# TREATMENT OF GLOMERULONEPHRITIS

**[0001]** This application claims the benefit of priority of U.S. Provisional Application No. 63/567,996, filed March 21, 2024, the disclosure of which is incorporated by reference in its entirety for all purposes.

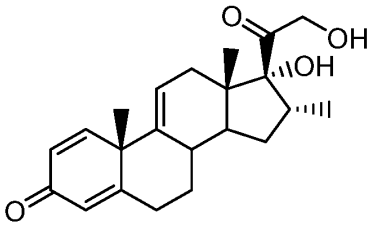
**[0002]** Anti-inflammatory steroids are the standard of care for glomerulonephritis, particularly lupus nephritis (LN), and their use has dramatically increased the rate of survival. However, use of glucocorticoids in LN is associated with increased risk of osteoporosis, osteonecrosis, cardiovascular events, and other serious side effects. Vamorolone, also known as VB-15, VBP-15, 16α-methyl-9,11-dehydroprednisolone, or 17α,21-dihydroxy-16α-methylpregna-1,4,9(11)-triene-3,20-dione, is a novel dissociative steroid and derivative of glucocorticoids (GCs) within the structural class of synthetic steroidal drugs, which includes prednisone, deflazacort, methylprednisolone, and dexamethasone. Vamorolone contains a delta 9,11 double bond in its chemical structure and is differentiated from other glucocorticoid/corticosteroid compounds, which, in their active form, contain a hydroxyl group in the 11β-position. Vamorolone was recently approved by FDA, EMA and MHRA for the treatment of Duchenne muscular dystrophy (DMD) via demonstration of compelling efficacy and improved safety in double-blind trials.

**[0003]** Due to its unique chemical structure, vamorolone shows three key structure/activity relationships that fully differentiates it from other corticosteroids. While vamorolone retains the transrepressive activities responsible for the anti-inflammatory effects of GCs, it is differentiated from traditional steroids because its interactions with the target GR leads to alterations in co-factor binding with less co-activator affinity and greater co-repressor affinity. Additionally, vamorolone is a potent antagonist of the mineralocorticoid receptor, which may contribute to improved renoprotection and increased safety, and is not subject to pro-drug/active drug modulation by 11β-hydroxysteroid dehydrogenase enzymes (HSD11B1, HSD11B2), as vamorolone lacks a hydroxyl/carbonyl group at the 11β position. This is of particular importance in the kidney, where HSD11B2, the enzyme responsible for inactivating traditional steroids, is highly expressed.

**[0004]** Long-term treatment with traditional corticosteroids, such as deflazacort and prednisone, may also present with a broad range of safety concerns that detract from a patient's quality of life. Accordingly, there remains a need in the art for treatments for glomerulonephritis in humans, particularly treatments that may be administered chronically.

## SUMMARY

**[0005]** Provided is a method of treatment of glomerulonephritis, comprising administering to the subject in need thereof between about 0.5 mg/kg/day and about 1 mg/kg/day of a compound having the structural formula

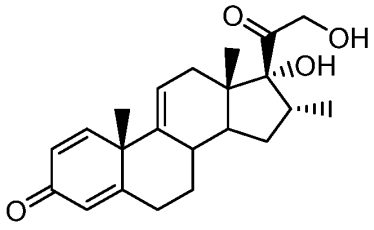



, or a salt thereof.

**[0006]** Also provided is a method of renoprotection in a subject in need thereof, comprising:

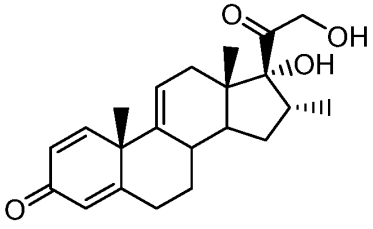
a) assaying a biological sample from the subject to identify the levels of one or more biomarkers; and

b) administering to the subject in need thereof an initial amount of between about 0.25 mg/kg/day and about 10 mg/kg/day of a compound having the structural formula



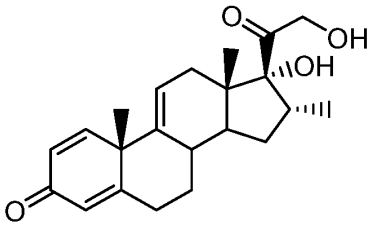

, or a salt thereof.

**[0007]** Also provided is a method of increasing the expression of a biomarker in a subject with glomerular nephritis currently being treated with prednisolone, comprising administering to the subject in need thereof between about 0.5 mg/kg and about 1 mg/kg of a compound having the structural formula




, or a salt thereof.

**[0008]** Also provided is a method of increasing the expression of one or more proteins chosen from growth hormone receptor, renin, IGFBP3, AHSG, and klotho in a subject with glomerular nephritis, comprising administering to the subject in need thereof between about 0.5 mg/kg and about 1 mg/kg of a compound having the structural formula

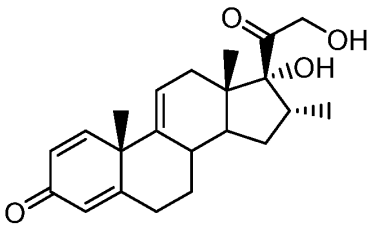


, or a salt thereof.

**[0009]** Also provided is a method of treatment of a subject with glomerular nephritis, comprising:

a) assaying a biological sample from the subject to identify the levels of one or more biomarkers;

b) administering to the subject in need thereof an initial amount of between about 0.5 mg/kg and about 1 mg/kg of a compound having the structural formula

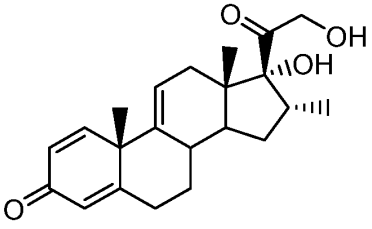


, or a salt thereof.

**[0010]** Also provided is a method of treatment of a subject with glomerular nephritis, comprising:

a) assaying a biological sample from the subject to identify the levels of one or more biomarkers;

b) administering to the subject in need thereof an initial amount of between about 0.5 mg/kg and about 1 mg/kg of a compound having the structural formula



, or a salt thereof;

c) collecting and assaying a new biological sample from the subject at one or more intervals and assaying for the levels of the one or more biomarkers; and

d) increasing or decreasing the amount of the compound in order to increase, decrease, or maintain the levels of the one or more biomarkers.

**[0011]** These and other aspects of the invention disclosed herein will be set forth in greater detail as the patent disclosure proceeds.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0012]** **FIG. 1A** depicts a graph showing the change in log2 concentrations from baseline over time of the collagen alpha-1(X) chain biomarker. **FIG. 1B** depicts a graph showing the change in log2 concentrations from baseline over time of the collagen alpha-2(XI) chain biomarker.

**[0013]** **FIG. 2A** depicts a graph showing the change in log2 concentrations from baseline over time of the chondrocalcin biomarker. **FIG. 2B** depicts a graph showing the change in log2 concentrations from baseline over time of the aggrecan core protein biomarker.

**[0014]** **FIG. 3A** depicts a graph showing the change in log2 concentrations from baseline over time of the biglycan biomarker. **FIG. 3B** depicts a graph showing the change in log2 concentrations from baseline over time of the alpha-L-fucoside fucohydrolase biomarker.

**[0015]** **FIG. 4A** depicts a graph showing the change in log2 concentrations from baseline over time of the HAPLN1 biomarker. **FIG. 4B** depicts a graph showing the change in log2 concentrations from baseline over time of the OSTM1 biomarker.

**[0016]** **FIG. 5A** depicts a graph showing the change in log2 concentrations from baseline over time of the growth hormone receptor biomarker. **FIG. 5B** depicts a graph showing the change in log2 concentrations from baseline over time of the IGF-1 biomarker.

**[0017]** **FIG. 6** depicts a graph showing the change in log2 concentrations from baseline over time of the IGFBP3 biomarker.

**[0018]** **FIG. 7A** depicts a graph showing the change in log2 concentrations from baseline over time of the renin biomarker. **FIG. 7B** depicts a graph showing the change in log2 concentrations from baseline over time of the angiotensinogen biomarker.

**[0019]** **FIG. 8A** depicts a graph showing the change in log2 concentrations from baseline over time of the klotho biomarker. **FIG. 8B** depicts a graph showing the change in log2 concentrations from baseline over time of the AHSG biomarker.

**[0020]** **FIG. 9** depicts the experimental timeline for the mouse study. Female WT (MRL) and lupus (MRL-*lpr*) mice arrived at 8 weeks of age. At 12 weeks, blood (submental) was

collected and daily treatment with vamorolone (vam; 30 mg/kg), prednisolone (pred; 10 mg/kg) or vehicle began. Mice were euthanized after 8 weeks of treatment.

**[0021]** **FIG. 10** depicts the survival results. Vamorolone (vam) and prednisolone (pred) improved survival in MRL-*lpr* mice. End of study (EOS), day=56. Note: all MRL mice reached EOS, not shown. Data presented as mean ± SEM.

**[0022]** **FIGS. 11A** and **11B** depict a clinical assessment of the study results. Vamorolone (vam) and prednisolone (pred) reduced lymphadenopathy in MRL-*lpr* mice. Both treatments appear to decrease development of skin lesions, with vamorolone having a better effect. Note: all MRL mice scored 0, not shown. Data presented as mean ± SEM.

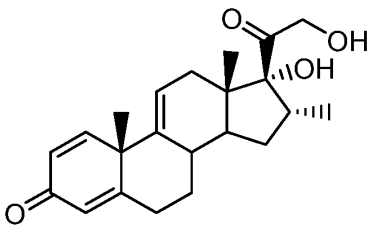
**[0023]** **FIGS. 12A–12C** depict BUN levels pre- and post-treatment. There were no differences in BUN levels at baseline between MRL and MRL-*lpr* mice. There was a significant decrease in BUN after prednisolone treatment and a trend for a decrease in vamorolone-treated mice. Data presented as mean ± SEM.

**[0024]** **FIGS. 13A–13D** depict kidney and spleen weight at euthanasia. Kidneys (averaged) and spleen weight from vehicle-treated MRL-*lpr* mice were significantly higher than MRL mice. Spleen weight was significantly decreased after treatment. Note: this only includes mice who made it to EOS. Data presented as mean ± SEM.

**[0025]** **FIG. 14** shows that vamorolone and prednisone reduce serum creatinine in MRL-*lpr* mice. Serum from blood collected at euthanasia was analyzed for creatinine levels after 8 weeks of treatment. Vamorolone and prednisolone reduced creatinine levels in MRL-*lpr* mice compared to vehicle. Veh, vehicle; vam, vamorolone; pred, prednisolone. *$p<0.05$.

## DETAILED DESCRIPTION

**[0026]** Provided is a method of treatment of glomerulonephritis, comprising administering to the subject in need thereof between about 0.25 mg/kg/day and about 10 mg/kg/day of a compound having the structural formula

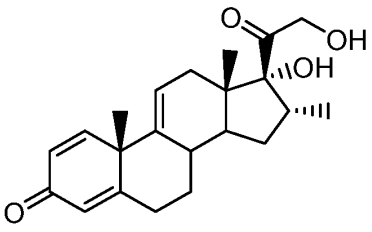



, or a salt thereof.

**[0027]** Also provided is a method of renoprotection in a subject in need thereof, comprising:

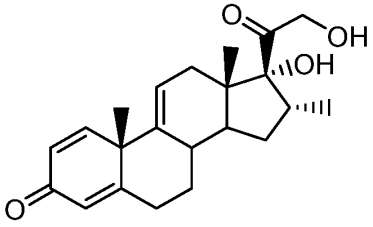
c) assaying a biological sample from the subject to identify the levels of one or more biomarkers; and

d) administering to the subject in need thereof an initial amount of between about 0.25 mg/kg/day and about 10 mg/kg/day of a compound having the structural formula



, or a salt thereof.

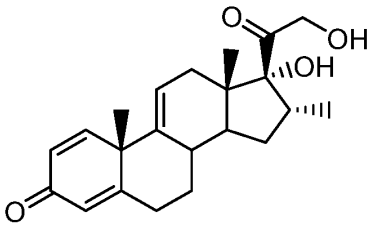
**[0028]** Also provided is a method of increasing the expression of a biomarker in a subject with glomerular nephritis currently being treated with prednisolone, comprising administering to the subject in need thereof between about 0.25 mg/kg/day and about 10 mg/kg/day of a compound having the structural formula



, or a salt thereof.

**[0029]** In some embodiments, the biomarker is chosen from collagen alpha-1(X), collagen alpha-2(XI), chondrocalcin, aggrecan, biglycan, alpha-l-fucosidase, HPLN1, and OSTM1.

**[0030]** Also provided is a method of increasing the expression of one or more proteins chosen from growth hormone receptor, renin, IGFBP3, AHSG, and klotho in a subject with glomerular nephritis, comprising administering to the subject in need thereof between about 0.25 mg/kg/day and about 10 mg/kg/day of a compound having the structural formula

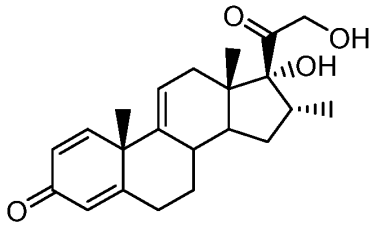



, or a salt thereof.

**[0031]** Also provided is a method of treatment of a subject with glomerular nephritis, comprising:

a) assaying a biological sample from the subject to identify the levels of one or more biomarkers;

b) administering to the subject in need thereof an initial amount of between about 0.25 mg/kg/day and about 10 mg/kg/day of a compound having the structural formula

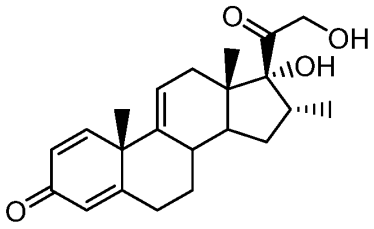



, or a salt thereof.

**[0032]** Also provided is a method of treatment of a subject with glomerular nephritis, comprising:

a) assaying a biological sample from the subject to identify the levels of one or more biomarkers;

b) administering to the subject in need thereof an initial amount of between about 0.25 mg/kg/day and about 10 mg/kg/day of a compound having the structural formula




, or a salt thereof;

c) collecting and assaying a new biological sample from the subject at one or more intervals and assaying for the levels of the one or more biomarkers; and

d) increasing or decreasing the amount of the compound in order to increase, decrease, or maintain the levels of the one or more biomarkers.

**[0033]** In some embodiments, the one or more biomarkers are independently chosen from renin, growth hormone, and aldosterone.

**[0034]** In some embodiments, the one or more biomarkers are independently chosen from collagen alpha-1(X), collagen alpha-2(XI), chondrocalcin, aggrecan, biglycan, alpha-l-fucosidase, HPLN1, OSTM1, growth hormone receptor, renin, IGFBP3, AHSG, klotho, renin, and aldosterone.

**[0035]** In some embodiments, the levels of one or more biomarkers are determined by an immunoassay, a spectrometric method, or an aptamer-based screen.

**[0036]** In some embodiments, the spectrometric method is chosen from is chosen from UV-visible spectroscopy, mass spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, proton NMR spectroscopy, nuclear magnetic resonance (NMR) spectrometry, gas chromatography, mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), correlation spectroscopy (COSY), nuclear Overhauser effect spectroscopy (NOESY), rotating-frame nuclear Overhauser effect spectroscopy (ROESY), time-of-flight LC-MS (LC-TOF-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and capillary electrophoresis-mass spectrometry.

**[0037]** In some embodiments, the aptamer-based screen is performed using slow off-rate modified aptamers (SOMAmers).

**[0038]** In some embodiments, the immunoassay is a bead-based immunoassay or a plate-based sandwich immunoassay.

**[0039]** In some embodiments, the initial dose of the compound is about 0.25, about 0.5, about 1, about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, or about 10 mg/kg/day.

**[0040]** In some embodiments, the method further comprises step c) collecting and assaying a new biological sample from the subject at one or more intervals and assaying for the levels of the one or more biomarkers.

**[0041]** In some embodiments, the intervals are about 12 weeks.

**[0042]** In some embodiments, the method further comprises step d) increasing or decreasing the amount of the compound to increase, decrease, or maintain the levels of the one or more biomarkers.

**[0043]** In some embodiments, the dose is increased or decreased by about 0.5, about 1, about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, or about 5 mg/kg/day.

**[0044]** In some embodiments, the method further comprises repeating steps c) and d) until a maintenance dose is achieved.

**[0045]** In some embodiments, the maintenance dose is between about 0.25 mg/kg/day and about 10 mg/kg/day.

**[0046]** In some embodiments, the glomerulonephritis is IgA nephropathy-associated glomerulonephritis, post-infectious glomerulonephritis, mesangial proliferative glomerulonephritis, membranoproliferative glomerulonephritis (MPGN), immune complex-mediated MPGN, complement-mediated MPGN, C3 glomerulonephritis, rapidly progressive glomerulonephritis, Goodpasture syndrome, or lupus nephritis.

**[0047]** In some embodiments, the lupus nephritis is minimal mesangial LN, mesangial proliferative LN, focal proliferative LN, diffuse proliferative LN, membranous LN, or advanced sclerosing LN.

**[0048]** In some embodiments, the administration is for at least 6 months.

**[0049]** In some embodiments, the administration is for at least 12 months.

**[0050]** In some embodiments, about 0.5 mg/kg/day of the compound is administered.

**[0051]** In some embodiments, about 1 mg/kg/day of the compound is administered.

**[0052]** In some embodiments, the compound is administered orally.

**[0053]** In some embodiments, the compound is administered as a solution or suspension.

**[0054]** In some embodiments, the solution or suspension comprises about 4 wt.% of the compound.

**[0055]** In some embodiments, the compound is administered as a tablet.

**[0056]** In some embodiments, the vamorolone, or a salt thereof, is administered via a titration scheme. In some embodiments, the goal of the titration scheme is to achieve an optimal level of disease control in which the patient is tolerating the treatment regimen, or has achieved satisfactory treatment, or, in the case of up-titration, until the maximum permitted dose is reached, or, in the case of down-titration, until administration of the vamorolone, or a salt thereof, is terminated.

**[0057]** In some embodiments, the vamorolone, or a salt thereof, is administered via a titration scheme that comprises the down-titration of the vamorolone, or a salt thereof, until a maintenance dose is administered.

**[0058]** In some embodiments, the down-titration scheme comprises:

administering an initial dose of the vamorolone, or a salt thereof,

monitoring the reduction of symptoms of glomerulonephritis and tolerability of the patient to the treatment,

administering a reduced dose of the vamorolone, or a salt thereof.

**[0059]** In some embodiments, the cycle of monitoring and reducing the dose that is administered is repeated until a maintenance dose is administered.

**[0060]** In some embodiments, the initial dose in a down-titration scheme is between about 0.25 mg/kg/day and 10 mg/kg/day. In some embodiments, the initial dose is about 0.25, about 0.5, about 1, about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, or about 10 mg/kg/day.

**[0061]** In some embodiments, the reduced dose is between about 0.25 mg/kg/day and 10 mg/kg/day. In some embodiments, the initial dose is about 0.25, about 0.5, about 1, about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, or about 10 mg/kg/day.

**[0062]** In some embodiments, the maintenance dose is between about between about 0.25 mg/kg/day and 10 mg/kg/day. In some embodiments, the initial dose is about 0.25, about 0.5, about 1, about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, or about 10 mg/kg/day.

**[0063]** In some embodiments, for each cycle of reduction, the dose is reduced by an increment of about 0.5, about 1, about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, or about 5 mg/kg/day.

**[0064]** In some embodiments, the vamorolone, or a salt thereof, is administered via a titration scheme that comprises the up-titration of the vamorolone, or a salt thereof, until a maintenance dose is administered.

**[0065]** In some embodiments, the up-titration scheme comprises:

administering an initial dose of the vamorolone, or a salt thereof,

monitoring the reduction of symptoms of the NF-κB-mediated disease and tolerability of the patient to the treatment,

administering an increased dose of the vamorolone, or a salt thereof.

**[0066]** In some embodiments, the cycle of monitoring and increasing the dose that is administered is repeated until a maintenance dose is administered.

**[0067]** In some embodiments, the initial dose for an up-titration scheme is between about 0.25 mg/kg/day and 10 mg/kg/day. In some embodiments, the initial dose is about 0.25, about

0.5, about 1, about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, or about 10 mg/kg/day.

**[0068]** In some embodiments, for each cycle, the dose is increased by an increment of about 0.5, about 1, about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, or about 5 mg/kg/day.

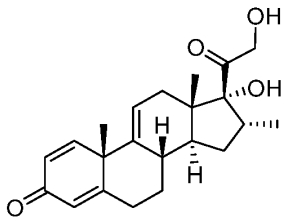
**[0069]** In some embodiments, the increased dose is between about 0.25 mg/kg/day and 10 mg/kg/day. In some embodiments, the initial dose is about 0.25, about 0.5, about 1, about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, or about 10 mg/kg/day.

**[0070]** In some embodiments, the maintenance dose is between about 0.25 mg/kg/day and 10 mg/kg/day. In some embodiments, the initial dose is about 0.25, about 0.5, about 1, about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, or about 10 mg/kg/day.

**Definitions**

**[0071]** As used in the present specification, the following words and phrases are generally intended to have the meanings as set forth below, except to the extent that the context in which they are used indicates otherwise.

**[0072]** As used herein, "vamorolone" refers to 17α,21-dihydroxy-16α-methylpregna-1,4,9(11)-triene-3,20-dione (also known as VBP15 or VB-15) and has the structure:




**[0073]** As used herein, the term "about" is intended to qualify the numerical values it modifies, denoting such a value as a variable within a margin of error. When no particular margin of error, such as a standard deviation to a mean value given in a chart or table of data, is recited, the term "about" should be understood to mean that range which would encompass the recited value and the range which would be included by rounding up or down to that figure as well, taking into account significant figures.

**[0074]** As used herein, "administering" means to provide a compound or other therapy, remedy, or treatment such that an individual internalizes a compound.

**[0075]** As used herein, the term "disease" is intended to be generally synonymous, and is used interchangeably with, the terms "disorder" and "condition" (as in medical condition), in that all reflect an abnormal condition of the human or animal body or of one of its parts that impairs normal functioning, is typically manifested by distinguishing signs and symptoms, and causes the human or animal to have a reduced duration or quality of life.

**[0076]** As used herein, "in need of treatment" and "in need thereof" when referring to treatment are used interchangeably to mean a judgment made by a caregiver (*e.g.,* physician, nurse, nurse practitioner, *etc.* in the case of humans; veterinarian in the case of animals, including non-human mammals) that an individual or animal requires or will benefit from treatment. This judgment is made based on a variety of factors in the realm of a caregiver's expertise, but that includes the knowledge that the individual or animal is ill, or will become ill, as the result of a disease, condition, or disorder that is treatable by the compounds of the invention. Accordingly, the compounds of the invention can be used in a protective or preventive manner; or compounds of the invention can be used to alleviate, inhibit or ameliorate the disease, condition, or disorder.

**[0077]** When ranges of values are disclosed, and the notation "from $n_1$ … to $n_2$" is used, where $n_1$ and $n_2$ are the numbers, then unless otherwise specified, this notation is intended to include the numbers themselves and the range between them. This range may be integral or continuous between and including the end values. Thus, by way of example, the range "from 2 to 6 carbons" is intended to include two, three, four, five, and six carbons since carbons come in integer units. Compare, by way of example, the range "from 1 to 3 μM (micromolar)," which is intended to include 1 μM, 3 μM, and everything in between to any number of significant figures (e.g., 1.255 μM, 2.1 μM, 2.9999 μM, etc.).

**[0078]** As used herein, "suspension" refers to a mixture of a solid in a liquid. In contrast, an "emulsion" refers to a mixture of two immiscible liquids.

**[0079]** As used herein, the term "therapeutically acceptable" refers to those compounds (or salts, prodrugs, tautomers, zwitterionic forms, etc.) suitable for use in contact with the tissues of patients without undue toxicity, irritation, and allergic response, are commensurate with a reasonable benefit/risk ratio and are effective for their intended use.

**[0080]** As used herein, the phrase "therapeutically effective" is intended to qualify the amount of active ingredients used to treat a disease or disorder. This amount will achieve the goal of reducing or eliminating the disease or disorder.

**[0081]** As used herein, “treating,” “treatment,” and the like means ameliorating a disease to reduce or eliminate its cause, its progression, its severity, or one or more of its symptoms, or otherwise beneficially alter the disease in a subject.

**[0082]** As used herein, a "dose" means the measured quantity of an active agent to be taken at one time by a patient.

**[0083]** As used herein, a “dosage” is the prescribed administration of a specific amount, number, and frequency of doses over a specific period of time.

**[0084]** As used herein, “down-titration” or “dose de-escalation” of a compound refers to decrease the amount of a compound to achieve a therapeutic effect that occurs before administration of the compound is terminated. Down-titration can be achieved in one or more dose increments, which may be the same or different.

**[0085]** As used herein, “up-titration” or “dose escalation” of a compound refers to increasing the amount of a compound to achieve a therapeutic effect that occurs before dose-limiting intolerability for the patient. Up-titration can be achieved in one or more dose increments, which may be the same or different.

**[0086]** As used herein, “maximum recommended total daily dose” or “maximum recommended daily dosage” or “maximum total daily dose” or “maximum daily dosage” or “total daily dosage” refers to the highest safe dosage of drug to be administered on a daily basis following dosage titration, i.e., the maintenance dose, as determined by a titration scheme, should not exceed the maximum recommended total daily dose.

**[0087]** Throughout this specification, unless the context requires otherwise, the word “comprise,” or variations such as “comprises” or “comprising” will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of elements or integers.

**[0088]** Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps, or group of compositions of matter shall be taken to encompass one and a plurality (*i.e.*, one or more) of those steps, compositions of matter, groups of steps, or groups of compositions of matter.

**[0089]** Each embodiment described herein is to be applied *mutatis mutandis* to each other embodiment unless specifically stated otherwise.

**[0090]** Those skilled in the art will appreciate that the invention(s) described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention(s) includes all such variations and modifications. The invention(s) also includes all the steps, features, compositions, and compounds referred to or

indicated in this specification, individually or collectively, and all combinations or any two or more steps or features unless specifically stated otherwise.

**[0091]** The present invention(s) is not limited in scope by the specific embodiments described herein, which are intended for exemplification only. Functionally equivalent products, compositions, and methods are clearly within the scope of the invention(s), as described herein.

**[0092]** It is appreciated that certain features of the invention(s), which are, for clarity, described in the context of separate embodiments, can also be provided in combination in a single embodiment. Conversely, various features of the invention(s), which are, for brevity, described in the context of a single embodiment, can also be provided separately or in any suitable subcombination.

## EXAMPLES

**[0093]** The following examples are included to demonstrate some embodiments of the disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in the examples represent techniques discovered by the inventors to function well in the practice of the disclosure. Those of skill in the art should, however, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure, therefore all matter set forth is to be interpreted as illustrative and not in a limiting sense.

### Example 1: Validation of serum biomarkers assays for proteins which are differentially modulated by treatment vamorolone compared to prednisone.

**[0094]** The SOMAscan platform is an aptamer-based high-throughput proteomics platform that uses Slow Off-rate Modified Aptamers (SOMAmers) for multiplexed protein analysis. SOMAmer reagents are modified short DNA sequences that contain functional side-chains that interact and bind to conformational epitopes and specific proteins, as well as a 5′-linker for compatibility with the screening platform. Because of their slow off-rate modification, SOMAmers can be used with more sensitivity when compared to antibodies.

**[0095]** A SOMAscan screen of 1,500 serum proteins was performed on clinical trial samples from a previously performed 48-week study of vamorolone, prednisone or placebo in Duchenne subjects. In the first 24-week double-blind period, 121 ambulant boys aged 4 to <7 years with DMD were randomized to receive vamorolone (low dose 2 mg/kg/day or high dose 6 mg/kg/day) or prednisone (0.75 mg/kg/day) or placebo. In the second period of 24 weeks, all participants received vamorolone treatment on either of the two dose levels. 238

samples were tested, with sample sets taken from three groups: those that transitioned from prednisone to vamorolone 6 mg/kg/day (PREDVAM6), those that transitioned from placebo to vamorolone 6 mg/kg/day (PLBVAM6), and those that received vamorolone 6 mg/kg/day throughout.

**[0096]** The screen identified proteins which were differentially modulated by vamorolone compared to prednisone, and thus are likely relevant to LN. These proteins are implicated in MR receptor antagonism, growth hormone receptor (GHR) signaling, and bone turnover:

- **MR receptor related**: renin, angiotensinogen (AGT), alpha-2-HS-glycoprotein (AHSG), and klotho.
- **GHR related**: growth hormone receptor, insulin-like growth factor I (IGF-1), and insulin-like growth factor-binding protein 3 (IGFBP3).
- **Bone related**: collagen alpha-1(X) chain (COAA1), collagen alpha-2(XI) chain (COL11A2), chondrocalcin, aggrecan, biglycan, alpha-l-fucosidase, hyaluronan and proteoglycan link protein 1 (HPLN1), and osteopetrosis-associated transmembrane protein 1 (OSTM1).

**[0097]** Prednisone created a noticeable decrease in the bone biomarkers at Week 12, which slowly recovered to baseline with the transition of patients to vamorolone. In contrast, vamorolone did not decrease these bone biomarkers (**FIGS. 1A–4B**). For the MR- and GHR-related biomarkers, vamorolone created noticeable increases in each. Prednisolone, conversely, only increased the levels of IGF-1 and AGT in relation to vamorolone, and in the case of renin caused a decrease compared to placebo (**FIGS. 5A–8B**).

**[0098]** The SOMAscan aptamer assay findings will be validated using available Meso Scale Discovery (MSD) antibody assays for 6 of these most relevant to LN in existing Duchenne muscular dystrophy clinical trial serum samples (n=220). These assays will become pharmacodynamic biomarkers in future clinical trials.

**Example 2: Preclinical efficacy study of vamorolone in a mouse model of lupus nephritis.**

**[0099]** The efficacy of vamorolone compared to prednisone was determined in a murine model of LN. Twelve-week-old female MRL-*lpr* mice with established disease were treated with Vehicle, 10 mg/kg/day prednisone, or vamorolone doses for 8 weeks. Serum creatinine and BUN were assessed at Baseline and Week 8. At study termination (Week 8), kidneys were collected and assessed for changes in histopathology activity index (**FIG. 9**).

**[0100]** **FIG. 10** shows that treatment with vamolorone and prednisone improved survival in MRL-*lpr* mice. **FIGS. 11A** and **11B** show that treatment with vamorolone (vam) and prednisolone (pred) reduced lymphadenopathy in MRL-*lpr* mice. Both treatments appear to decrease development of skin lesions, with vamorolone having a better effect. **FIGS. 12A–12C** show that there were no differences in BUN levels at baseline between MRL and MRL-*lpr* mice. There was a significant decrease in BUN after prednisolone treatment and a trend for a decrease in vamorolone-treated mice. **FIGS. 13A–13D** show that kidneys (averaged) and spleen weight from vehicle-treated MRL-*lpr* mice were significantly higher than MRL mice. Spleen weight was significantly decreased after treatment. **FIG. 14** shows that vamorolone and prednisone reduce serum creatinine in MRL-*lpr* mice.

**[0101]** Those results show that the unique renoprotective effects of vamorolone (klotho induction; MR antagonism), coupled with the reduction in safety concerns (loss of bone morbidities; less mood disturbance) may lead to vamorolone replacing corticosteroids as standard of care in LN.

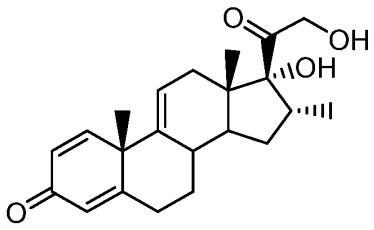
*Other Embodiments*

**[0102]** The detailed description set forth above is provided to aid those skilled in the art in practicing the present disclosure. However, the disclosure described and claimed herein is not to be limited in scope by the specific embodiments herein disclosed because these embodiments are intended as an illustration of several aspects of the disclosure. Any equivalent embodiments are intended to be within the scope of this disclosure. Indeed, various modifications of the disclosure in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description, which do not depart from the spirit or scope of the present inventive discovery. Such modifications are also intended to fall within the scope of the appended claims.

## CLAIMS

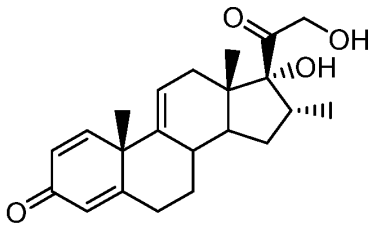
What is claimed is:

1. A method of treatment of a subject with glomerular nephritis, comprising:
    a) assaying a biological sample from the subject to identify the levels of one or more biomarkers; and
    b) administering to the subject in need thereof an initial amount of between about 0.25 mg/kg/day and about 10 mg/kg/day of a compound having the structural formula




, or a salt thereof.

2. A method of renoprotection in a subject in need thereof, comprising:
    a) assaying a biological sample from the subject to identify the levels of one or more biomarkers; and
    b) administering to the subject in need thereof an initial amount of between about 0.25 mg/kg/day and about 10 mg/kg/day of a compound having the structural formula

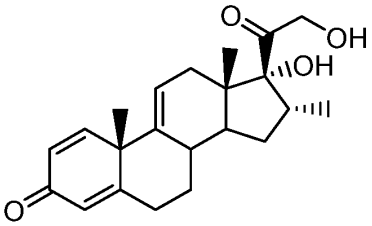



, or a salt thereof.

3. The method of claim 1, wherein the one or more biomarkers are independently chosen from collagen alpha-1(X), collagen alpha-2(XI), chondrocalcin, aggrecan, biglycan, alpha-l-fucosidase, HPLN1, OSTM1, growth hormone receptor, renin, IGFBP3, AHSG, klotho, renin, and aldosterone.
4. The method of any one of the preceding claims, wherein the levels of one or more biomarkers are determined by an immunoassay, a spectrometric method, or an aptamer-based screen.
5. The method of claim 4, wherein the spectrometric method is chosen from is chosen from UV-visible spectroscopy, mass spectroscopy, nuclear magnetic resonance

(NMR) spectroscopy, proton NMR spectroscopy, nuclear magnetic resonance (NMR) spectrometry, gas chromatography, mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), correlation spectroscopy (COSY), nuclear Overhauser effect spectroscopy (NOESY), rotating-frame nuclear Overhauser effect spectroscopy (ROESY), time-of-flight LC-MS (LC-TOF-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and capillary electrophoresis-mass spectrometry.

6. The method of claim 4, wherein the aptamer-based screen is performed using slow off-rate modified aptamers (SOMAmers).
7. The method of claim 4, wherein the immunoassay is a bead-based immunoassay or a plate-based sandwich immunoassay.
8. The method of any one of claims 1 to 7, further comprising step c) collecting and assaying a new biological sample from the subject at one or more intervals and assaying for the levels of the one or more biomarkers.
9. The method of claim 8, wherein the intervals are about 12 weeks.
10. The method of claim 9, further comprising step d) increasing or decreasing the amount of the compound to increase, decrease, or maintain the levels of the one or more biomarkers.
11. A method of treatment of a subject with glomerular nephritis, comprising:
    a) assaying a biological sample from the subject to identify the levels of one or more biomarkers;
    b) administering to the subject in need thereof an initial amount of between about 0.5 mg/kg and about 1 mg/kg of a compound having the structural formula




, or a salt thereof;

    c) collecting and assaying a new biological sample from the subject at one or more intervals and assaying for the levels of the one or more biomarkers; and
    d) increasing or decreasing the amount of the compound in order to increase, decrease, or maintain the levels of the one or more biomarkers.

12. The method of claim 10 or 11, wherein the dose is increased or decreased by about 0.5, about 1, about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, or about 5 mg/kg/day.

13. The method of claim 12, further comprising repeating steps c) and d) until a maintenance dose is achieved.

14. The method of claim 13, wherein the maintenance dose is between about 0.25 mg/kg/day and about 10 mg/kg/day.

15. The method of any one of claims 1 to 14, wherein the glomerulonephritis is IgA nephropathy-associated glomerulonephritis, post-infectious glomerulonephritis, mesangial proliferative glomerulonephritis, membranoproliferative glomerulonephritis (MPGN), immune complex-mediated MPGN, complement-mediated MPGN, C3 glomerulonephritis, rapidly progressive glomerulonephritis, Goodpasture syndrome, or lupus nephritis (LN).

16. The method of claim 15, wherein the lupus nephritis is minimal mesangial LN, mesangial proliferative LN, focal proliferative LN, diffuse proliferative LN, membranous LN, or advanced sclerosing LN.

17. The method of any one of claims 1 to 16, wherein the administration is for at least 6 months.

18. The method of claim 17, wherein the administration is for at least 12 months.

19. The method of any one of claims 1 to 18, wherein the compound is administered orally.

20. The method of claim 19, wherein the compound is administered as a solution or suspension.

21. The method of claim 20, wherein the solution or suspension comprises about 4 wt.% of the compound.

22. The method of claim 19, wherein the compound is administered as a tablet.