-CFZ (17.5 mL/kg); or empty liposome (15 mL/kg).). Survival was then monitored over a seven day period. The survival rates of mice, the liposomal compositions, and dosing used for treatment were as shown in Table 4.

[00267] **Table 4**

Group	Composition # (Dose Volume)	Dose (mg/kg)	Overall Mortality (No. dead/total)
CFZ alone (10 mg/kg MTD)	2 m g/mL SBE-CD Composition (150 μ L)	15	5/5
Empty Liposome	Composition A (300 μ L)	0	0/5
L-CFZ	Composition B (200 μ L)	10	0/5
L-CFZ	Composition C (150 uL)	15	0/5
L-CFZ	Composition C (200 uL)	20	0/5
L-CFZ	Composition C (250 uL)	25	0/5
L-CFZ	Composition C (300 uL)	30	1/5
L-CFZ	Composition C (350 uL)	35	4/5
Empty PEG Liposome	Composition D (300 μ L)	0	0/5
pL-CFZ	Composition E (100 uL)	10	0/5
pL-CFZ	Composition E (150 uL)	15	0/5
pL-CFZ	Composition E (200 uL)	20	5/5
pL-CFZ	Composition E (250 uL)	25	5/5

[00268] Male Sprague Dawley rats (5/cohort) weighing approximately 250-300 grams were dosed intravenously with the following: 8 mg/kg CFZ (SBE-CD composition) (5 mL/kg); 8 mg/kg L-CFZ (4 mL/kg); 10 mg/kg L-CFZ (5 mL/kg); 12.5 mg/kg L-CFZ (6 mL/kg); or empty liposome (5 mL/kg). Survival was then monitored over a seven day period. The survival rates of dosed rats were as shown in Table 5.

[00269]**Table 5**

Group	Composition # (mL/kg)	Dose (mg/kg)	Overall Mortality (No. dead/total)
CFZ (7 mg/kg MTD)	SBE-CD Composition (5 mL/kg)	8	2/5
Empty Liposome	Composition A (5 mL/kg)	0	0/5
L-CFZ	Composition C (4 mL/kg)	8	0/5
L-CFZ	Composition C (5 mL/kg)	10	0/5
L-CFZ	Composition C (6 mL/kg)	12.5	3/5

[00270] Liposomal carfilzomib significantly enhanced tolerability by increasing the maximum tolerated dose (MTD) of carfilzomib in mice by approximately 2.5 fold for the liposomal carfilzomib compositions and by 50% with PEGylated liposomal carfilzomib compositions compared to SBE-CD based carfilzomib composition. Only a slight increase in the MTD in rats was observed with liposomal carfilzomib L-CFZ (10 mg/kg) compared to carfilzomib (7 mg/kg) formulated in SBE-CD.

[00271] These data demonstrate that liposomal compositions comprising peptide epoxyketone compounds significantly enhanced tolerability by increasing the maximum tolerated dose (MTD) of a peptide epoxyketone compound.

Example 5

Pharmacodynamic Response of CFZ Liposomal Compositions

[00272] The pharmacodynamic response of carfilzomib formulated in SBE-CD (CFZ SBE-CD) (using an injectable composition of carfilzomib formulated in 10% sulfobutylether beta cyclodextrin (SBE-CD), 10 mM Citrate, pH 3.5 (see, *e.g.*, U.S. Patent Publication Nos. 2011/0236428)), empty pL (Composition D, FIG.1), liposomes comprising CFZ (L-CFZ, Composition C, FIG. 1), and pegylated liposomes comprising (pL-CFZ, Composition E, FIG. 1) was evaluated in BALB/C mice following a single intravenous bolus administration.

[00273] The mice (three mice per time point) were administered a dose of 10 mg/kg in a dose volume of 5 mL/kg. Blood samples and tissues for pharmacodynamic testing were taken at 1, 8, and 24 hours after administration of each composition. The pharmacodynamic response was determined by measurement of proteasome activity in whole blood (primarily erythrocytes) (see FIG. 2A), adrenal (see FIG. 2B), liver (see FIG. 2C), and heart (see FIG.

2D), using a fluorogenic substrate (LLVY-AMC; as described by Lightcap ES, McCormack TA, Pien CS, et al., Clin. Chem. 46:673-683 (2000)) to quantitate the chymotrypsin-like activity of the proteasome. All samples were normalized to the CFZ vehicle, and the vehicle time point was 1 hour post dose. Three tissue samples were evaluated per time point for each tissue from each mouse.

[00274] A single intravenous dose of 10 mg/kg resulted in rapid proteasome inhibition of >80% within 1 hour in whole blood and all tissues. Similar and complete recovery from proteasome inhibition was observed 24 hours post-dose in all tissues tested except for the blood and heart and occurred with a $t_{1/2}$ of 8-24 hours for all compositions. The slower recovery observed in the heart with both the liposomes and pegylated liposomes suggest that the heart tissue may act as a depot. As expected, there was no recovery of proteasome activity in blood due to the irreversible binding of carfilzomib and the lack of the erythrocytes to synthesize new proteasome.

[00275] These observations indicate that inhibition of proteasome activity in whole blood and tissues is rapid, similar across compositions and correlates with rapid absorption. The liposomal compositions did not adversely affect biodistribution of CFZ.

Example 6

Circulation Half-Life of Liposomal CFZ

[00276] Circulation half-life of liposomal CFZ was evaluated in 7-8 week old female BALB/c mice (3/timepoint) following a single i.v. injection of either 5 mg/kg CFZ formulated in 10% sulfobutylether beta cyclodextrin, 10 mM Citrate, pH 3.5 or 15 mg/kg of liposomal carfilzomib compositions.

[00277] When CFZ was delivered in the composition containing SBE-CD at 5 mg/kg, plasma concentration rapidly declines with time and drops to below the limit of quantitation (BLOQ; limit of quantitation - LOQ) after 60 minutes (Table 6). The plasma half-life ($t_{1/2}$) was about 20 minutes.

[00278]**Table 6**

Plasma Levels of CFZ Using SBE-CD Composition I.V. Bolus 5mg/kg in BALB/c Mice		
Time (min)	Plasma Conc. (uM)	
	Mean	STD
0	0.0	0
2	10.379	0.844
5	1.732	0.431
10	0.310	0.064
20	0.176	0.040
30	0.061	0.011
60	0.042	0.032
LOQ = 1 ng/mL (MW = 719.4)		

[00279] When delivered in liposomal compositions at 15 mg/kg (using L-CFZ, Composition-C, or pL-CFZ-Chol, Composition G, (FIG. 1) with a dose volume of 150 μ L), detectable CFZ was observed at 6 hours post-dosing (Table 7).

[00280]**Table 7**

Mean Plasma Levels of Liposomal CFZ IV bolus at 15 mg/kg in BALB/c Mice				
Time (min)	Plasma Conc. (uM)		Plasma Conc. (uM)	
	Liposomal CFZ		PEGylated Liposomal CFZ	
	Mean	STD	Mean	STD
predose	BLOQ	BLOQ	BLOQ	BLOQ
2	102	21	79.0	22.7
5	51.4	9.1	42.5	8.4
10	22.2	6.2	9.81	3.48
30	1.26	0.48	0.183	0.045
60	0.143	0.018	0.0537	0.0200
120	0.0424	0.0134	0.0111	0.0010
240	0.0125	0.0010	0.0152	0.0070
360	0.0129	0.0023	0.0122	0.0100

[00281] The $t_{1/2}$ was 140 and 201 minutes, respectively, for liposomal CFZ and pegylated liposomal CFZ compositions, respectively. The data for liposomal CFZ compositions versus CFZ SBE-CD, clearly demonstrate the ability of liposome to significantly enhance the circulation half-life of peptide epoxyketone compounds.

[00282] Circulation half-life of liposomal CFZ was also evaluated in male Sprague-Dawley rats (3/timepoint) weighing approximately 250-300 grams following a single i.v. injection of 8 mg/kg CFZ formulated in 10% sulfobutylether beta cyclodextrin, 10 mM

Citrate, pH 3.5, or 8 mg/kg liposomal CFZ. Similar to mice, a rapid decline in plasma concentration was observed in rats when CFZ SBE-CD was delivered (Table 8), with a plasma t_{1/2} of 17 minutes. When CFZ was delivered in liposomal compositions (L-CFZ, Composition C, FIG.1, with a dose volume of 4 mL/kg) at the same dose level, detectable CFZ was observed at 4 hours post-dosing (Table 8), with a t_{1/2} of about 50 minutes.

[00283]**Table 8**

Plasma Levels of CFZ Using SBE-CD Composition or Liposomal CFZ (IV bolus at 8 mg/kg) in Rats				
Time (min)	SBE-CD Composition		Liposomal-CFZ	
	Plasma Conc. (uM)			
	Mean	STD	Mean	STD
predose	BLOQ	BLOQ	BLOQ	BLOQ
0.1	42.9	4.4	ND	ND
1	3.93	0.36	ND	ND
2	1.90	0.26	36.9	4.8
5	0.651	0.115	21.0	1.8
15	0.0505	0.0030	0.583	0.072
30	0.0189	0.0030	0.139	0.050
60	0.0072	0.0020	0.058	0.029
120	ND	ND	0.012	0.003
240	ND	ND	0.004	0.001
420	ND	ND	BLOQ	BLOQ

[00284]

The data presented in Table 9 and Table 10 demonstrate that, compared to CFZ SBE-CD, the exposure (AUC) to liposomal CFZ compositions (L-CFZ Composition C, FIG. 1) and pegylated liposomal CFZ compositions (pL-CFZ Composition E, FIG. 1), was increased about 5 to 7 and 20-fold in mice and rats, respectively.

[00285]**Table 9**

Mean AUC Levels of CFZ Using SBE-CD Composition or Liposomal CFZ (IV bolus at 15 mg/kg) in Mice				
Species	Dose (mg/kg)	Composition	AUC_{inf} (min*μmol/L)	Relative Increase AUC_{liposome}/ AUC_{SBE-CD}
Mouse	15	SBE-CD	130.5	-
Mouse	15	L-CFZ-C	942.8	7
Mouse	15	pL-CFZ-E	623.3	5

[00286]**Table 10**

Mean AUC Levels of CFZ Using SBE-CD Composition or Liposomal CFZ (IV bolus at 8 mg/kg) in Rats				
Species	Dose (mg/kg)	Composition	AUCinf (min*μmol/L)	Relative Increase AUC-liposome/ AUC-SBE-CD
Rat	8	SBE-CD	14.5	-
Rat	8	L-CFZ-C	297.7	20

[00287] These data demonstrate the extended duration of exposure to peptide epoxyketone compounds in liposomal composition versus the non-liposomal SBE-CD composition.

Example 7

Preparation Of Thin Lipid Film And Use In Preparing Liposomes Comprising An Aqueous Core Loaded With Peptide Epoxyketone Compounds Complexed With SBE-CD

[00288] To make the PEGylated liposomal film, the following materials at their indicated ratios were added to a suitably sized round bottom flask. Total lipids typically comprise the lipids EPC, DSPC, DPPC, and/or SPH with PEG-modified lipids (e.g., PEG-modified phospholipids) in weight ratio (lipids:PEG-modified lipids) of from 0.9:0.1 to 0.75:0.25, or when cholesterol is added the lipid to PEG-modified lipid to cholesterol weight ratio (lipid:PEG-modified lipid:cholesterol) of from 0.83:0.083:0.083 to 0.57:0.14:0.29.

[00289] To dissolve the lipids, an appropriate volume of organic solvent (e.g., chloroform:MeOH (60:40 v/v)), enough to achieve between 10-20 mg/mL dissolved lipid, was added to the flask. The flask was attached to a rotary evaporator spinning at 100 rpm and immersed in a water bath set above the highest gel-liquid crystal phase transition (T_c) temperature of the lipids used. Although the T_c for egg phosphatidylcholine is below room temperature (-15°C to -7°C), the temperature bath used for EPC was approximately 45°C to 50°C . For DSPC, DPPC, and mPEG-DSPE, the water bath temperature should be set greater than 55°C , 41°C , and 50°C , respectively. If there is no phase transition temperature, the water bath temperature is set between 35 - 45°C .

[00290] The flask was allowed to rotate in the water bath for approximately 1 minute to equilibrate. A slow vacuum was pulled, to as low as < 10 Torr, to obtain a thin dry film on the walls of the flask without precipitation (typically for about 30 minutes). To remove any

residual solvent, the flask was subjected to high vacuum at room temperature for a few hours or overnight.

[00291] Carfilzomib (CFZ) was solubilized in an aqueous solution by complexation with sulfobutylether beta cyclodextrin (SBE-CD). Excess carfilzomib was added to an aqueous solution of 20% SBE-CD and 20 mM citric acid. The solution pH was adjusted to approximately pH 2.5 with 1N HCl, if needed to solubilize CFZ. The mixture was sonicated for approximately 10 minutes and stirred using a magnetic stir bar for not less than an hour prior to filtration through a 0.2 µm filter to remove excess undissolved drug. After filtration the solution pH was adjusted to between pH 3.5 and 5. This aqueous solution of CFZ complexed with SBE-CD was used to rehydrate the thin lipid film.

[00292] Once the vesicles were rehydrated the unencapsulated free drug was removed by centrifugation at 31000 rpm for 30 minutes and washing with PBS or by being dialyzed using a membrane with a MWCO of 8-10kD in PBS for up to 48 hours.

[00293] Table 11 presents nominal concentrations of the components of exemplary liposomal compositions comprising liposomes comprising an aqueous core loaded with CFZ complexed with SBE-CD.

[00294] **Table 11**

Composition Designation	Lipid composition	Drug content *
apL	12.5 mg/mL EPC, 3.3 mg/mL cholesterol, 1.3 mg/mL mPEG-DSPE	0.9 mg/mL
apL-9	25 mg/mL EPC, 6.3 mg/mL cholesterol, 2.5 mg/mL mPEG-DSPE	1.3 mg/mL
apL-11 (for 15 mg/kg dosing)	12.5 mg/mL egg SPH, 3.3 mg/mL cholesterol, 1.3 mg/mL mPEG-DSPE	0.8 mg/mL
apL-11 (for 5 mg/kg dosing)	12.5 mg/mL egg SPH, 3.3 mg/mL cholesterol, 1.3 mg/mL mPEG-DSPE	0.2 mg/mL

*Total drug content may include unencapsulated drug that was not removed during processing.

Example 8

Pharmacodynamic Response of CFZ Liposomal Compositions Comprising Liposome Comprising An Aqueous Core Loaded With Peptide Epoxyketone Compounds Complexed With SBE-CD

[00295] The pharmacodynamic response of liposomal compositions comprising liposomes comprising an aqueous core loaded with CFZ complexed with SBE-CD was evaluated in BALB/C mice following a single intravenous bolus administration.

[00296] The pharmacodynamic response of injectable carfilzomib formulated in SBE-CD (non-liposomal; see, *e.g.*, U.S. Patent Publication Nos. 2011/0236428) or liposomal compositions comprising liposomes comprising an aqueous core loaded with CFZ complexed with SBE-CD was evaluated in BALB/C mice (apL-11 (for 15 mg/kg dosing), Example 7; and apL-11 (for 5 mg/kg dosing), Example 7) following a single intravenous bolus administration. The mice (three mice per time point) were administered a dose of 5 or 10 mg/kg of non-liposomal carfilzomib or 5 or 15 mg/kg of liposomal carfilzomib as a solution in a dose volume of 5 mL/kg. Blood samples and tissues for pharmacodynamic testing were taken at 0, 1, 8, and 14 hours after administration of the non-liposomal composition at 10 mg/kg; 0, 1, 4, 6, 8 and 24 hours after administration of the non-liposomal composition at 5 mg/kg; and 0, 1, 4, 6, and 24 hours after administration of the liposomal compositions at 5 mg/kg and 15 mg/kg. Three tissue samples were evaluated per time point for each tissue from each mouse. The pharmacodynamic response was determined by measurement of proteasome activity in whole blood (primarily erythrocytes), adrenal, liver, and heart using a fluorogenic substrate (LLVY-AMC; as described by Lightcap ES, McCormack TA, Pien CS, et al., Clin. Chem. 46:673-683 (2000)) to quantitate the chymotrypsin-like activity of the proteasome. All samples were normalized to the corresponding vehicle without CFZ, and the vehicle sample time point measurement was 1 hour post dose.

[00297] A single dose of injectable carfilzomib formulated in SBE-CD (non-liposomal) at either 5 or 10 mg/kg or liposomal compositions comprising liposomes comprising an aqueous core loaded with CFZ complexed with SBE-CD (apL-11) at either 5 or 15 mg/kg resulted in a rapid inhibition of proteasome activity within 1 hour in whole blood and all other tissues. Greater inhibition of proteasome activity was observed at the 15 mg/kg dose which resulted in >80% inhibition of proteasome activity at 1 hour in all tissue: whole blood (primarily erythrocytes) (*see* FIG. 3A), heart (*see* FIG. 3B), liver (*see* FIG. 3C), and adrenal (*see* FIG. 3D). Similar and near complete recovery from proteasome inhibition was observed 24 hours post-dose in all tissues tested except for the blood and heart and occurred with at t_{1/2} of 6-24 hours for both the 5 and 10 mg/kg dose levels of injectable carfilzomib formulated in SBE-CD (non-liposomal). Delayed recovery of proteasome activity in the adrenals was observed with liposomal compositions comprising liposomes comprising an aqueous core loaded with CFZ complexed with SBE-CD at both 5 and 15 mg/kg; this result suggests long term exposure of CFZ. As expected, there was no recovery of proteasome activity in blood due to the irreversible binding of carfilzomib and the lack of the erythrocytes to synthesize new proteasome.

[00298] These observations indicate that inhibition of proteasome activity in whole blood and tissues was rapid and similar across between the non-liposomal formulation and liposomal compositions comprising peptide epoxyketone compounds. Further, the delay in the recovery of proteasome activity in the adrenals with liposomal compositions comprising liposomes comprising an aqueous core loaded with peptide epoxyketone compound complexed with SBE-CD suggests extended exposure with the liposomal composition versus the non-liposomal composition. Further, the liposomal compositions comprising peptide epoxyketone compounds did not adversely affect biodistribution of the peptide epoxyketone compounds.

Example 9

Circulation Half-Life of Liposomal CFZ

[00299] Circulation half-life of liposomal CFZ was evaluated in 7-8 week old female BALB/c mice (3/timepoint) following a single i.v. injection of the following: 5 mg/kg CFZ formulated in 10% sulfobutylether beta cyclodextrin (CAPTISOL®), 10 mM Citrate, pH 3.5 (CFZ SBE-CD; non-liposomal); 5 mg/kg of liposomal carfilzomib compositions apL-11 (Example 7; apL-11 (for 5 mg/kg dosing)) and 15 mg/kg of liposomal carfilzomib compositions apL-11 (Example 7; apL-11 (for 15 mg/kg dosing)).

[00300] As shown in FIG. 4, the plasma concentration of injectable carfilzomib SBE-CD composition (non-liposomal) declined rapidly following intravenous, bolus administration due to rapid and extensive metabolism (FIG. 4: line with open squares containing an X corresponds to administration of 5 mg/kg of an injectable carfilzomib SBE-CD composition (non-liposomal)). The half-life of carfilzomib dosed at 5 mg/kg was about 20 minutes and carfilzomib was not detectable after 1 hour post-dose.

[00301] When delivered in liposomal compositions, the duration of exposure to carfilzomib was greatly extended (FIG. 4, line with solid squares correspond to administration of 5 mg/kg of apL11 (Example 7), a pegylated liposomal composition of carfilzomib wherein the aqueous core of the pegylated liposomes comprises carfilzomib and SBE-CD; line with solid circles correspond to administration of 15 mg/kg of apL11 (Example 7), a pegylated liposomal composition of carfilzomib wherein the aqueous core of the pegylated liposomes comprises carfilzomib and SBE-CD). Total drug (encapsulated and released) was detectable for up to 24 hours post-dose. This is consistent with the observed delay in proteasome recovery in tissues.

[00302] The data for liposomal CFZ compositions versus CFZ SBE-CD (non-liposomal), demonstrate the ability of liposomal compositions to significantly enhance the circulation half-life of peptide epoxyketone compounds. Further, the data show the ability to provide extended duration of exposure to peptide epoxyketone compounds in liposomal compositions versus the non-liposomal SBE-CD composition.

Example 10

Preparation of Liposomes Comprising An Aqueous Core Loaded With Peptide Epoxyketone Using pH Control and An Ethanol Injection Method

[00303] To make the liposomal compositions using an ethanol injection method, the following materials at their indicated ratios were used. Total lipids typically comprise the lipids EPC, DSPC, DPPC, and/or SPH, and can further comprise PEG-modified lipids (e.g., PEG-modified phospholipids) and/or cholesterol in weight ratio (lipids:PEG-modified lipids or lipids:cholesterol) of 0.9:0.1 to 0.75:0.25, or when cholesterol is added the lipid to PEG-modified lipid to cholesterol weight ratio (lipid:PEG-modified lipid:cholesterol) can be from 0.83:0.083:0.083 to 0.57:0.14:0.29.

[00304] Other materials used in the ethanol injection method include the following: absolute Ethanol; 1N HCl; Carfilzomib (crystalline or amorphous); Hamilton Syringe Gastight, 22 gauge; Dialysis kit, Spectra/Por® Float-A-Lyzer® G2 (Spectrum Laboratories Inc., Rancho Dominguez, CA) molecular weight cut off (MWCO) 8-10 kD; Water for Injection (WFI); and Phosphate buffer saline 1X (PBS).

[00305] A lipid/ethanol solution was prepared as follows: 2 mL of ethanol containing 125 mg/mL egg sphingomyelin, 31.25 mg/mL cholesterol, 12.5 mg/mL mPEG-DSPE. If needed, the lipid/ethanol solution was sonicated several minutes to facilitate dissolution.

[00306] An aqueous solution of CFZ was prepared as follows: 10 mL of a 0.1M HCl aqueous solution was prepared (~pH 1) and CFZ in excess of solubility was added. The aqueous solution was sonicated in heated water bath (~ 30°C) for 20-30 minutes. Approximate carfilzomib solubility at pH 1 is 1.8 mg/mL. Undissolved excess drug was removed by filtering through a 0.2 µm filter to yield a visibly clear solution.

[00307] Alternatively, a supersaturated solution of carfilzomib is prepared by dissolving amorphous carfilzomib in 0.1M HCl solution with 6% (v/v) ethanol as a cosolvent followed by sonication in a warm water bath until solution becomes clear.

[00308] Liposomes were formed by rapid injection of 1 mL of the lipid-ethanol solution into 9 mL of the aqueous solution of CFZ with stirring using a magnetic stir bar.

Stirring was continued for 5-10 minutes. The solution pH was ~pH 1. The resulting solution was dialyzed against phosphate buffer saline (or WFI) using the Dialysis kit (Spectra/Por® Float-A-Lyzer® G2) MWCO 8-10 kD for 12 to 16 hours. The bulk dialysis solution was replaced with fresh PBS or WFI after approximately 6-8 hours. Solution pH of the dialyzed liposome containing formulation was about pH 3 to 3.5. The pH of the aqueous solution comprising the liposomes was adjusted with sodium hydroxide to between pH 3.5 to a physiologic pH, ~ pH 6.8.

[00309] Table 12 presents nominal concentrations of the components of exemplary liposomal compositions.

[00310] **Table 12**

Composition Designation	Lipid composition	Drug content *
apL-15	25 mg/mL egg SPH, 6.3 mg/mL cholesterol, 2.5 mg/mL mPEG-DSPE	0.6 mg/mL
apL-11b	12.5 mg/mL EPC, 3.1 mg/mL cholesterol, 1.2 mg/mL mPEG-DSPE	1 mg/mL

*Total drug content may include unencapsulated drug that was not removed during processing.

Example 11

Liposomes Comprising Entrapped Peptide Epoxyketone Induce Anti-tumor Response

[00311] To evaluate the anti-cancer effect of liposomal compositions comprising liposomes comprising peptide epoxyketone compounds, an exemplary liposomal composition was tested in a mouse xenograft tumor model.

[00312] The liposomal composition was made by the methods described in Example 1 and Example 2. The liposomal composition was as follows: pL6 = 2 mg/mL CFZ, 12.5 mg/mL Sphingomylin, 3.2 mg/mL cholesterol, 1.3 mg/mL mPEG-DSPE.

[00313] First, the pharmacodynamic response of injectable carfilzomib formulated in SBE-CD (non-liposomal; see, *e.g.*, U.S. Patent Publication Nos. 2011/0236428) and the pL-6 a liposomal composition comprising liposomes loaded with CFZ was evaluated in BALB/C mice following a single intravenous bolus administration.

[00314] The mice (three mice per time point) were administered a dose of 10 mg/kg carfilzomib formulated in SBE-CD (non-liposomal) or 15 mg/kg of pL-6 a liposomal composition comprising liposomes loaded with CFZ as a solution in a dose volume of 5 mL/kg. Blood samples and tissues for pharmacodynamic testing were taken at 0, 1, 4, 6 and 24 hours after administration of the liposomal formulation and 0, 1, 8, and 24 hours for the

non-liposomal formulation. Three tissue samples were evaluated per time point for each tissue from each mouse. The pharmacodynamic response was determined by measurement of proteasome activity in whole blood (primarily erythrocytes), adrenal, liver, and heart using a fluorogenic substrate (LLVY-AMC; as described by Lightcap ES, McCormack TA, Pien CS, et al., Clin. Chem. 46:673-683 (2000)) to quantitate the chymotrypsin-like activity of the proteasome. All samples were normalized to the corresponding vehicle without CFZ, and the vehicle sample time point measurement was 1 hour post dose.

[00315] A single dose of injectable carfilzomib formulated in SBE-B-CD (non-liposomal) at 10 mg/kg (MTD) or liposomal compositions comprising liposomes comprising entrapped CFZ (pL-6) at 15 mg/kg resulted in a rapid inhibition of > 80% of proteasome activity within 1 hour in whole blood and all other tissues: whole blood (primarily erythrocytes) (*see* FIG. 5A), heart (*see* FIG. 5B), liver (*see* FIG. 5C), and adrenal (*see* FIG. 5D). Similar and near complete recovery from proteasome inhibition was observed 24 hours post-dose in all tissues tested except for the blood and heart and occurred with a $t_{1/2}$ of 6-24 hours for the non-liposomal injectable CFZ. Delayed recovery of proteasome activity in the adrenals was observed with the liposomal composition pL-6 suggesting long-term exposure of CFZ. As expected, there was no recovery of proteasome activity in blood due to the irreversible binding of carfilzomib and the lack of the erythrocytes to synthesize new proteasome.

[00316] These observations indicate that inhibition of proteasome activity in whole blood and tissues is rapid and similar across compositions. The delay in recovery of proteasome activity in the adrenals suggests extended exposure with the liposomal composition. The liposomal compositions did not adversely affect biodistribution of CFZ.

[00317] Second, circulation half-life of liposomal CFZ was evaluated in 7-8 week old female BALB/c mice (3/timepoint) following a single i.v. injection of the following: injectable carfilzomib formulated in SBE-CD (non-liposomal) administered at 5 mg/kg; and the pL-6 a liposomal composition comprising liposomes loaded with CFZ administered at 15 mg/kg.

[00318] As shown in FIG. 6, the plasma concentration of injectable carfilzomib SBE-CD composition (non-liposomal) declined rapidly following intravenous, bolus administration and was below the limit of quantitation after 1 hour. The half-life was about 20 minutes. This is due to rapid and extensive metabolism (FIG. 6, line with open circles).

[00319] When delivered in the pL-6 liposomal composition, the duration of exposure to carfilzomib was greatly extended (FIG. 6, line with solid squares). Plasma concentration

of total drug (encapsulated and released) declined slowly and was detectable for up to 24 hours post-dose. This is consistent with the observed delay in proteasome recovery in tissues.

[00320] The data for the liposomal CFZ compositions versus CFZ SBE-CD (non-liposomal) demonstrate the ability of liposomal compositions to significantly enhance the circulation half-life of peptide epoxyketone compounds. Further, the data show the ability to provide extended duration of exposure to peptide epoxyketone compounds in liposomal compositions versus the non-liposomal SBE-CD composition.

[00321] Third, the anti-tumor response of injectable carfilzomib formulated in SBE-CD (non-liposomal) and the pL-6 liposomal composition comprising liposomes loaded with CFZ was evaluated in mice. Tumors were established by s.c. injection of RL cells (human non-Hodgkin's B cell lymphoma cells; passage number <9 and viability >95% at the time of implantation) in the right flank of BNX mice (n = 8 - 10 per group). For RL studies, cell suspensions containing 1×10^7 cells in a volume of 0.1 mL were injected. Mice were randomized into treatment groups and dosing initiated when tumors reached $\sim 100 \text{ mm}^3$ (RL). Tumors were measured thrice weekly by recording the longest perpendicular diameters and tumor volumes were calculated using the equation $V \text{ (in mm}^3\text{)} = (\text{length} \times \text{width}^2)/2$.

[00322] BNX mice bearing established human tumor xenograft derived from RL cells were treated with either non-liposomal carfilzomib or liposomal carfilzomib. Drug was administered on either a weekly (QW) schedule or a schedule of two consecutive daily doses administered each week (QDx2). The group sizes were N=8-10 mice/group.

[00323] The results are presented in FIG. 7. The data presented in the figure demonstrate that weekly IV administration of liposomal compositions comprising carfilzomib (FIG. 7, liposomal composition 15 mg/kg, QW, open triangles) and QDx2 administration of liposomal compositions (FIG. 7, liposomal composition, 10 mg/kg CFZ, QDx2, solid circles) induced anti-tumor responses similar to injectable carfilzomib formulated in SBE-CD (non-liposomal; FIG. 7, QDx2, 5 mg/kg) administered on a Day 1/Day 2 schedule (i.e., QDx2). Statistical comparisons between treatment groups and vehicle controls were made by one-way ANOVA and Bonferroni post-hoc analysis (significance was $p < 0.001$). The data show that the liposomal composition administered at 15 mg/kg once a week was as efficacious as a liposomal or non-liposomal composition administered QDx2.

[00324] These data demonstrate that liposomal compositions comprising peptide epoxyketone compounds maintain efficacy at a reduced dosing frequency relative to a non-liposomal formulation.

[00325] As is apparent to one of skill in the art, various modification and variations of the above embodiments can be made without departing from the spirit and scope of this invention. Such modifications and variations are within the scope of this invention.

What is claimed is:

1. A pharmaceutical liposomal composition comprising:
liposome entrapped peptide epoxyketone compound, wherein (i) liposomes of the liposomal composition comprise one or more lipids, the lipids comprising at least one phospholipid selected from the group consisting of L- α -phosphatidylcholine, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, and sphingomyelin; (ii) the liposomes comprising a weight ratio of peptide epoxyketone compound:lipid of between about 0.01:1 and about 1:1; and (iii) the liposomes have an average size of between about 0.05 microns and about 0.5 microns; and
an aqueous solution comprising the liposomes.
2. The pharmaceutical liposomal composition of claim 1, wherein the weight ratio of peptide epoxyketone compound:lipid is between about 0.05:1 and about 0.5:1.
3. The pharmaceutical liposomal composition of any preceding claim, wherein the liposomes have an average size of between about 0.05 microns and about 0.2 microns.
4. The pharmaceutical liposomal composition of any preceding claim, wherein the lipids of the liposomes comprise between about 20 and about 100 weight percent phospholipid.
5. The pharmaceutical liposomal composition of any preceding claim, wherein the lipids of the liposomes further comprise between about 10 and about 50 weight percent cholesterol.
6. The pharmaceutical liposomal composition of any preceding claim, wherein the lipids of the liposomes further comprise between about 1 and about 20 weight percent of a hydrophilic polymer-derivatized lipid.
7. The pharmaceutical liposomal composition of claim 6, wherein the hydrophilic polymer of the hydrophilic polymer-derivatized lipid is a polyethylene glycol.

8. The pharmaceutical liposomal composition of claim 6 or claim 7, wherein the lipid of the hydrophilic polymer-derivatized lipid is a cholesterol or a phospholipid.

9. The pharmaceutical liposomal composition of claim 8, wherein the hydrophilic polymer-derivatized lipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000].

10. The pharmaceutical liposomal composition of any preceding claim, wherein the lipids of the liposomes comprise L- α -phosphatidylcholine.

11. The pharmaceutical liposomal composition of any preceding claim, wherein the lipids of the liposomes comprise sphingomyelin.

12. The pharmaceutical liposomal composition of any preceding claim, wherein the lipids of the liposomes comprise 1,2-distearoyl-*sn*-glycero-3-phosphocholine.

13. The pharmaceutical liposomal composition of any preceding claim, wherein the lipids of the liposomes comprise 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

14. The pharmaceutical liposomal composition of any preceding claim, wherein the lipids of the liposomes comprise 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine.

15. The pharmaceutical liposomal composition of any preceding claim, wherein the lipids of the liposomes further comprise about 0.001 to about 5 weight percent α -tocopherol.

16. The pharmaceutical liposomal composition of any preceding claim, wherein the aqueous solution further comprises a buffer.

17. The pharmaceutical liposomal composition of any preceding claim, wherein the aqueous solution further comprises an agent to maintain isotonicity.

18. The pharmaceutical liposomal composition of any preceding claim, wherein the liposomal composition comprises liposomes comprising the peptide epoxyketone compound and a solubilizing agent in an internal aqueous core of the liposomes.

19. The pharmaceutical liposomal composition of claim 18, wherein the solubilizing agent, is a compound.

20. The pharmaceutical liposomal composition of claim 19, wherein the solubilizing agent is a compound, the compound is a cyclodextrin, and the liposomes of the liposomal composition comprise the peptide epoxyketone compound complexed with the cyclodextrin in the internal aqueous core of the liposomes.

21. The pharmaceutical liposomal composition of claim 20, wherein the cyclodextrin is a sulfobutylether-betacyclodextrin or a hydroxypropyl-betacyclodextrin.

22. The pharmaceutical liposomal composition of claim 21, wherein the cyclodextrin is a sulfobutylether-betacyclodextrin.

23. The pharmaceutical liposomal composition of claim 21, wherein the cyclodextrin is a hydroxypropyl-betacyclodextrin.

24. The pharmaceutical liposomal composition of any preceding claim, wherein the aqueous solution of liposomal composition is adjusted to between about pH 3.5 and about pH 7.0.

25. The pharmaceutical liposomal composition of claim 24, wherein the aqueous solution of liposomal composition is adjusted to a human physiological pH.

26. The pharmaceutical liposomal composition of any preceding claim, wherein the peptide epoxyketone compound is selected from the group consisting of compound II, compound III, compound IV, and compound V.

27. The pharmaceutical liposomal composition of any preceding claim, wherein the peptide epoxyketone compound is carfilzomib (compound V).

28. A dry pharmaceutical composition formed by drying the pharmaceutical liposomal composition of any preceding claim.

29. A dry pharmaceutical composition comprising:

one or more lipids, the lipids comprising at least one phospholipid selected from the group consisting of L- α -phosphatidylcholine, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, and sphingomyelin; and a weight ratio of peptide epoxyketone compound:lipid of between about 0.01:1 and about 1:1.

30. The dry pharmaceutical composition of claim 29, further comprising additional excipients.

31. The dry pharmaceutical formulation of claim 30, wherein the additional excipients comprise one or more excipient selected from the group consisting of a cryoprotectant agent, a sugar, a glass transition modifying agent, and combinations thereof.

32. A method of making a pharmaceutical liposomal composition comprising:

preparing a dried film comprising (i) a peptide epoxyketone compound and one or more lipids, the lipids comprising at least one phospholipid selected from the group consisting of L- α -phosphatidylcholine, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, and sphingomyelin, and (ii) a weight ratio of peptide epoxyketone compound:lipid of between about 0.01:1 and about 1:1; and

rehydrating the dried film with an aqueous solution to form a liposomal composition comprising liposomes and the aqueous solution.

33. The method of claim 32, wherein the weight ratio of peptide epoxyketone compound:lipid is between about 0.05:1 and about 0.5:1.

34. The method of claim 32 or claim 33, further comprising sizing the liposomes to have an average size of between about 0.05 microns and about 0.5 microns.

35. The method of claim 34, comprising sizing the liposomes to have an average size of between about 0.05 microns and about 0.2 microns.

36. The method of any one of claims 32-35, wherein the lipids of the dried film comprise between about 20 and about 100 weight percent phospholipid.

37. The method of any of claims 32-36, wherein the lipids of the dried film further comprise between about 10 and about 50 weight percent cholesterol.

38. The method of any of claims 32-37, wherein the lipids of the dried film further comprise between about 1 and about 20 weight percent of a hydrophilic polymer-derivatized lipid.

39. The method of claim 38, wherein the hydrophilic polymer of the hydrophilic polymer-derivatized lipid is a polyethylene glycol.

40. The method of claim 38 or claim 39, wherein the lipid of the hydrophilic polymer-derivatized lipid is a cholesterol or a phospholipid.

41. The method of claim 40, wherein the hydrophilic polymer-derivatized lipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000].

42. The method of any of claims 32-41, wherein the lipids of the dried film comprise L- α -phosphatidylcholine.

43. The method of any of claims 32-42, wherein the lipids of the dried film comprise sphingomyelin.

44. The method of any of claims 32-43, wherein the lipids of the dried film comprise 1,2-distearoyl-*sn*-glycero-3-phosphocholine.

45. The method of any of claims 32-44, wherein the lipids of the dried film comprise 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

46. The method of any of claims 32-45, wherein the lipids of the dried film comprise 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine.

47. The method of any of claims 32-46, wherein the lipids of the dried film further comprise about 0.001 to about 5 weight percent α -tocopherol.

48. The method of any of claims 32-47, further comprising adding a buffer to the aqueous solution.

49. The method of any of claims 32-48, further comprising adding an agent to maintain isotonicity to the aqueous solution.

50. The method of any of claims 32-49, further comprising adjusting the pH of the aqueous solution to between about pH 3.5 and about pH 7.0.

51. The method of any of claims 32-49, further comprising adjusting the pH of the aqueous solution to a human physiological pH.

52. The method of any of claims 32-51, wherein the peptide epoxyketone compound is selected from the group consisting of compound II, compound III, compound IV, and compound V.

53. The method of any of claims 32-50, wherein the peptide epoxyketone compound is carfilzomib (compound V).

54. A pharmaceutical liposomal composition made by the method of any of claims 32-53.

55. A method of making a pharmaceutical liposomal composition comprising:
preparing a dried film comprising one or more lipids, the lipids comprising at least one phospholipid selected from the group consisting of L- α -phosphatidylcholine, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, and sphingomyelin; and
rehydrating the dried film with an aqueous solution comprising a peptide epoxyketone compound and one or more solubilizing agent to form a liposomal composition comprising liposomes dispersed in the aqueous solution.

56. The method of claim 55, wherein the one or more solubilizing agent, is selected from the group consisting of a compound, a pH adjusting agent, a cosolvent, and a combination thereof.

57. The method of claim 56, wherein the one or more solubilizing agent comprises a compound and the compound is a cyclodextrin.

58. The method of claim 57, wherein the cyclodextrin is a sulfobutylether-betacyclodextrin or a hydroxypropyl-betacyclodextrin.

59. The method of any of claims 56-58, wherein the one or more solubilizing agent comprises a pH adjusting agent and the aqueous solution has a pH of between about pH 0.5 and about pH 3.0.

60. The method of claim 59, wherein the pH of the aqueous solution is about pH 1.0.

61. The method of any of claims 56-60, wherein the one or more solubilizing agent comprises a cosolvent.

62. The method of any of claims 55-61, further comprising dialysis, desalting, buffer exchange, or gel filtration.

63. The method of any of claims 55-62, wherein the liposomes of the liposomal composition comprise a weight ratio of peptide epoxyketone compound:lipid of between about 0.01:1 and about 1:1.

64. The method of any of claims 55-63, further comprising sizing the liposomes to have an average size of between about 0.05 microns and about 0.5 microns.

65. The method of claim 64, comprising sizing the liposomes to have an average size of between about 0.05 microns and about 0.2 microns.

66. The method of any one of claims 55-65, wherein the lipids of the dried film comprise between about 20 and about 100 weight percent phospholipid.

67. The method of any of claims 55-66, wherein the lipids of the dried film further comprise between about 10 and about 50 weight percent cholesterol.

68. The method of any of claims 55-67, wherein the lipids of the dried film further comprise between about 1 and about 20 weight percent of a hydrophilic polymer-derivatized lipid.

69. The method of claim 68, wherein the hydrophilic polymer of the hydrophilic polymer-derivatized lipid is a polyethylene glycol.

70. The method of claim 68 or claim 69, wherein the lipid of the hydrophilic polymer-derivatized lipid is a cholesterol or a phospholipid.

71. The method of claim 70, wherein the hydrophilic polymer-derivatized lipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000].

72. The method of any of claims 55-71, wherein the lipids of the dried film comprise L- α -phosphatidylcholine.

73. The method of any of claims 55-72, wherein the lipids of the dried film comprise sphingomyelin.

74. The method of any of claims 55-73, wherein the lipids of the dried film comprise 1,2-distearoyl-*sn*-glycero-3-phosphocholine.

75. The method of any of claims 55-74, wherein the lipids of the dried film comprise 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

76. The method of any of claims 55-75, wherein the lipids of the dried film comprise 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine.

77. The method of any of claims 55-76, wherein the lipids of the dried film further comprise about 0.001 to about 5 weight percent α -tocopherol.

78. The method of any of claims 55-77, further comprising, after forming the liposomal composition, removing peptide epoxyketone compound from non-encapsulated aqueous solution.

79. The method of claim 78, wherein dialysis, ultracentrifugation, or gel filtration is used for removing the peptide epoxyketone compound from the non-encapsulated aqueous solution.

80. The method of any of claims 55-79, further comprising, after forming the liposomal composition, adjusting the aqueous solution to a pH of between about pH 3.5 and about pH 7.0.

81. The method of any of claims 55-79, further comprising, after forming the liposomal composition, adjusting the aqueous solution to a human physiological pH.

82. The method of any of claims 55-81, further comprising, after forming the liposomal composition, adding an agent to the aqueous solution to maintain isotonicity.

83. The method of any of claims 55-82, wherein the peptide epoxyketone compound is selected from the group consisting of compound II, compound III, compound IV, and compound V.

84. The method of any of claims 55-83, wherein the peptide epoxyketone compound is carfilzomib (compound V).

85. A pharmaceutical liposomal composition made by the method of any of claims 55-84.

86. A method of making a pharmaceutical liposomal composition comprising:
preparing a lipid solution comprising a solvent and one or more lipids, the lipids comprising at least one phospholipid selected from the group consisting of L- α -phosphatidylcholine, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, 1,2-dipalmitoyl-*sn*-

glycero-3-phosphocholine, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, and sphingomyelin; and

injecting the lipid solution into an aqueous solution comprising a peptide epoxyketone compound and one or more solubilizing agent to form a liposomal composition comprising liposomes dispersed in the aqueous solution.

87. The method of claim 86, wherein the solvent is an organic solvent.

88. The method of claim 87, wherein the solvent is ethanol.

89. The method of any of claims 86-88, wherein the one or more solubilizing agent is a compound, a pH adjusting agent, a cosolvent, or a combination thereof.

90. The method of claim 89, wherein the one or more solubilizing agent comprises a compound and the compound is a cyclodextrin.

91. The method of claim 90, wherein the cyclodextrin is a sulfobutylether-betacyclodextrin or a hydroxypropyl-betacyclodextrin.

92. The method of any of claims 89-91, wherein the one or more solubilizing agent comprises a pH adjusting agent and the aqueous solution has a pH of between about pH 0.5 and about pH 3.0.

93. The method of claim 92, wherein the pH of the aqueous solution is about pH 1.0.

94. The method of any of claims 89-93, wherein the solubilizing agent comprises a cosolvent.

95. The method of any of claims 86-94, further comprising dialysis, desalting, buffer exchange, or gel filtration.

96. The method of any of claims 86-95, wherein the liposomes of the liposomal composition comprise a weight ratio of peptide epoxyketone compound:lipid of between about 0.01:1 and about 1:1.

97. The method of any of claims 86-96, further comprising sizing the liposomes to have an average size of between about 0.05 microns and about 0.5 microns.

98. The method of claim 97, comprising sizing the liposomes to have an average size of between about 0.05 microns and about 0.2 microns.

99. The method of any one of claims 86-98, wherein the lipids of the liposomes of the liposomal composition comprise between about 20 and about 100 weight percent phospholipid.

100. The method of any of claims 86-99, wherein the lipids of the liposomes of the liposomal composition further comprise between about 10 and about 50 weight percent cholesterol.

101. The method of any of claims 86-100, wherein the lipids of the liposomes of the liposomal composition further comprise between about 1 and about 20 weight percent of a hydrophilic polymer-derivatized lipid.

102. The method of claim 101, wherein the hydrophilic polymer of the hydrophilic polymer-derivatized lipid is a polyethylene glycol.

103. The method of claim 101 or claim 102, wherein the lipid of the hydrophilic polymer-derivatized lipid is a cholesterol or a phospholipid.

104. The method of claim 103, wherein the hydrophilic polymer-derivatized lipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000].

105. The method of any of claims 86-104, wherein the lipids of the liposomes of the liposomal composition comprise L- α -phosphatidylcholine.

106. The method of any of claims 86-105, wherein the lipids of the liposomes of the liposomal composition comprise sphingomyelin.

107. The method of any of claims 86-106, wherein the lipids of the liposomes of the liposomal composition comprise 1,2-distearoyl-*sn*-glycero-3-phosphocholine.

108. The method of any of claims 86-107, wherein the lipids of the liposomes of the liposomal composition comprise 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

109. The method of any of claims 86-108, wherein the lipids of the liposomes of the liposomal composition comprise 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine.

110. The method of any of claims 86-109, wherein the lipids of the liposomes of the liposomal composition further comprise about 0.001 to about 5 weight percent α -tocopherol.

111. The method of any of claims 86-110, further comprising, after forming the liposomal composition, removing peptide epoxyketone compound from non-encapsulated aqueous solution.

112. The method of claim 111, wherein dialysis, ultracentrifugation, or gel filtration is used for removing peptide epoxyketone compound from the non-encapsulated aqueous solution.

113. The method of any of claims 86-112, further comprising, after forming the liposomal composition, adjusting the aqueous solution to a pH of between about pH 3.5 and about pH 7.0.

114. The method of any of claims 86-112, further comprising, after forming the liposomal composition, adjusting the aqueous solution to a human physiological pH.

115. The method of any of claims 86-114, further comprising, after forming the liposomal composition, adding an agent to the aqueous solution to maintain isotonicity.

116. The method of any of claims 86-115, wherein the peptide epoxyketone compound is selected from the group consisting of compound II, compound III, compound IV, and compound V.

117. The method of any of claims 86-116, wherein the peptide epoxyketone compound is carfilzomib (compound V).

118. A pharmaceutical liposomal composition made by the method of any of claims 86-117.

119. A method of treating multiple myeloma in a subject in need of treatment, comprising:

administering a therapeutically effective amount of a pharmaceutical liposomal composition any one of claims 1-27, 54, 85, or 118.

120. The method of claim 119, further comprising simultaneous, sequential, or separate administration of a therapeutically effective amount of a chemotherapeutic agent.

121. A method of treating a solid tumor in a subject in need of treatment, comprising:
administering a therapeutically effective amount of a pharmaceutical liposomal composition any one of claims 1-27, 54, 85, or 118.

122. The method of claim 121, further comprising simultaneous, sequential, or separate administration of a therapeutically effective amount of a chemotherapeutic agent.

123. A method of treating a disease or condition in a subject in need of treatment, comprising:

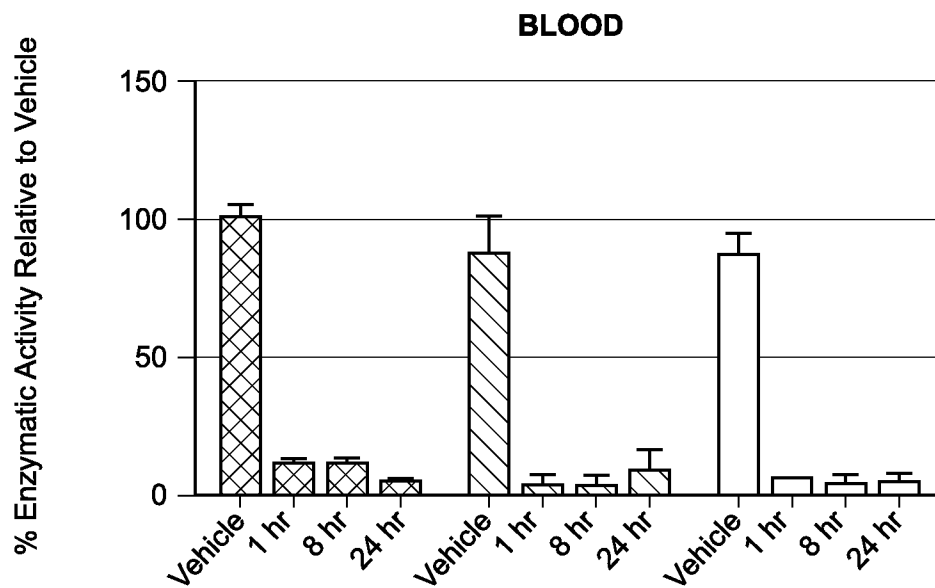
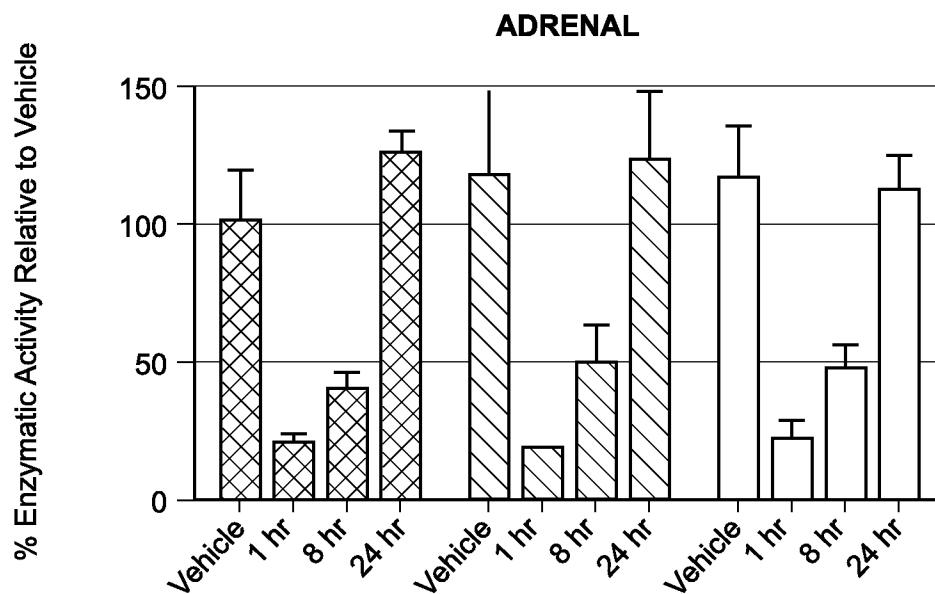
administering a therapeutically effective amount of a pharmaceutical liposomal composition any one of claims 1-27, 54, 85, or 118.

124. The method of claim 123, further comprising simultaneous, sequential, or separate administration of a therapeutically effective amount of a chemotherapeutic agent, a cytokine, a steroid, or an immunotherapeutic agent.

Lot	Composition	CFZ (mg)	EPC (mg)	mPEG (mg)	Cholesterol (mg)	Drug: Lipid Ratio (%)	Lipid Conc (mg/mL)	mPEG Conc (mg/mL)	Cholesterol Conc. (mg/mL)	Rehydration Volume (mL)	Theoretical Conc (mg/mL)	Avg Conc (mg/mL)	n =	% Diff	SD	% RSD	Used In:
6005-27B	A	0	104.1	0	0	0	13.0	0	0	8	0	0	N/A	N/A	N/A	N/A	Table 4
6005-29C	B	5.8	111.3	0	0	5%	13.9	0	0	8	0.73	0.81	3	12	0.008	0.95	Table 4
6005-35B	D	0	91.39	10.31	0	0	11.4	1.3	0	8	0	0	1	N/A	N/A	N/A	Table 4/Example 5
6005-45A	C	17.2	112.3	0	0	13%	14.0	0	0	8	2.15	N/A	N/A	N/A	N/A	N/A	Table 4
6005-45B	C	16.5	105.5	0	0	14%	13.2	0	0	8	2.06	N/A	N/A	N/A	N/A	N/A	Table 4
6005-45A/B Pooled	C	N/A	N/A	N/A	0	N/A	N/A	0	0	N/A	N/A	1.97	3	N/A	0.004	0.2	Table 4
6005-45C	E	17	94.6	12.7	0	15%	11.8	1.6	0	8	2.13	N/A	N/A	N/A	N/A	N/A	Table 4
6005-45D	E	16	93.9	11.5	0	15%	11.7	1.4	0	8	2.00	N/A	N/A	N/A	N/A	N/A	Table 4
6005-45C/D Pooled	E	N/A	N/A	N/A	0	N/A	N/A	0	0	N/A	N/A	2.06	3	N/A	0.104	5.0300	Table 4
6005-53A	C	22.6	130.6	0	0	15%	13.1	0	0	10	2.26	2.31	3	2	0.015	0.63	Table 5
6005-53D	C	41.9	251.8	0	0	14%	12.6	0	0	20	2.10	2.10	3	0	0.012	0.59	Table 5
6005-53A/D Pooled	C	N/A	N/A	N/A	0	N/A	N/A	0	0	N/A	N/A	2.04	3	N/A	0.108	5.29	Table 5
6005-53C	A	0	129.8	0	0	0	13.0	0	0	10	0	0	N/A	N/A	N/A	N/A	Table 5
6005-67B	C	41.7	252.9	0	0	14%	12.6	0	0	20	2.09	2.10	3	1	0.006	0.27	Table 8/9
6005-69A	C	21.3	126.9	0	0	14%	12.7	0	0	10	2.13	2.18	3	2	0.02	0.96	Table 7/9
6005-69B	A	0	125.9	0	0	0	12.6	0	0	10	0	0	1	N/A	N/A	N/A	Ex 6
6005-71D	F	0	124.5	13.2	31.4	0	12.5	1.3	3.1	10	0	0	1	N/A	N/A	N/A	Ex 6
6005-71E	G	20.0	127.2	13.6	31.6	14%	12.7	1.4	3.2	10	2.0	1.97	3	1.5	.01	0.24	Table 7/9

FIG. 1

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**FIG. 2A****FIG. 2B**

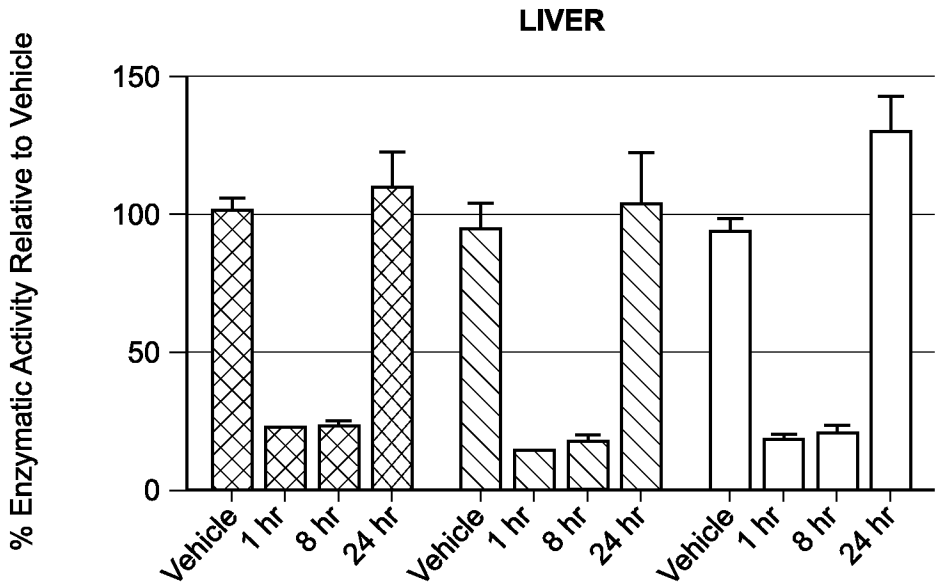


FIG. 2C

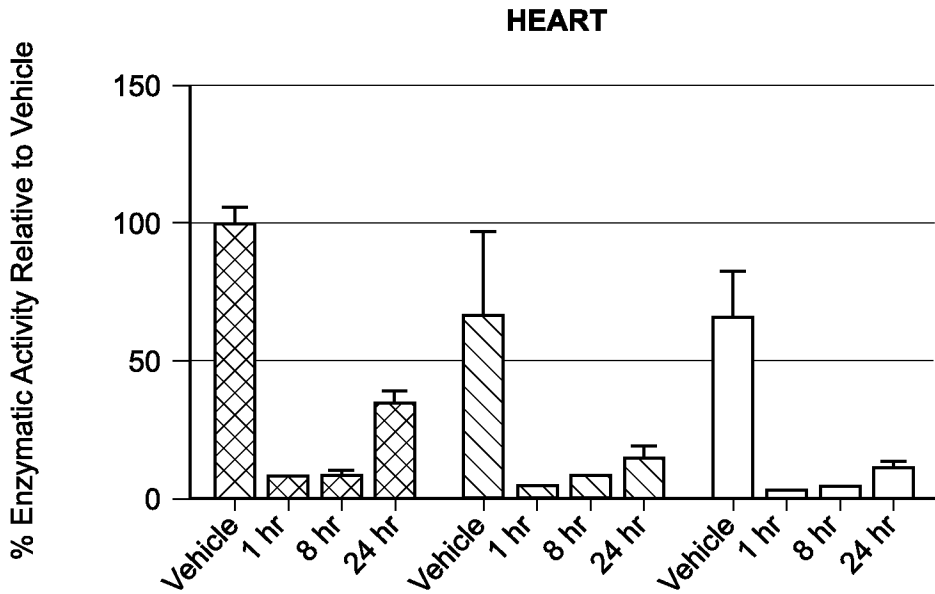


FIG. 2D

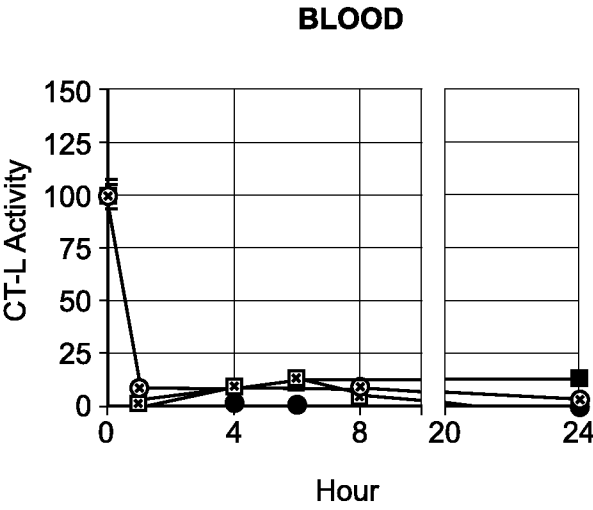


FIG. 3A

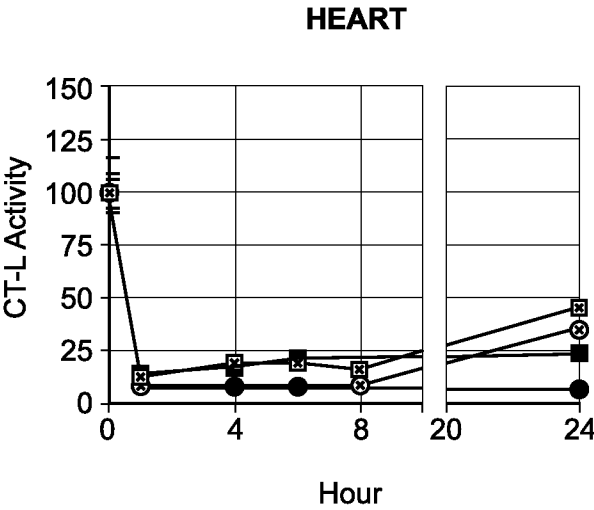


FIG. 3B

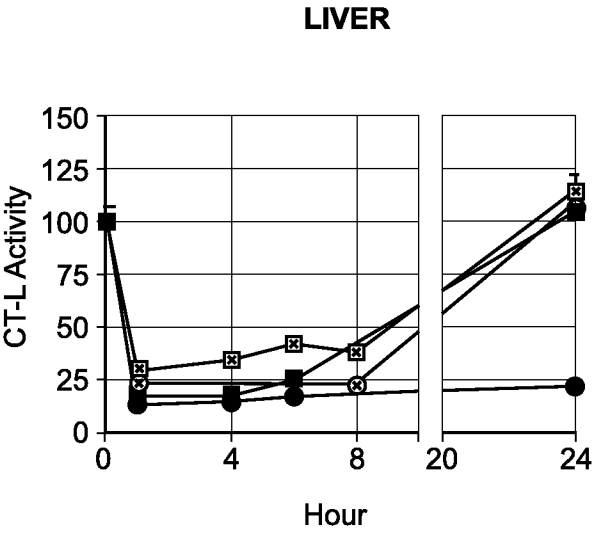


FIG. 3C

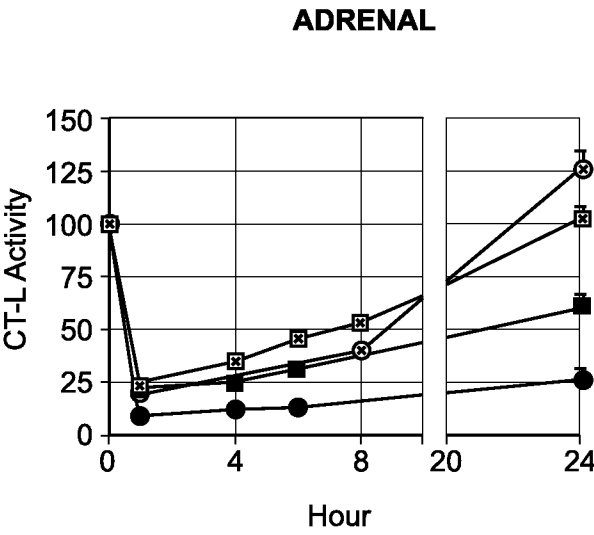


FIG. 3D

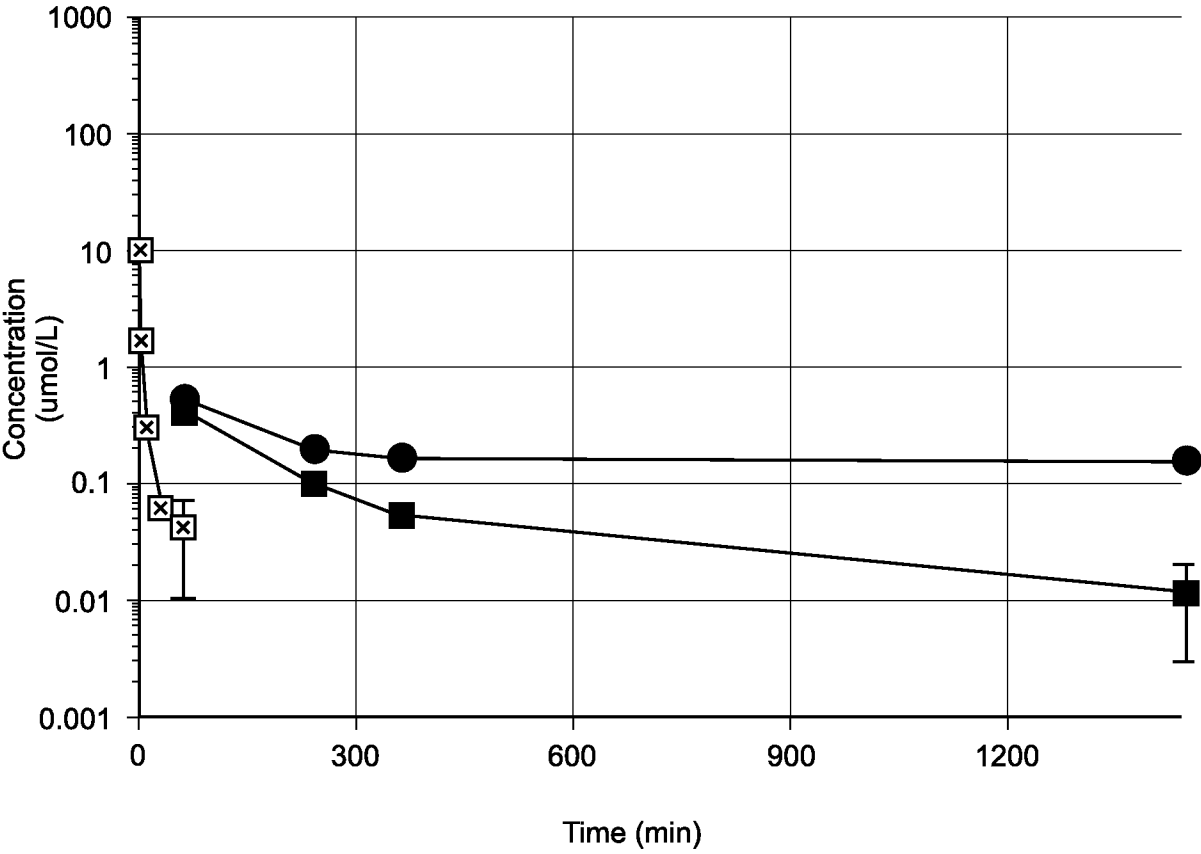


FIG. 4

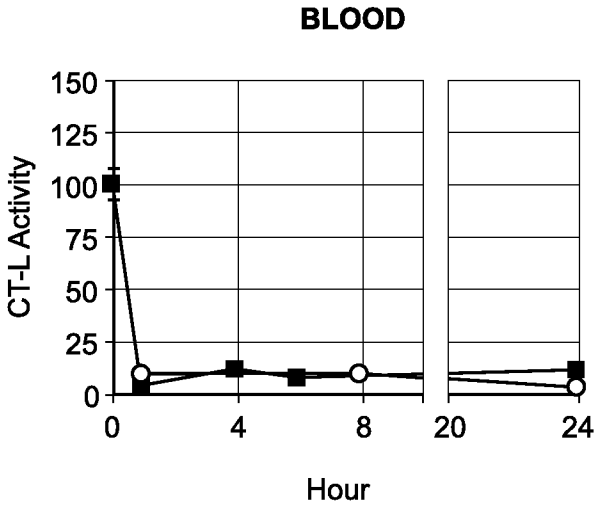


FIG. 5A

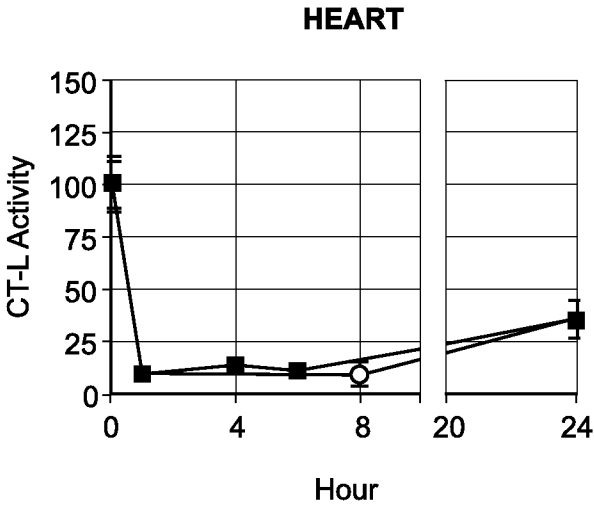


FIG. 5B

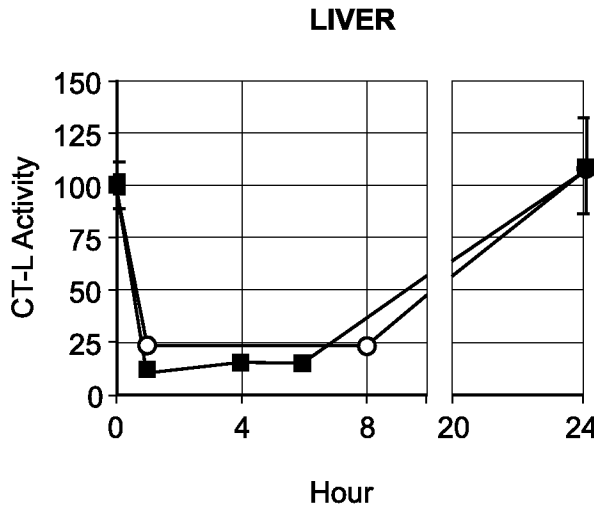


FIG. 5C

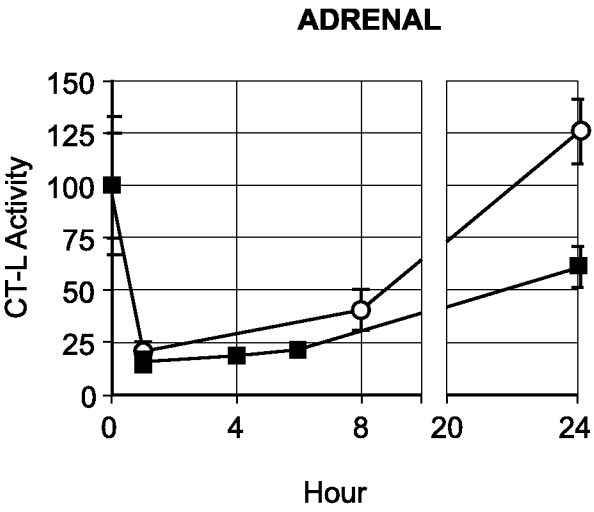
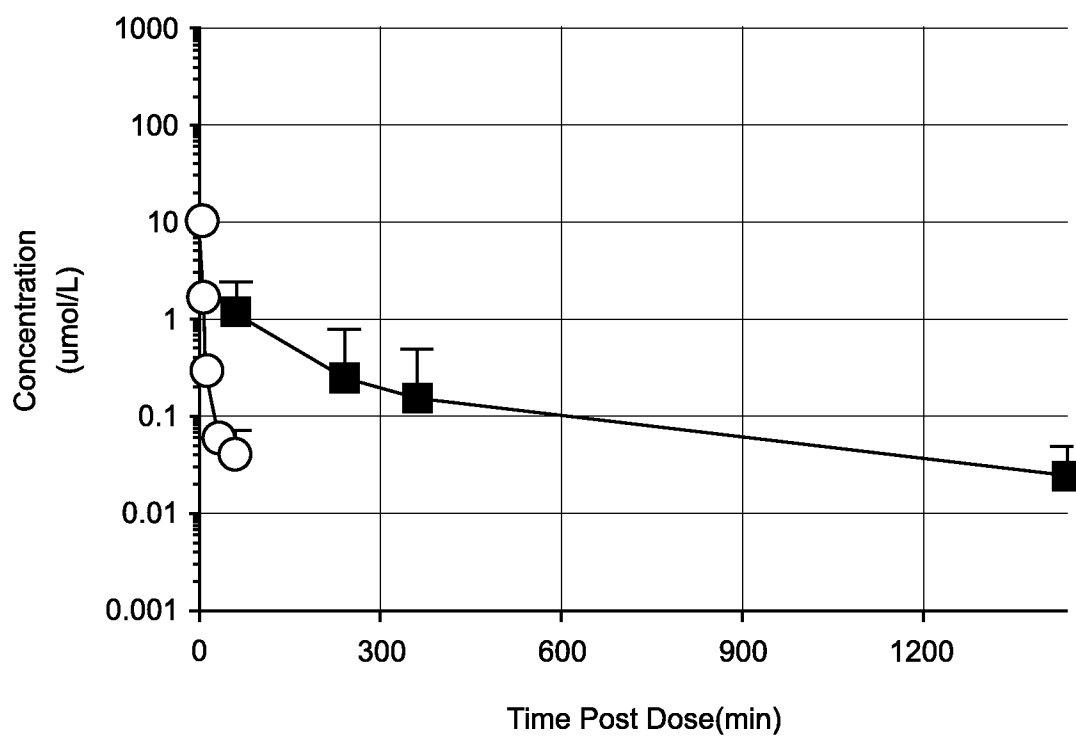


FIG. 5D

**FIG. 6**

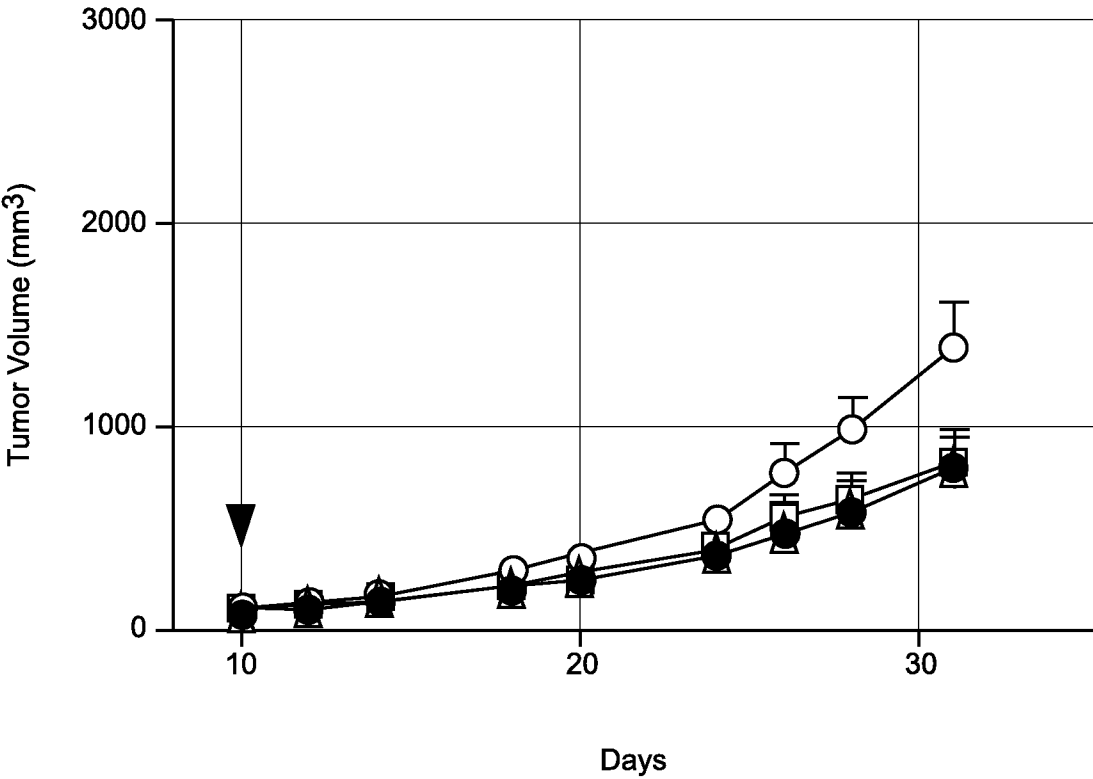


FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/050853

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 9/127 (2013.01)

USPC - 424/450

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61K 9/127, 47/40 (2013.01)

USPC - 264/4.1; 424/1.21, 9.321, 450; 436/829

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

CPC - A61K9/127, 1271, 1273 (2013.01)

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

Orbit, Google Patents, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,004,534 A (LANGER et al) 21 December 1999 (21.12.1999) entire document	1-3, 29-35, 55-60
Y	US 2011/0236428 A1 (KIRK et al) 29 September 2011 (29.09.2011) entire document	1-3, 29-35, 55-60, 86-91
Y	US 4,687,661 A (KIKUCHI et al) 18 August 1987 (18.08.1987) entire document	86-91
A	US 2009/0004145 A1 (RAMESH) 01 January 2009 (01.01.2009) entire document	1-3, 29-35, 55-60, 86-91

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

11 November 2013

Date of mailing of the international search report

05 DEC 2013

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/050853

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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.: 4-28, 36-54, 61-85, 92-124
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

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.:

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.