



(51) International Patent Classification:

A61K 31/18 (2006.01) C07D 213/44 (2006.01)
A61K 31/437 (2006.01) A61P 13/12 (2006.01)

(21) International Application Number:

PCT/US2018/055244

(22) International Filing Date:

10 October 2018 (10.10.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/570,778 11 October 2017 (11.10.2017) US

(71) Applicant: CHEMOCENTRYX, INC. [—/US]; 850
Maude Avenue, Mountain View, CA 94043 (US).(72) Inventors: MIAO, Zhenhua; c/o 850 Maude Avenue,
Mountain View, CA 94043 (US). SCHALL, Thomas, J.;
c/o 850 Maude Avenue, Mountain View, CA 94043 (US).
SINGH, Rajinder; 1832 Hillman Avenue, Belmont, CA
94002 (US).(74) Agent: KEZER, William, B. et al.; Mintz Levin Cohn Fer-
ris Glovsky and Popeo, P.C., One Financial Center, Boston,
MA 02111 (US).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP,
KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,
OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,

SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: TREATMENT OF FOCAL SEGMENTAL GLOMERULOSCLEROSIS WITH CCR2 ANTAGONISTS

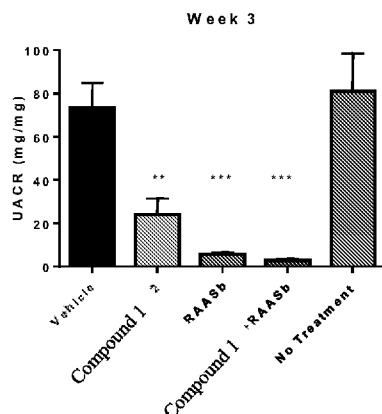


FIG. 4B

(57) Abstract: Provided herein are methods of treating focal segmental glomerulosclerosis, said methods include administering to a subject in need thereof a therapeutically effective amount of a CCR2 antagonist. In some embodiments, the CCR2 antagonist is used in monotherapy. In some embodiments, the CCR2 antagonist is used in combination therapy. In some embodiments, the additional therapeutic agent is a RAAS blocker and/or an endothelin receptor inhibitor.

**TREATMENT OF FOCAL SEGMENTAL GLOMERULOSCLEROSIS
WITH CCR2 ANTAGONISTS**

CROSS-REFERENCES TO RELATED APPLICATIONS

- 5 **[0001]** This application is an application claiming benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 62/570,778 filed October 11, 2017, which is incorporated herein by reference in its entirety.

**STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER
FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT**

- 10 **[0002]** NOT APPLICABLE

**REFERENCE TO A "SEQUENCE LISTING," A TABLE, OR A COMPUTER
PROGRAM LISTING APPENDIX SUBMITTED ON A COMPACT DISK**

- [0003]** NOT APPLICABLE

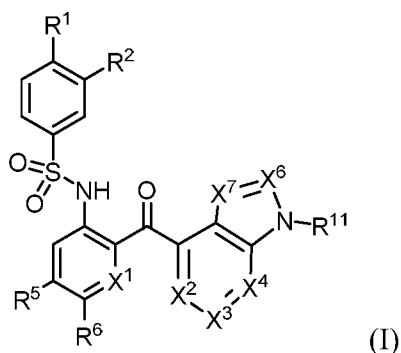
BACKGROUND OF THE INVENTION

- 15 **[0004]** Focal segmental glomerulosclerosis (FSGS) comprises a group of uncommon disorders that present with marked proteinuria, nephrotic syndrome, progressive renal failure and characteristic glomerular lesions on histopathology. The current standard of care for patients with FSGS include immunosuppressive drugs such as glucocorticoids followed by calcineurin inhibitors, if needed, for intolerance or inadequate response to glucocorticoids. Renin-
20 angiotensin-aldosterone (RAAS) blockers are also used to control proteinuria, an important signature of FSGS. Existing treatments, however, achieved only limited success. Despite best care, treatment failure is common and FSGS is causal in a significant proportion of end stage renal disease. Thus, an unmet need exists for novel disease modifying treatments for FSGS.

BRIEF SUMMARY OF THE INVENTION

[0005] Provided herein are methods of treating focal segmental glomerulosclerosis, said methods comprising administering to a subject in need thereof a therapeutically effective amount of a CCR2 antagonist.

5 [0006] In some embodiments, the CCR2 antagonist is a compound of Formula I



or a pharmaceutically acceptable form thereof, wherein

R¹ and R² are each independently selected from the group consisting of hydrogen,

halogen, C₁₋₈ alkyl, CN, or C₁₋₈ haloalkyl, provided that at least one of R¹ or R² is

10 other than hydrogen;

R⁵ is halogen or C₁₋₈ alkyl;

R⁶ is hydrogen or C₁₋₈ alkyl;

X¹ and X² are each independently is CR⁷, N, or NO;

X⁴ is N or NO;

15 X³ is CR⁷;

X⁶ and X⁷ are each independently selected from CR⁷, N, and NO;

R⁷ is independently selected from the group consisting of hydrogen, halogen, C₁₋₈ alkyl,

C₂₋₈ alkenyl, C₂₋₈ alkynyl, -CN, -NO₂, -OR⁸, -OC(O)R⁸, -CO₂R⁸, -C(O)R⁸, -

C(O)NR⁹R⁸, -OC(O)NR⁹R⁸, -NR¹⁰C(O)R⁸, -NR¹⁰C(O)NR⁹R⁸, -NR⁹R⁸,

20 -NR¹⁰CO₂R⁸, -SR⁸, -S(O)R⁸, -S(O)₂R⁸, -S(O)₂NR⁹R⁸, -NR¹⁰S(O)₂R⁸, C₆₋₁₀ aryl,

5- to 10-membered heteroaryl and 3-to 10-membered heterocyclyl;

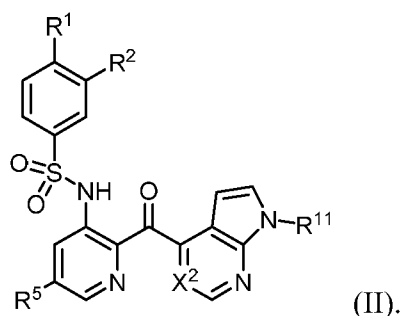
each R⁸, R⁹ and R¹⁰ is independently selected from the group consisting of hydrogen, C₁₋₈

alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, aryl, or heteroaryl; or R⁹ and R⁸ or R¹⁰ and R⁸,

together with the atom(s) to which they are attached, form a 5-, 6-, or 7-membered ring;

R¹¹ is selected from the group consisting of hydrogen, C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, C₆₋₁₀ aryl, 5- to 10-membered heteroaryl and 3- to 10-membered heterocycle.

5 [0007] In some embodiments, the CCR2 antagonist is a compound of Formula II



[0008] In some embodiments, the CCR2 antagonists are administered in combination with an additional therapeutic agent.

BRIEF DESCRIPTION OF THE DRAWINGS

10 [0009] **FIG. 1** illustrates the titration of Compound 1 and the inhibition of radio-labeled mJE binding to WEHI cells. Compound 1 was added at the indicated concentrations and competed with the binding of murine CCL2 (JE) to the cells, as described in Methods. The IC₅₀ was determined to be 270nM.

15 [0010] **FIG. 2** illustrates the pharmacokinetic profile of Compound 1 in mice. Compound 1 was administered by s.c. injection and the concentration in the blood was determined. Measured levels for 30mg/kg (black circles) and 90mg/kg (black squares) are shown.

[0011] **FIG. 3** Compound 1 improves renal function in adriamycin challenged mice. Mice were challenged with Adriamycin as described in Methods. Test compound treatment was begun one hour prior to the Adriamycin challenge. Urine was collected for measurement of albumin and creatinine at the indicated time points (**A**, week 1; **B**, week 2), as described in Methods. Serum creatinine (**C**) and BUN (**D**) were measured at time of the terminal bleed after two weeks

of treatment. Error bars represent standard error of the mean. N= 10/group at week 1, and N=8 at week 2.

[0012] **FIG. 4** Compound 1 improves renal function in partially nephrectomized mice. Mice underwent a 5/6 nephrectomy as described in Methods. Three weeks post-surgery the mice were randomized to the groups indicated above for the study period of 4 weeks. Urine was collected for measurement of albumin and creatinine at week 1 (**A**) and week 3 (**B**), as described in Methods. Error bars represent standard error of the mean. P<0.05: *; P<0.01: **; P<0.001: ***; P value compared to vehicle; P<0.01: ##P value compared to RAASb

[0013] **FIG. 5** Compound 1 attenuates the rise in serum creatinine (**A**) and BUN (**B**) in partially nephrectomized mice. Measurements were made at the 4-week time point. Error bars represent standard error of the mean. P<0.05: *; P<0.01: **; P value compared to vehicle.

[0014] **FIG. 6** Histological analysis of kidneys from the 5/6 nephrectomy model. The kidneys (8 animals/treated groups) were harvested 5 weeks after initiation of treatment, and fixed and stained as described in Methods. The kidneys were harvested 5 weeks after treatment began, and fixed and stained as described in Methods. Vehicle treatment is shown panel **A**; Compound 1 treatment is shown in panel **B**; RAAS blocker is shown in panel **C**; and combination of Compound 1 & RAAS blocker are shown in panel **D**. The magnification is 100X. The black circle indicate areas of hyaline deposition, black arrows denote tubular collapse, and black arrowhead denotes tubular dilation.

[0015] **FIG. 7** Histological analysis of kidneys from the 5/6 nephrectomy model. The kidneys (8 animals/treated groups) were harvested 5 weeks after initiation of treatment, and fixed and stained as described in Methods. Vehicle treatment is shown in panel **A**; Compound 1 treatment is shown panel **B**; RAAS blocker is shown in panel **C**; and combination of Compound 1 & RAAS blocker are shown panel **D**. The magnification is 400X. The black circle indicate areas of hyaline deposition, black arrows denote glomerular sclerosis, and black arrowhead denotes mesangial expansion.

[0016] **FIG. 8** Relative efficacy of CCR2, RAAS, and Endothelin Receptor Blockade in the 5/6 Nephrectomy Model. Mice underwent a 5/6 nephrectomy as described in Methods. Three weeks post-surgery the mice were randomized to the indicated groups for the study period of 4

weeks. Urine was collected for measurement of albumin and creatinine at week 1 (A) and week 4 (B), as described in Methods. Error bars represent standard error of the mean. N= 10/group.

DETAILED DESCRIPTION OF THE INVENTION

I. General

5 [0017] Provided herein are methods of treating focal segmental glomerulosclerosis using a CCR2 antagonist. CCR2 antagonists may be administered as a monotherapy or in combination. In some embodiments, CCR2 antagonists are administered as a monotherapy. In some
10 embodiments, CCR2 antagonists are administered in combination with a Renin-angiotensin-aldosterone (RAAS) blocker. In some embodiments, CCR2 antagonists are administered in combination with an endothelin receptor antagonist. In some embodiments, CCR2 antagonists are administered in combination with a Renin-angiotensin-aldosterone (RAAS) blocker and an endothelin receptor antagonist.

II. Definitions

[0018] As used herein the term "alkyl", by itself or as part of another substituent, means,
15 unless otherwise stated, a straight or branched chain hydrocarbon radical, having the number of carbon atoms designated (*i.e.* C₁₋₈ means one to eight carbons). Examples of alkyl groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. The term "alkenyl" refers to an unsaturated alkyl group having one or more double bonds. Similarly, the term "alkynyl" refers to an unsaturated alkyl group
20 having one or more triple bonds. Examples of such unsaturated alkyl groups include vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1-and 3-propynyl, 3-butynyl, and the higher homologs and isomers. The term "cycloalkyl" refers to hydrocarbon rings having the indicated number of ring atoms (*e.g.*, C₃₋₆cycloalkyl) and being fully saturated or having no more than one double bond between ring vertices. "Cycloalkyl" is
25 also meant to refer to bicyclic and polycyclic hydrocarbon rings such as, for example, bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane, etc. The term "heterocycloalkane", "heterocycloalkyl", or "heterocyclyl" refers to a cycloalkyl group that contain from one to five heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally

oxidized, and the nitrogen atom(s) are optionally quaternized. The heterocycloalkane may be a monocyclic, a bicyclic or a polycyclic ring system. Non limiting examples of heterocycloalkane groups include pyrrolidine, imidazolidine, pyrazolidine, butyrolactam, valerolactam, imidazolidinone, hydantoin, dioxolane, phthalimide, piperidine, 1,4-dioxane, morpholine, thiomorpholine, thiomorpholine-S-oxide, thiomorpholine-S,S-oxide, piperazine, pyran, pyridone, 3-pyrroline, thiopyran, pyrone, tetrahydrofuran, tetrahydrothiophene, quinuclidine, and the like. A heterocycloalkane group can be attached to the remainder of the molecule through a ring carbon or a heteroatom.

[0019] As used herein, the term "alkylene" by itself or as part of another substituent means a divalent radical derived from an alkane, as exemplified by $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$. Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred in the present disclosure. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having four or fewer carbon atoms. Similarly, "alkenylene" and "alkynylene" refer to the unsaturated forms of "alkylene" having double or triple bonds, respectively. The term "heteroalkylene" refers to an alkylene group in which one or two carbon atoms are replaced by N, O, or S.

[0020] As used herein, a wavy line, " \sim ", that intersects a single, double or triple bond in any chemical structure depicted herein, represent the point attachment of the single, double, or triple bond to the remainder of the molecule.

[0021] As used herein, the terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "haloalkyl" and "haloalkoxy," are meant to include monohalo- and polyhalo- versions of alkyl and alkoxy, respectively. For example, the term " C_{1-4} haloalkyl" is meant to include trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

[0022] As used herein, the term "aryl" or "aromatic ring" means, unless otherwise stated, a polyunsaturated, typically aromatic, hydrocarbon group which can be a single ring or multiple rings (up to three rings) which are fused together or linked covalently. Similarly,

the terms "heteroaryl" and "heteroaromatic ring" refer to aryl groups (or rings) that contain from one to five heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group or heteroaromatic ring can be attached to the remainder of the molecule through a heteroatom. Non-limiting examples of aryl groups include phenyl, naphthyl and biphenyl, while non-limiting examples of heteroaryl groups include pyridyl, pyridazinyl, pyrazinyl, pyrimidinyl, triazinyl, quinolinyl, quinoxalyl, quinazolinyl, cinnolinyl, phthalazinyl, benzotriazinyl, purinyl, benzimidazolyl, benzopyrazolyl, benzotriazolyl, benzisoxazolyl, isobenzofuryl, isoindolyl, indoliziny, benzotriazinyl, thienopyridinyl, thienopyrimidinyl, pyrazolopyrimidinyl, imidazopyridines, benzothiazolyl, benzofuranyl, benzothienyl, indolyl, quinolyl, isoquinolyl, isothiazolyl, pyrazolyl, indazolyl, pteridinyl, imidazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, thiadiazolyl, pyrrolyl, thiazolyl, furyl, thienyl and the like. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below.

[0023] As used herein, the term "heteroatom" is meant to include oxygen (O), nitrogen (N), sulfur (S) and silicon (Si).

[0024] As used herein the term "oxo" refers to a double bonded oxygen (=O).

[0025] As used herein, the term "pharmaceutically acceptable salts" is meant to include salts of the active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present disclosure contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of salts derived from pharmaceutically-acceptable inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic, manganous, potassium, sodium, zinc and the like. Salts derived from pharmaceutically-acceptable organic bases include salts of primary, secondary and tertiary amines, including substituted amines, cyclic amines, naturally-occurring amines and the like, such as arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-

ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine and the like. When compounds of the present disclosure contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, malonic, benzoic, succinic, suberic, fumaric, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, for example, Berge, S.M., et al, “Pharmaceutical Salts”, *Journal of Pharmaceutical Science*, **1977**, *66*, 1-19). Certain specific compounds of the present disclosure contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

[0026] The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present disclosure.

[0027] In addition to salt forms, the present disclosure provides compounds which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present disclosure. Additionally, prodrugs can be converted to the compounds of the present disclosure by chemical or biochemical methods in an *ex vivo* environment. For example, prodrugs can be slowly converted to the compounds of the present disclosure when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent.

[0028] Certain compounds of the present disclosure can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are intended to be encompassed within the scope of the present disclosure. Certain compounds of the present disclosure may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present disclosure and are intended to be within the scope of the present disclosure.

[0029] Certain compounds of the present disclosure possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers, regioisomers and individual isomers (e.g., separate enantiomers) are all intended to be encompassed within the scope of the present disclosure. When a stereochemical depiction is shown, it is meant to refer the compound in which one of the isomers is present and substantially free of the other isomer. 'Substantially free of' another isomer indicates at least an 80/20 ratio of the two isomers, more preferably 90/10, or 95/5 or more. In some embodiments, one of the isomers will be present in an amount of at least 99%.

[0030] The compounds of the present disclosure may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. Unnatural proportions of an isotope may be defined as ranging from the amount found in nature to an amount consisting of 100% of the atom in question. For example, the compounds may incorporate radioactive isotopes, such as for example tritium (^3H), iodine-125 (^{125}I) or carbon-14 (^{14}C), or non-radioactive isotopes, such as deuterium (^2H) or carbon-13 (^{13}C). Such isotopic variations can provide additional utilities to those described elsewhere with this application. For instance, isotopic variants of the compounds of the disclosure may find additional utility, including but not limited to, as diagnostic and/or imaging reagents, or as cytotoxic/radiotoxic therapeutic agents. Additionally, isotopic variants of the compounds of the disclosure can have altered pharmacokinetic and pharmacodynamic characteristics which can contribute to enhanced safety, tolerability or efficacy during treatment. All isotopic variations of the compounds of the present disclosure, whether radioactive or not, are intended to be encompassed within the scope of the present disclosure. Substitution with heavier isotopes such as deuterium, *i.e.* ^2H , may afford certain therapeutic advantages resulting from greater metabolic stability. For example, *in vivo* half-life may increase or dosage requirements may be reduced.

[0031] As used herein, the terms “subject”, “patient” or “individual” are used herein interchangeably to include a human or animal. For example, the animal subject may be a mammal, a primate (*e.g.*, a monkey), a livestock animal (*e.g.*, a horse, a cow, a sheep, a pig, or a goat), a companion animal (*e.g.*, a dog, a cat), a laboratory test animal (*e.g.*, a mouse, a rat, a guinea pig, a bird), an animal of veterinary significance, or an animal of economic significance.

[0032] As used herein, the term “urine albumin-to-creatinine ratio” refers to the ratio of albumin to creatinine as measured in a subject’s urine sample (*e.g.* (urine albumin / urine creatinine)). This is a common measure used to determine kidney function, because when the kidneys are functioning properly, little to no albumin is found in the urine and creatinine is normally released into the urine at a relatively constant rate.

[0033] As used herein, the term “therapeutically effective amount” means the amount of the subject compound that will elicit the biological or medical response of a cell, tissue, system, or animal, such as a human, that is being sought by the researcher, veterinarian, medical doctor or other treatment provider.

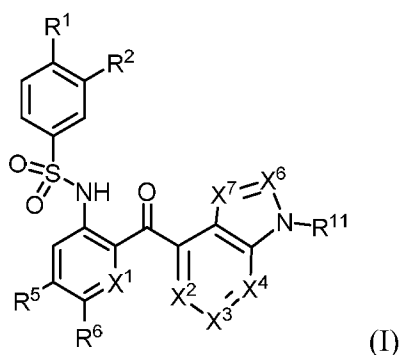
[0034] As used herein, the term “selective CCR2 antagonist” refers to a highly discriminatory compound that inhibits normal CCR2 activity with little or no cross reactivity on non-targeted proteins such as CCR1, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, FPRL2, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CXCR7, C3aR, and/or C5aR. In some embodiments, “selective CCR2 antagonists” have an IC₅₀ that is at least 10, 100, 500, 1,000, 5,000, 10,000 or more times lower than for that of proteins such as CCR1, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, FPRL2, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CXCR7, C3aR, and/or C5aR when measured in the assay types described in Example 3 of this application. In some embodiments, “selective CCR2 antagonists” do not inhibit the activity of CCR1, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, FPRL2, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CXCR7, C3aR, and/or C5aR at concentrations of 1 μ M or below in assay types described in Example 3 of this application. The above-mentioned proteins are considered to be “not inhibited” when they maintain 100%, 99%, 95%, 90%, or 85% of their activity under the referenced conditions with a selective CCR2 antagonist.

[0035] As used herein, the term "composition" as used herein is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts. By "pharmaceutically acceptable" it is meant the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

III. Methods of treating focal segmental glomerulosclerosis

[0036] Provided method of treating focal segmental glomerulosclerosis, said method comprising administering to a subject in need thereof a therapeutically effective amount of a CCR2 antagonist.

[0037] In some embodiments, the CCR2 antagonist is a compound of Formula I



(I)

or a pharmaceutically acceptable form thereof, wherein

R¹ and R² are each independently selected from the group consisting of hydrogen,

halogen, C₁₋₈ alkyl, CN, or C₁₋₈ haloalkyl, provided that at least one of R¹ or R² is other than hydrogen;

R⁵ is halogen or C₁₋₈ alkyl;

R⁶ is hydrogen or C₁₋₈ alkyl;

X¹ and X² are each independently is CR⁷, N, or NO;

X⁴ is N or NO;

X³ is CR⁷;

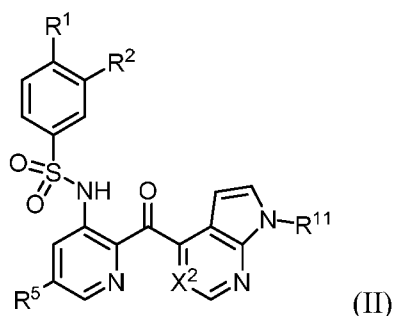
X⁶ and X⁷ are each independently selected from CR⁷, N, and NO;

R^7 is independently selected from the group consisting of hydrogen, halogen, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, $-CN$, $-NO_2$, $-OR^8$, $-OC(O)R^8$, $-CO_2R^8$, $-C(O)R^8$, $-C(O)NR^9R^8$, $-OC(O)NR^9R^8$, $-NR^{10}C(O)R^8$, $-NR^{10}C(O)NR^9R^8$, $-NR^9R^8$, $-NR^{10}CO_2R^8$, $-SR^8$, $-S(O)R^8$, $-S(O)_2R^8$, $-S(O)_2NR^9R^8$, $-NR^{10}S(O)_2R^8$, C_{6-10} aryl, 5- to 10-membered heteroaryl and 3- to 10-membered heterocyclyl;

each R^8 , R^9 and R^{10} is independently selected from the group consisting of hydrogen, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, aryl, or heteroaryl; or R^9 and R^8 or R^{10} and R^8 , together with the atom(s) to which they are attached, form a 5-, 6-, or 7-membered ring;

R^{11} is selected from the group consisting of hydrogen, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-10} aryl, 5- to 10-membered heteroaryl and 3- to 10-membered heterocycle.

[0038] In some embodiments, the compound of Formula I has the structure of Formula II



or a pharmaceutically acceptable form thereof.

[0039] In some embodiments of the compound of Formula II,

R^1 and R^2 are each independently selected from the group consisting of halogen, C_{1-8} alkyl, or C_{1-8} haloalkyl, provided that at least one of R^1 or R^2 is other than hydrogen;

R^5 is halogen or C_{1-8} alkyl;

X^2 is CR^7 or N;

R^7 is independently selected from the group consisting of hydrogen, halogen, C_{1-8} alkyl, $-CN$, $-NO_2$, $-OR^8$, and $-NR^9R^8$;

R^{11} is hydrogen or C_{1-8} alkyl.

[0040] In some embodiments of the compound of Formula II,

R¹ is halogen

R² C₁₋₈ haloalkyl;

R⁵ is C₁₋₈ alkyl;

5 X² is CR⁷ or N;

R⁷ is independently selected from the group consisting of hydrogen, halogen, C₁₋₈ alkyl;

R¹¹ is hydrogen or C₁₋₈ alkyl.

[0041] In some embodiments of the compound of Formula II,

R¹ is chloro

10 R² trifluoromethyl;

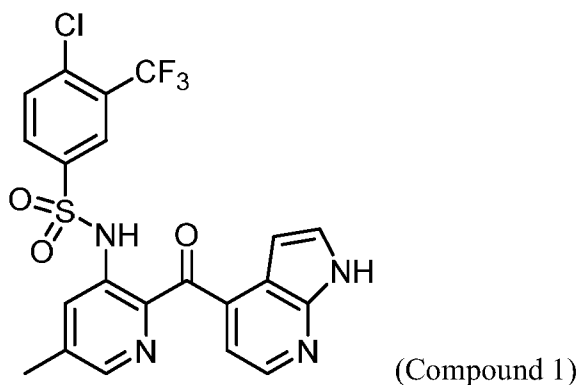
R⁵ is methyl;

X² is CR⁷ or N;

R⁷ is independently selected from the group consisting of hydrogen, halogen, C₁₋₈ alkyl;

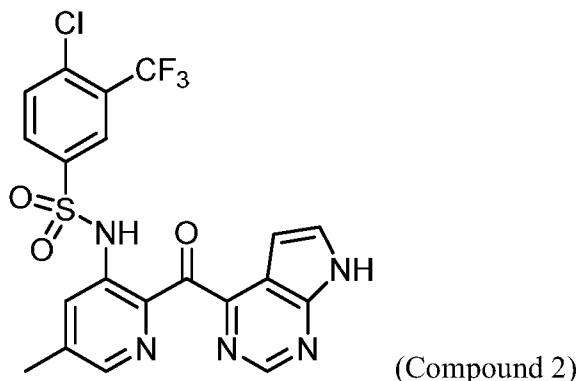
R¹¹ is hydrogen or C₁₋₈ alkyl.

15 [0042] In some embodiments, the compound of Formula II has the structure of Compound 1



or a pharmaceutically acceptable form thereof.

[0043] In some embodiments, the compound of Formula II has the structure of Compound 2



or a pharmaceutically acceptable form thereof.

[0044] In some embodiments, the CCR2 antagonist is a selective CCR2 antagonist. Selective CCR2 antagonists are highly discriminatory compounds that have little or no cross reactivity with other chemokine receptors. In some embodiments, selective CCR2 antagonists have an IC_{50} that is at least 10, 100, 500, 1,000, 5,000, 10,000 or more times lower than for that of proteins such as CCR1, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, FPRL2, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CXCR7, C3aR, and/or C5aR when measured in the assay types described in Example 3 of this application. In some embodiments, selective CCR2 antagonists do not inhibit the activity of CCR1, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, FPRL2, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CXCR7, C3aR, and/or C5aR at concentrations of 1 μ M or below in assay types described in Example 3 of this application. The above-mentioned proteins are considered to be “not inhibited” when they maintain 100%, 99%, 95%, 90%, or 85% of their activity under the referenced conditions with a selective CCR2 antagonist. In some embodiments, the above-mentioned proteins maintain 100% of their activity under the referenced conditions with a selective CCR2 antagonist. In some embodiments, the above-mentioned proteins maintain 99% of their activity under the referenced conditions with a selective CCR2 antagonist. In some embodiments, the above-mentioned proteins maintain 95% of their activity under the referenced conditions with a selective CCR2 antagonist. In some embodiments, the above-mentioned proteins maintain 90% of their activity under the referenced conditions with a selective CCR2 antagonist. In some embodiments, the above-mentioned proteins maintain 85% of their activity under the referenced conditions with a selective CCR2 antagonist.

[0045] Individuals with normal kidney function have little or no albumin in the urine. On the other hand, creatinine is normally released into the urine at a constant rate. Thus, the albumin to creatinine ration in a subject's urine can be used as a metric of kidney function. Accordingly, in some embodiments, administration of the CCR2 antagonist to the subject in need thereof reduces a urine albumin-to-creatinine ratio in said subject.

[0046] In some embodiments, the urine albumin-to-creatinine ratio in said subject is reduced by at least 20% after 3 weeks of administering the CCR2 antagonist. In some embodiments, the urine albumin-to-creatinine ratio in said subject is reduced by at least 30% after 3 weeks of administering the CCR2 antagonist. In some embodiments, the urine albumin-to-creatinine ratio in said subject is reduced by at least 40% after 3 weeks of administering the CCR2 antagonist. In some embodiments, the urine albumin-to-creatinine ratio in said subject is reduced by at least 50% after 3 weeks of administering the CCR2 antagonist. In some embodiments, the urine albumin-to-creatinine ratio in said subject is reduced by at least 60% after 3 weeks of administering the CCR2 antagonist

[0047] In the treatment or prevention of focal segmental glomerulosclerosis an appropriate dosage level will generally be about 0.001 to 100 mg per kg patient body weight per day which can be administered in single or multiple doses. Preferably, the dosage level will be about 0.01 to about 25 mg/kg per day; more preferably about 0.05 to about 10 mg/kg per day. A suitable dosage level may be about 0.01 to 25 mg/kg per day, about 0.05 to 10 mg/kg per day, or about 0.1 to 5 mg/kg per day. Within this range the dosage may be 0.005 to 0.05, 0.05 to 0.5, 0.5 to 5.0, or 5.0 to 50 mg/kg per day. For oral administration, the compositions are preferably provided in the form of tablets containing 1.0 to 1000 milligrams of the active ingredient, particularly 1.0, 5.0, 10.0, 15.0, 20.0, 25.0, 50.0, 75.0, 100.0, 150.0, 200.0, 250.0, 300.0, 400.0, 500.0, 600.0, 750.0, 800.0, 900.0, and 1000.0 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. The compounds may be administered on a regimen of 1 to 4 times per day, preferably once or twice per day. In some embodiments, the CCR2 antagonist is administered daily.

[0048] It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the

activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, hereditary characteristics, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

5 IV. Combination Therapy

[0049] In some embodiments, methods of treating focal segmental glomerulosclerosis include administering the CCR2 antagonists described herein as part of combination therapy.

[0050] In some embodiments, the additional therapeutic agent is a Renin-angiotensin-aldosterone (RAAS) blocker. In some embodiments, the additional therapeutic agent is an
10 endothelin receptor antagonist. In some embodiments, the additional therapeutic agent is a Renin-angiotensin-aldosterone (RAAS) blocker, and an endothelin receptor antagonist.

[0051] RAAS blockers include Renin inhibitors, ACE inhibitors, and angiotensin receptor blockers. As such, in some embodiments, the RAAS blocker is a renin inhibitor. In some
15 embodiments, the RAAS blocker is an ACE inhibitor. In some embodiments, the RAAS blocker is an angiotensin receptor blocker (ARB).

[0052] In some embodiments, the renin inhibitor is selected from the group consisting of aliskiren, remikiren, H-142, SPP635, SPP1148, SPP676, SPP1234, or combinations thereof.

[0053] In some embodiments, the ACE inhibitor is selected from the group consisting of benazepril (tradename Lotensin®), captopril (tradename Capoten®), enalapril (tradename
20 Vasotec®), fosinopril (tradename Monopril®), lisinopril (tradename Prinivil®, Zestril®), perindopril (tradename Aceon®), quinapril (tradename Accupril®), ramipril (tradename Altace®), trandolapril (tradename Mavik®), or combinations thereof.

[0054] In some embodiments, the angiotensin receptor blocker (ARB) is selected from the group consisting of eprosartan (tradename Teveten®), candesartan (tradename Atacand®),
25 irbesartan (tradename Avapro®), losartan (tradename Cozaar®), olmesartan (tradename Benicar®), telmisartan (tradename Micardis®), valsartan (tradename Diovan®), CGP-42112A, DuP753, saralasin, sarthran, or combinations thereof. In some embodiments, the angiotensin receptor blocker (ARB) is selected from the group consisting of sparsentan, eprosartan

(tradename Teveten®), candesartan (tradename Atacand®), irbesartan (tradename Avapro®), losartan (tradename Cozaar®), olmesartan (tradename Benicar®), telmisartan (tradename Micardis®), valsartan (tradename Diovan®), CGP-42112A, DuP753, saralasin, sarthran, or combinations thereof. In some embodiments, the angiotensin receptor blocker (ARB) is

5 sparsentan. A person of skill in the art will recognize that sparsentan is a dual-acting receptor antagonist for endothelin (A type) receptors and angiotensin II receptors.

[0055] In some embodiments, the endothelin receptor antagonist is selected from the group consisting of sparsentan, bosentan, macitentan, ambrisentan, sitazentan, aprocitentan, and artasentan. In some embodiments, the endothelin receptor antagonist is selected from the group
10 consisting of bosentan, macitentan, ambrisentan, sitazentan, aprocitentan, and artasentan. In some embodiments, the endothelin receptor antagonist is sparsentan. A person of skill in the art will recognize that sparsentan is a dual-acting receptor antagonist for endothelin (A type) receptors and angiotensin II receptors.

[0056] In some embodiments, the amount of additional therapeutic agent is sub-therapeutic
15 when administered alone. Those of skill in the art will appreciate that "combinations" can involve combinations in treatments (i.e., two or more drugs can be administered as a mixture, or at least concurrently or at least introduced into a subject at different times but such that both are in the bloodstream of a subject at the same time). Additionally, compositions of the current disclosure may be administered prior to or subsequent to a second therapeutic regimen.

20 [0057] The weight ratio of the CCR2 antagonists of the present disclosure to the second active ingredient may be varied and will depend upon the effective dose of each ingredient. Generally, an effective dose of each will be used.

V. Pharmaceutical Compositions

[0058] The pharmaceutical compositions for the administration of the compounds of this
25 disclosure may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active ingredient into association with the carrier which constitutes one or more accessory ingredients. In general, the pharmaceutical compositions are prepared by uniformly and intimately bringing

the active ingredient into association with a liquid carrier or a finely divided solid carrier or both, and then, if necessary, shaping the product into the desired formulation. In the pharmaceutical composition the active object compound is included in an amount sufficient to produce the desired effect.

- 5 **[0059]** The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions and self-emulsifications as described in U.S. Patent No. 6,451,339, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of
- 10 pharmaceutical compositions. Such compositions may contain one or more agents selected from sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with other non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be, for example, inert diluents such as cellulose,
- 15 silicon dioxide, aluminum oxide, calcium carbonate, sodium carbonate, glucose, mannitol, sorbitol, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example PVP, cellulose, PEG, starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated enterically or otherwise by known
- 20 techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by the techniques described in the U.S. Pat. Nos. 4,256,108; 4,166,452; and 4,265,874 to form osmotic therapeutic tablets for control release.
- 25 **[0060]** Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil. Additionally, emulsions can be prepared with a non-water miscible ingredient such as oils and stabilized with surfactants
- 30 such as mono-diglycerides, PEG esters and the like.

[0061] Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents
5 may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylenoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a
10 hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl, p-hydroxybenzoate, one or more coloring agents, one or more
flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

[0062] Oily suspensions may be formulated by suspending the active ingredient in a vegetable
15 oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

[0063] Dispersible powders and granules suitable for preparation of an aqueous suspension by
20 the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

[0064] The pharmaceutical compositions of the disclosure may also be in the form of oil in
25 water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids

and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

[0065] Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, and flavoring and coloring agents. Oral solutions can be prepared in combination with, for example, cyclodextrin, PEG and surfactants.

[0066] The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, axed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

[0067] The compounds of the present disclosure may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols. Additionally, the compounds can be administered via ocular delivery by means of solutions or ointments. Still further, transdermal delivery of the subject compounds can be accomplished by means of iontophoretic patches and the like.

[0068] For topical use, creams, ointments, jellies, solutions or suspensions containing the compounds of the present disclosure are employed. As used herein, topical application is also meant to include the use of mouth washes and gargles.

[0069] The pharmaceutical compositions and methods of the present disclosure may further comprise other therapeutically active compounds as noted herein, such as those applied in the treatment of the above mentioned pathological conditions.

[0070] In one embodiment, the present disclosure provides a composition consisting of a pharmaceutically acceptable carrier and a compound of the disclosure.

VI. Kits

[0071] Also provided herein are kits comprising pharmaceutical compositions of the compound of Formula I or the compound of Formula II, and including kits for combination therapy.

[0072] In some aspects, the present invention provides a kit that includes the compound of Formula I or the compound of Formula II. Some of the kits described herein include a label describing a method of administering the compound of Formula I or the compound of Formula II. Some of the kits described herein include a label describing a method of administering the compound of Formula I or the compound of Formula II in combination with one or more (e.g., one, two three, one to two, or one to three) additional therapeutic agents. Some of the kits described herein include a label describing a method of treating focal segmental glomerulosclerosis. In some embodiments, the kits described herein include a label describing a method of reducing urine albumin excretion.

[0073] The compositions of the present invention, including but not limited to, compositions comprising Compound 1 and or Compound 2 in a bottle, jar, vial, ampoule, tube, or other container-closure system approved by the United States Food and Drug Administration (FDA) or other regulatory body, which may provide one or more dosages containing the compounds. The package or dispenser may also be accompanied by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, the notice indicating approval by the agency. In certain aspects, the kit may include a formulation or composition as described herein, a container closure system including the formulation or a dosage unit form including the formulation, and a notice or instructions describing a method of use as described herein.

Examples

[0074] The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results.

5 Materials & Methods

Cells and Reagents

[0075] WEHI-274.1 and ThP1 cells were from ATCC (Rockville, MD). Human monocytes and neutrophils were isolated from healthy volunteers (Stanford Blood Center, Palo Alto, CA) using MACS separation reagents (Miltenyi, Germany). The CCR2 antagonist Compound 1 was
10 discovered and synthesized at ChemoCentryx and stored as a dry powder until the time of formulation for *in vivo* use. The compound was formulated in 1% hydroxylpropyl methylcellulose (HPMC) (Sigma-Aldrich, St Louis, MO) in water for subcutaneous (s.c.) injection at the indicated concentration. Candesartan (AK Scientific, Union City, CA) and its vehicle were dosed orally once daily at 5 mg/kg in water. Recombinant chemokines were
15 acquired from R&D Systems (Minneapolis, MN). [125I]-CCL2 was from PerkinElmer (Boston, MA). Human plasma and mouse plasma were from Bioreclamation (Hicksville, NY)

Mice

[0076] Balb/c, 129s and 129X1/SvJ mice were purchased from Jackson Laboratories (Bar Harbor, Maine) and housed at the ChemoCentryx animal facility in accordance with guidelines
20 described in the Guide and Use of Laboratory Animals of the National Research Council. All studies were approved by the ChemoCentryx Institutional Animal Care and Use Committee. All animal studies were conducted under the protocol entitled "Kidney Disease and Diabetes Models in Mice", number CCX176-2008.

Mouse Pharmacokinetic (PK) Study

[0077] Compound 1 was formulated in 1% HPMC in water at 6 and 18 mg/mL concentrations,
25 respectively. Five male 129s mice per dose group were injected s.c. with 30 and 90 mg/kg of Compound 1. Blood was drawn at 0.5, 2, 5, 4, 8, 12, and 24 hours post-dosing. Compound 1 drug level was analyzed by LC-MS/MS at ChemoCentryx with plasma samples.

Adriamycin Induced FSGS Model

[0078] These experiments were performed using female Balb/c mice (Jackson Laboratories, Bar Harbor, Maine). The mice were kept on standard chow and had free access to water. At age of 10 weeks, 7.5 mg/kg Adriamycin (Selleck Chemicals, Houston, TX) or saline (control) was injected via tail vein in isoflurane-anesthetized animals (day 0). Compound 1 and/or vehicle were dosed subcutaneously once daily at 90 mg/kg formulated in 1% HPMC. The RAAS blocker Candesartan (AK Scientific, Union City, CA) and its vehicle were dosed orally once daily at 5 mg/kg, formulated in water. All dosing started 2 hours prior Adriamycin challenge. The mice were housed individually in metabolic cages for quantitative collection of urinary albumin and creatinine.

5/6 Nephrectomy Model

[0079] 5/6 nephrectomy mice on the 129X1/SvJ background were obtained from Jackson Laboratories, and were received at ChemoCentryx after the surgery was performed at JAX. Under isoflurane anesthesia, two-thirds of the left kidney mass was removed at 5-6 weeks of age. Then, after 7 to 10 days, a right unilateral nephrectomy was performed. The mice were fed a standard chow and had free access to water. Three weeks after the 5/6 nephrectomy, the mice were grouped to ensure a similar starting UAER in each treatment group. Compound 1 or its vehicle were dosed subcutaneously once daily formulated in 1% HPMC. The RAAS blocker candesartan (AK Scientific, Union City, CA) and its vehicle were dosed orally once daily at 5 mg/kg formulated in water. Sparsentan, the dual-acting receptor antagonist for endothelin (A type) and angiotensin II receptors (Type 1) (Murugesan et al, 2005) was synthesized at ChemoCentryx and was dosed orally twice daily at 90 mg/kg formulated in 1% HPMC. The mice were housed individually in metabolic cages for quantitative collection of urinary albumin and creatinine.

In Vitro Experiments

[0080] Chemotaxis, calcium mobilization, and radio-ligand binding assays were conducted as previously described (Sullivan et al, 2013; Walters et al, 2010). Inhibition values (IC₅₀) were calculated using non-linear regression with a one-site competition model (GraphPad Prism, GraphPad Software, La Jolla, CA). A2 values for assessment of potency in chemotaxis assays

denotes the concentration of the antagonist required to right-shift by 2- fold the chemokine dose-response curve. Receptor engagement indices (REIs) were calculated by dividing the plasma concentration of Compound 1 by the measured potency in the chemotaxis assay in 100% mouse serum. Urinary albumin was measured by ELISA (Bethyl Labs, Montgomery, TX). Urinary and serum creatinine was measured by LC-MS/MS at ChemoCentryx. The urinary albumin excretion rate (UAER) was calculated as micrograms per 24 h. Blood urea nitrogen (BUN) was measured by Antech Diagnostics (Morrisville, NC). The albumin to creatinine ratio (ACR) was calculated as micrograms of albumin per milligram of creatinine.

Histology

[0081] The kidneys were collected, fixed in formalin, embedded in paraffin, and cut into 5- μ m-thick sections. Sections were stained with Haematoxylin and Eosin (H&E) (Sigma-Aldrich, St Louis, MO) and PAS (Periodic Acid Schiff) (Sigma-Aldrich, St Louis, MO) and using standard protocols. Glomerular hypertrophy, mesangial expansion, glomerular sclerosis, and tubular structure were determined by examination of sections by an observer blinded to the treatment groups.

Statistical Methods

[0082] Differences between treatment groups were analyzed using Student's t-test.

Example 1: Inhibition of CCL2 binding in WEHI-274 murine monocyte cell line using Compound 1

[0083] To determine if CCR2 plays a key role in FSGS we examined the ability of Compound 1 to block radio-labeled CCR2 ligand, mJE (CCL2) binding in the WEHI-274 murine monocyte cell line that endogenously expresses CCR2. As shown in **FIG. 1**, Compound 1 inhibited mJE binding in WEHI 274 cells with an IC_{50} of 270 nM.

Example 2: Pharmacokinetics of Compound 1 in mice

[0084] To determine if Compound 1 was suitable for *in vivo* experiments, we analyzed its pharmacokinetic profile in mice. As shown in **FIG. 2**, a single s.c. injection of Compound 1 at 90mg/kg provided a consistent level of Compound 1 in the blood, with a concentration of

approximately 3.5 μ M, 24 hours post-dosing. We therefore concluded that a once daily s.c. administration of Compound 1 would provide more than adequate CCR2 coverage.

Example 3: Selectivity of Compound 1 inhibition

[0085] To determine the selectivity of Compound 1, we performed a variety of binding-competition and functional assays on cell lines that either endogenously expressed certain chemokine receptors, or were individually transfected with various receptors for other chemokines or chemotactic complement fragments. As summarized in **Table 1**, Compound 1 did not inhibit any activity in a diverse selection of other receptors even at concentrations well above one micromolar.

Table 1: Selectivity Data for Compound 1 on Diverse Family of Human Chemokine and Chemoattractant Receptors

Receptor	Assay Type	Compound 1 Potency [nM]
CCR2	ThP1 Cell Chemotaxis	0.5
CCR1	Monocyte Chemotaxis	>10,000
CCR3	293-CCR3 Calcium Mobilization	>10,000
CCR4	Activated Lymphocyte Calcium Mobilization	>10,000
CCR5	L1.2-CCR5 Chemotaxis	>8,000
CCR6	Activated Lymphocyte Calcium Mobilization	>8,000
CCR7	Activated Lymphocyte Calcium Mobilization	>10,000
CCR8	293-CCR8 Calcium Mobilization	>10,000

CCR9	Molt4 Serum Chemotaxis	>10,000
CCR10	293-CCR10 Calcium Mobilization	>10,000
FPRL2	Neutrophil Calcium Mobilization	>10,000
CXCR1	Neutrophil Calcium Mobilization	>10,000
CXCR2	Neutrophil Calcium Mobilization	>10,000
CXCR3	Activated Lymphocyte Calcium Mobilization	>10,000
CXCR4	Activated Lymphocyte Calcium Mobilization	>10,000
CXCR5	Baf3-CXCR5 Calcium Mobilization	>10,000
CXCR6	Activated Lymphocyte Buffer Chemotaxis	>10,000
CXCR7	293-CXCR7 Radio-ligand Binding	>10,000
C3aR	Neutrophil Calcium Mobilization	>10,000
C5aR	Neutrophil Calcium Mobilization	>10,000

Shown are the IC₅₀ values for inhibition of the specified responses.

Example 4: Adriamycin Induced FSGS Model

[0086] To evaluate the therapeutic potential of Compound 1 for FSGS, an Adriamycin induced FSGS model was used. Adriamycin is an oncolytic antibiotic that induces proteinuria and glomerulosclerosis in rodents after a single infusion. Rapid reduction in urinary albumin excretion rate (UAER) (mg/day) by Compound 1 alone, or in combination with RAAS blockade in Adriamycin nephropathy model is observed (**FIG. 3**). Compound 1 as a single agent had achieved a marked reduction in urine albumin-to-creatinine ratio (UACR) by two weeks after the

Adriamycin infusion. Adriamycin treated mice displayed significant reduction in UAER in response to Compound 1 alone or in combination with RAAS blockade **Table 2**). The level of protection was equal to or better than that of RAAS blockade. Combined treatment with Compound 1 and RAAS blocker achieved a statistically significant decrease in UACR with respect to vehicle, accompanied by similar improvements in serum creatinine and BUN levels (**FIG. 3**).

Table 2: Reduction in UAER (mg/day) by Compound 1 alone, or in combination with

RAAS blockade in Adriamycin nephropathy model

	Week 1	Week2
Vehicle	75.87	89.99
Compound 1	26.15, p = 0.06	24.19, p = 0.032
RAAS Blocker	39.46, p = 0.16	66.87
Compound 1+RAAS Blocker	20.39, p = 0.027	23, p = 0.030

Example 5: 5/6 Nephrectomy Model

[0087] To corroborated the above findings, another FSGS model mechanistically distinct from the Adriamycin approach was tested, the 5/6 nephrectomy model. Beginning three weeks after the completion of surgery, the mice were treated with Compound 1 or RAAS blocker either alone or in combination. As above, renal function was quantified by measuring the UACR, UAER serum creatinine and BUN levels.

[0088] As a mono-therapy, Compound 1 markedly reduced UACR, which was apparent one week after the start of treatment (**FIG. 4**). This inhibition persisted throughout the four-week study, with reductions of 72% and 57% at weeks three and four, respectively. As expected, RAAS blockade also significantly decreased the UACR. Notably, the addition of Compound 1 to the RAAS blockade achieved a further, statistically significant reduction in UACR, an additive effect consistent with two distinct mechanisms of action and similar results were obtained for UAER (**Table 4**).

Table 3: Reduction in UAER (mg/day) by Compound 1 alone, or in combination with RAAS blockade in 5/6 nephrectomy model

RAAS blockade in 5/6 nephrectomy model	Week 1	Week2	Week3
Vehicle	26.82	17.21	39.55
Compound 1	15.68, p = 0.30	6.72, p = 0.07	11.75, p = 0.001
RAAS Blocker	8.27, p = 0.03	3.62, p = 0.03	3.32, p < 0.0001
Compound 1+RAAS Blocker	2.04, p = 0.005	1.45, p = 0.009	1.68, p < 0.0001
No Treatment	31.88	20.58	21.08

¹ Compound 1, 90 mg/kg² RAAS Blocker, 5 mg/kg**Table 4:** Reduction in UAER (mg/day) by combination of Compound 1 with RAAS blockade comparing with ET1/AT2 dual inhibitor in 5/6 nephrectomy model

	Week 1	Week 2	Week 4
Vehicle	20.7	23.8	23.3
Compound 1	7.1, p = 0.07	11.0, p = 0.1	2.8, p = 0.02
RAAS Blocker	9.4, p = 0.27	7.7, p = 0.06	1.7, p = 0.02
Compound 1+RAAS Blocker	2.1, p = 0.012	1.95, p = 0.002	1.5, p = 0.001
ET1/AT2 Dual Blocker	2.6, p=0.036	1.5, p=0.01	1.15, p=0.001

10 [0089] Compound 1 was associated with marked reductions in both creatinine and BUN with respect to vehicle control at the four-week time point (**FIG. 5**). The degree to which Compound 1 monotherapy reduced these parameters was greater or equal to that observed with RAAS blockade alone.

Example 6: Histological Analysis of Kidneys from the 5/6 Nephrectomy Model

15 [0090] Fixed kidney sections were obtained from 5/6 nephrectomized mice for pathology. The sections were stained and evaluated to determine whether the Compound 1-mediated improvements in renal function were associated with anatomical changes. As shown in **FIG. 6** (100x magnification) reductions in tubular dilation and hyaline deposits (with respect to vehicle

control) were evident in the kidney remnants from mice treated with Compound 1 alone or in combination with RAAS blockade. At higher magnification (400X), changes in the glomeruli were apparent, including decreased glomerular sclerosis, mesangial expansion, hyaline deposits and tubular collapse (**FIG. 7**).

5 **Example 7: Comparing CCR2 inhibition to Endothelin Receptor Inhibition in the 5/6 Nephrectomy Model**

[0091] Endothelin has also been implicated in FSGS, and recent clinical trials have featured a dual-acting receptor antagonist for endothelin (A type) and angiotensin II receptors (Type 1) (Murugesan et al, 2005). We used the 5/6 nephrectomy model to determine how Compound 1
10 compared to this antagonist in providing renal protection in FSGS-like disease. The combination of Compound 1 and RAAS blockade was as effective as the combination of endothelin receptor inhibition plus RAAS blockade, as determined by both UACR and UAER (**FIG. 8**).

Specifically, UACR was reduced by administration of Compound 1 alone (64.8%) or RAAS blocker alone (73.4%), at week one. Addition of the CCR2 antagonist to RAAS blockade further
15 reduced the UACR (92.7% reduction vs vehicle, $p=0.02$; $p < .045$ versus RAAS blockade alone), which was comparable to the combination of endothelin receptor plus RAAS blockade (93.5% reduction vs vehicle, $p=0.019$). These results indicate that CCR2 inhibition and endothelin receptor inhibition are equally effective when combined with RAAS blockade in this model of FSGS.

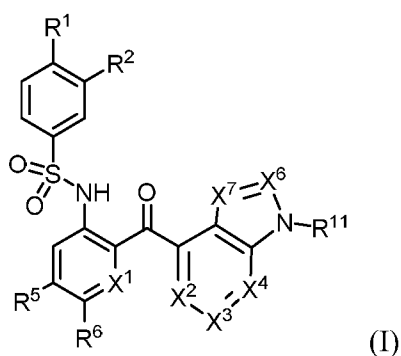
20 [0092] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, one of skill in the art will appreciate that certain changes and modifications may be practiced within the scope of the appended claims. In addition, each reference provided herein is incorporated by reference in its entirety to the same extent as if each reference was individually incorporated by reference.

25 Where a conflict exists between the instant application and a reference provided herein, the instant application shall dominate.

WHAT IS CLAIMED IS:

1. A method of treating focal segmental glomerulosclerosis, said method comprising administering to a subject in need thereof a therapeutically effective amount of a CCR2 antagonist.

2. The method of claim 1, wherein the CCR2 antagonist is a compound of Formula I



or a pharmaceutically acceptable form thereof, wherein

R^1 and R^2 are each independently selected from the group consisting of hydrogen, halogen, C_{1-8} alkyl, CN, or C_{1-8} haloalkyl, provided that at least one of R^1 or R^2 is other than hydrogen;

R^5 is halogen or C_{1-8} alkyl;

R^6 is hydrogen or C_{1-8} alkyl;

X^1 and X^2 are each independently is CR^7 , N, or NO;

X^4 is N or NO;

X^3 is CR^7 ;

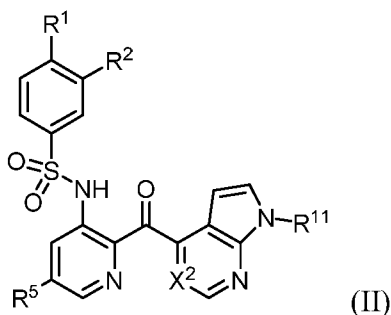
X^6 and X^7 are each independently selected from CR^7 , N, and NO;

R^7 is independently selected from the group consisting of hydrogen, halogen, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, $-CN$, $-NO_2$, $-OR^8$, $-OC(O)R^8$, $-CO_2R^8$, $-C(O)R^8$, $-C(O)NR^9R^8$, $-OC(O)NR^9R^8$, $-NR^{10}C(O)R^8$, $-NR^{10}C(O)NR^9R^8$, $-NR^9R^8$, $-NR^{10}CO_2R^8$, $-SR^8$, $-S(O)R^8$, $-S(O)_2R^8$, $-S(O)_2NR^9R^8$, $-NR^{10}S(O)_2R^8$, C_{6-10} aryl, 5- to 10-membered heteroaryl and 3- to 10-membered heterocyclyl;

each R⁸, R⁹ and R¹⁰ is independently selected from the group consisting of hydrogen, C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, aryl, or heteroaryl; or R⁹ and R⁸ or R¹⁰ and R⁸, together with the atom(s) to which they are attached, form a 5-, 6-, or 7-membered ring;

R¹¹ is selected from the group consisting of hydrogen, C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, C₆₋₁₀ aryl, 5- to 10-membered heteroaryl and 3- to 10-membered heterocycle.

3. The method of claim 2, wherein the compound of Formula I has the structure of Formula II



or a pharmaceutically acceptable form thereof.

4. The method of claim 3, wherein
 R¹ and R² are each independently selected from the group consisting of halogen, C₁₋₈ alkyl, or C₁₋₈ haloalkyl, provided that at least one of R¹ or R² is other than hydrogen;
 R⁵ is halogen or C₁₋₈ alkyl;
 X² is CR⁷ or N;
 R⁷ is independently selected from the group consisting of hydrogen, halogen, C₁₋₈ alkyl, –CN, –NO₂, –OR⁸, and –NR⁹R⁸;
 R¹¹ is hydrogen or C₁₋₈ alkyl.

5. The method of claim 3, wherein
 R¹ is halogen
 R² C₁₋₈ haloalkyl;
 R⁵ is C₁₋₈ alkyl;

X^2 is CR^7 or N;

R^7 is independently selected from the group consisting of hydrogen, halogen, C_{1-8} alkyl;

R^{11} is hydrogen or C_{1-8} alkyl.

6. The method of claim 3, wherein

R^1 is chloro

R^2 trifluoromethyl;

R^5 is methyl;

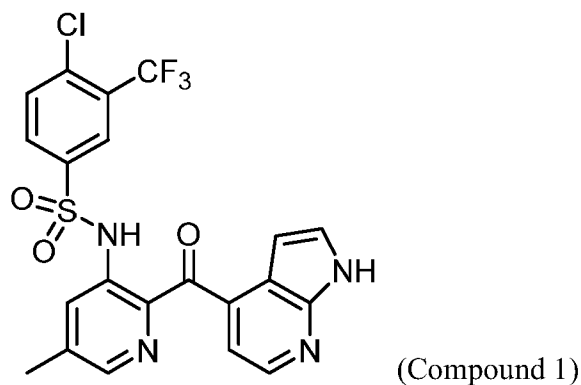
X^2 is CR^7 or N;

R^7 is independently selected from the group consisting of hydrogen, halogen, C_{1-8} alkyl;

R^{11} is hydrogen or C_{1-8} alkyl.

7. The method of claim 3, wherein the compound of Formula II has the

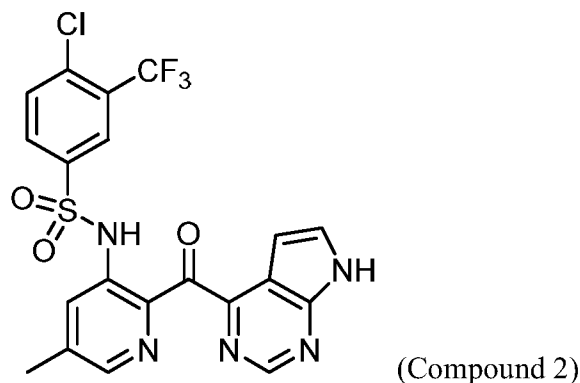
structure of Compound 1



or a pharmaceutically acceptable form thereof.

8. The method of claim 3, wherein the compound of Formula II has the

structure of Compound 2



or a pharmaceutically acceptable form thereof.

9. The method of any one of claims **1** to **8**, wherein the CCR2 antagonist is administered daily.

10. The method of any one of claims **1** to **8**, wherein administration of the CCR2 antagonist to the subject in need thereof reduces a urine albumin-to-creatinine ratio in said subject.

11. The method of claim **10**, wherein the urine albumin-to-creatinine ratio in said subject is reduced by at least 20% after 3 weeks of administering the CCR2 antagonist as compared to the albumin to creatinine ratio in said subject prior to treatment.

12. The method of claim **10**, wherein the urine albumin-to-creatinine ratio in said subject is reduced by at least 30% after 3 weeks of administering the CCR2 antagonist as compared to the albumin to creatinine ratio in said subject prior to treatment.

13. The method of claim **10**, wherein the urine albumin-to-creatinine ratio in said subject is reduced by at least 40% after 3 weeks of administering the CCR2 antagonist as compared to the albumin to creatinine ratio in said subject prior to treatment.

14. The method of claim **10**, wherein the urine albumin-to-creatinine ratio in said subject is reduced by at least 50% after 3 weeks of administering the CCR2 antagonist as compared to the albumin to creatinine ratio in said subject prior to treatment.

1 **15.** The method of claim **10**, wherein the urine albumin-to-creatinine ratio in
2 said subject is reduced by at least 60% after 3 weeks of administering the CCR2 antagonist as
3 compared to the albumin to creatinine ratio in said subject prior to treatment.

1 **16.** The method of any one of claims **1** to **15**, wherein the CCR2 antagonist is
2 a selective CCR2 antagonist.

1 **17.** The method of any one of claims **1** to **16**, wherein the CCR2 antagonist is
2 administered as a monotherapy.

1 **18.** The method of any one of claims **1** to **16**, wherein the CCR2 antagonist is
2 administered in combination with one or more additional therapeutic agents.

1 **19.** The method of claim **18**, wherein the additional therapeutic agent is a
2 Renin-angiotensin-aldosterone (RAAS) blocker.

1 **20.** The method of claim **19**, wherein the RAAS blocker is a renin inhibitor.

1 **21.** The method of claim **20**, wherein the renin inhibitor is selected from the
2 group consisting of aliskiren, remikiren, H-142, SPP635, SPP1148, SPP676, and SPP1234.

1 **22.** The method of claim **19**, wherein the RAAS blocker is an ACE inhibitor.

1 **23.** The method of claim **22**, wherein the ACE inhibitor is enalapril, captopril,
2 enalapril, fosinopril, lisinopril, perindopril, quinapril, ramipril, and trandolapril.

1 **24.** The method of claim **19**, wherein the RAAS blocker an angiotensin
2 receptor blocker (ARB).

1 **25.** The method of claim **24**, wherein the ARB is selected from the group
2 consisting of sparsentan, eprosartan, candesartan, irbesartan, losartan, olmesartan, telmisartan,
3 valsartan, CGP-42112A, DuP753, saralasin, and sarthran.

1 **26.** The method of claim **24**, wherein the ARB is selected from the group
2 consisting of eprosartan, candesartan, irbesartan, losartan, olmesartan, telmisartan, valsartan,
3 CGP-42112A, DuP753, saralasin, and sarthran.

1 **27.** The method of claim **24**, wherein the ARB is candesartan.

1 **28.** The method of any one of claims **18** to **27**, wherein the additional
2 therapeutic agent is an endothelin receptor antagonist.

1 **29.** The method of claim **28**, wherein the endothelin receptor antagonist is
2 selected from the group consisting of sparsentan, bosentan, macitentan, ambrisentan, sitazentan,
3 aproцитentan, and artasentan.

1 **30.** The method of claim **28**, wherein the endothelin receptor antagonist is
2 sparsentan.

1 / 6

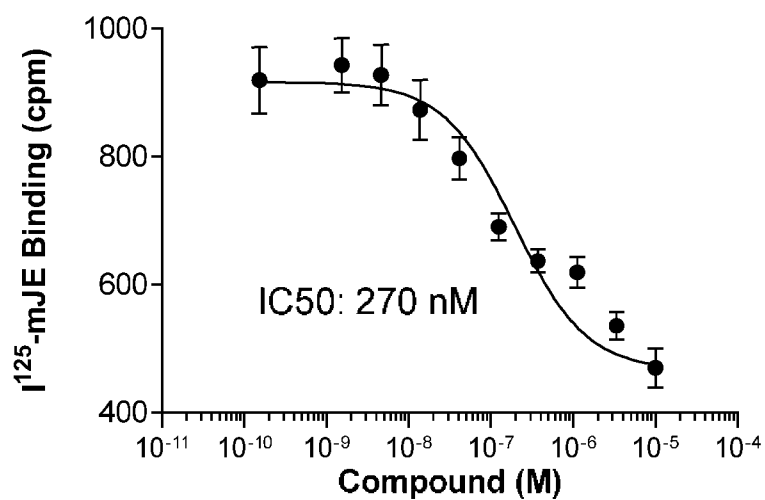


FIG. 1

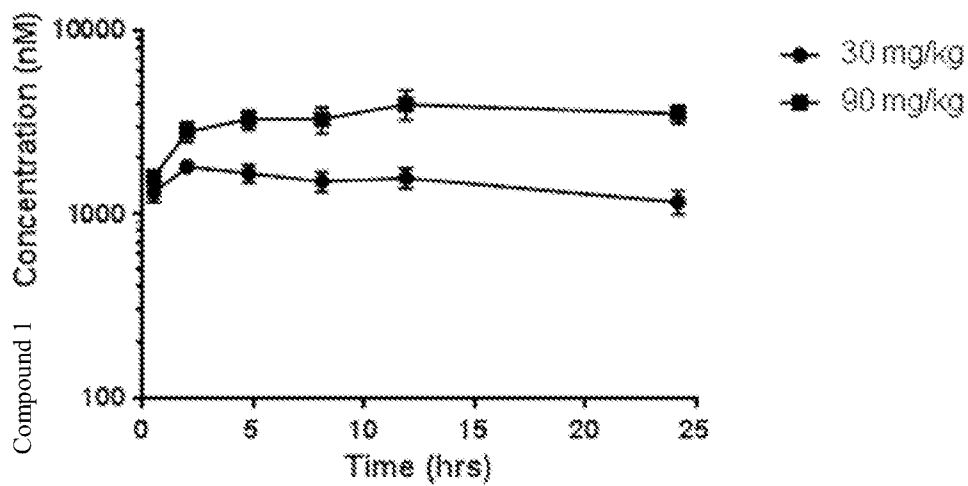


FIG. 2

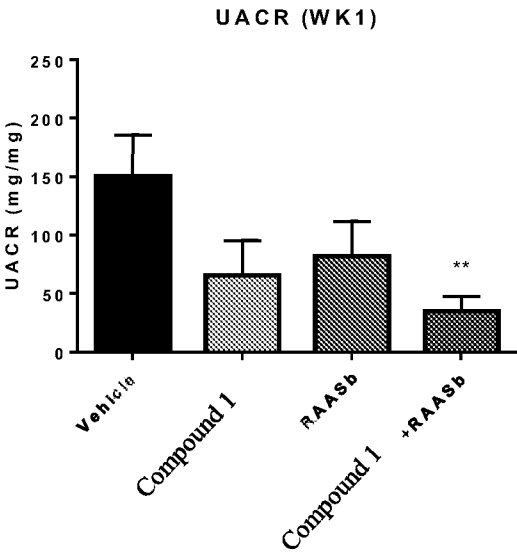


FIG. 3A

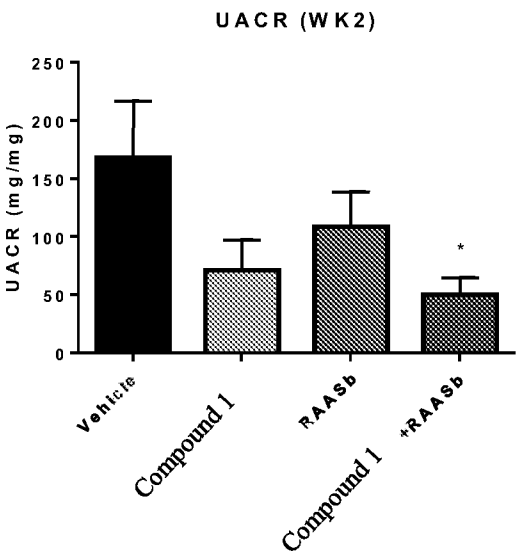


FIG. 3B

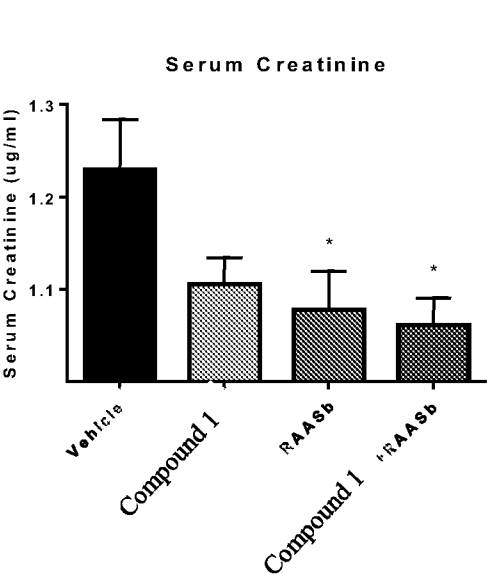


FIG. 3C

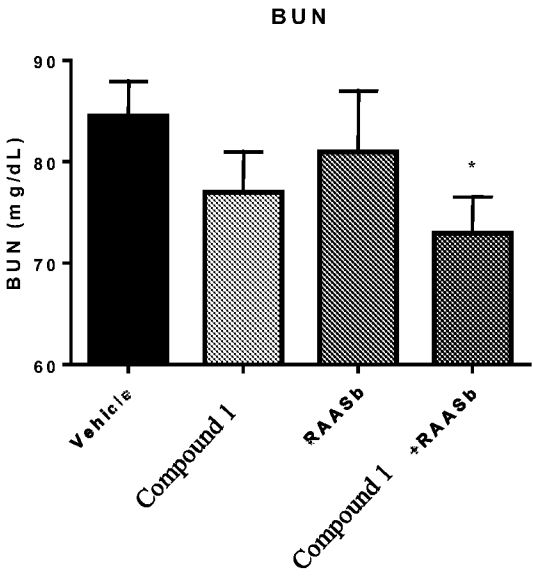


FIG. 3D

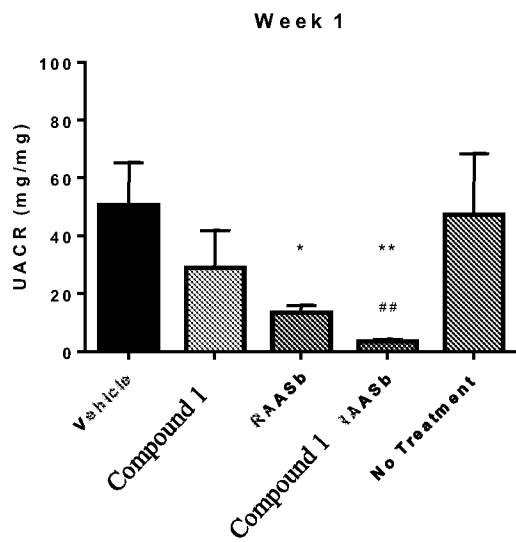


FIG. 4A

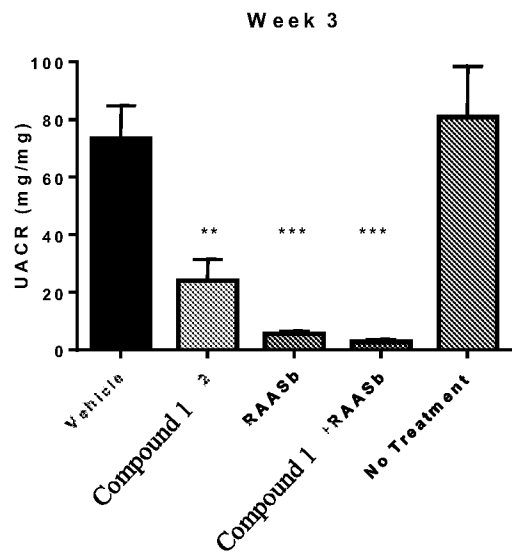


FIG. 4B

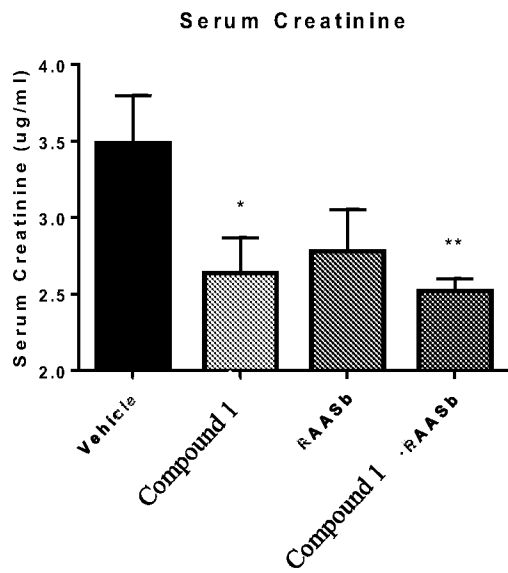


FIG. 5A

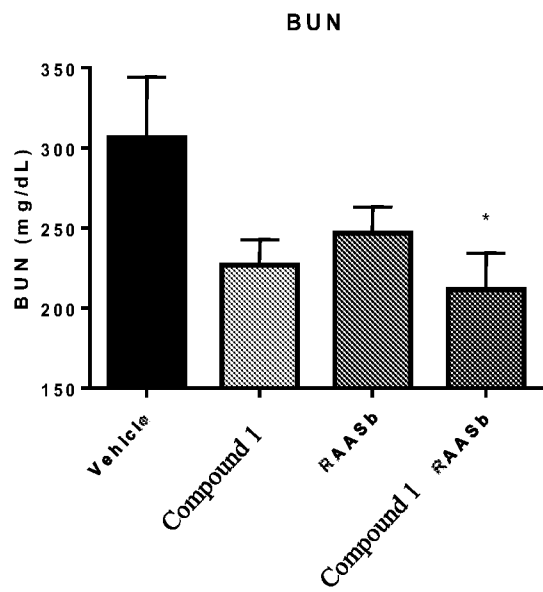


FIG. 5B

Vehicle

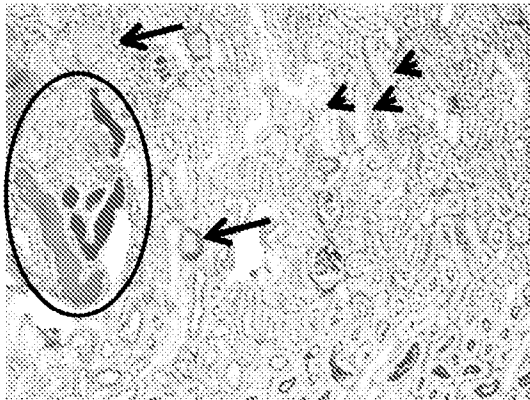


FIG. 6A

Compound 1

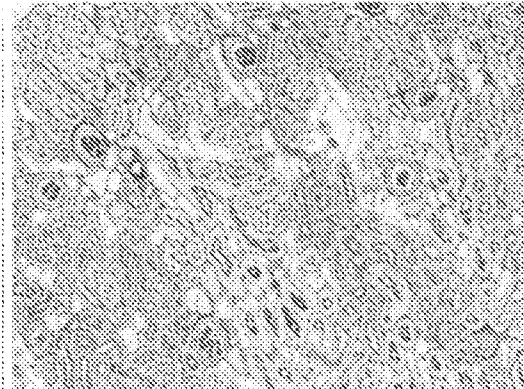


FIG. 6B

RAAS Blocker

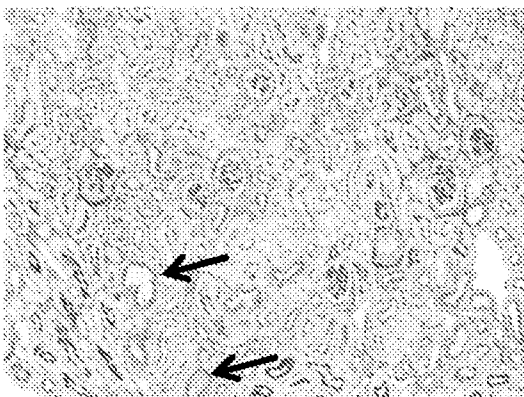


FIG. 6C

Compound 1+RAAS Blocker

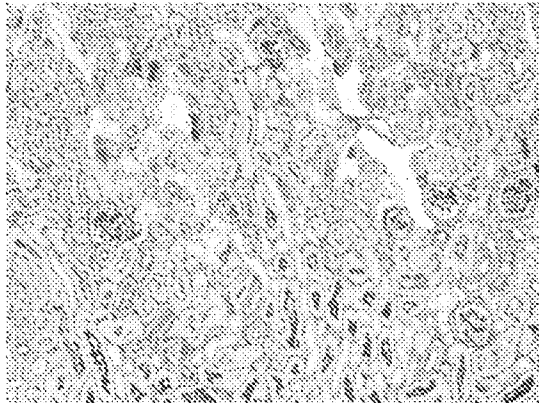


FIG. 6D

Vehicle

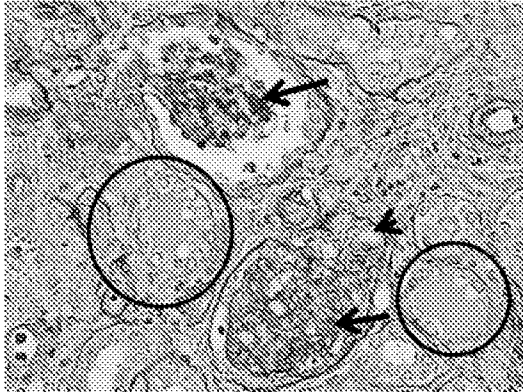


FIG. 7A

Compound 1

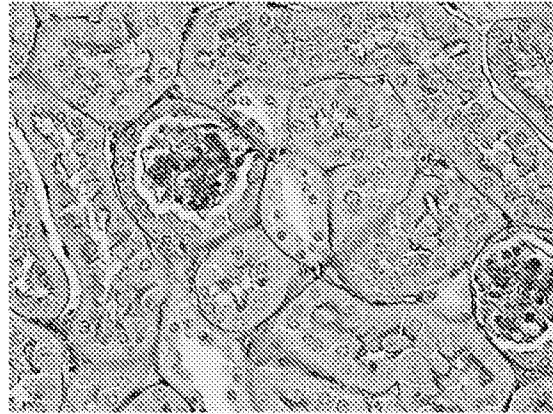


FIG. 7B

RAAS Blocker

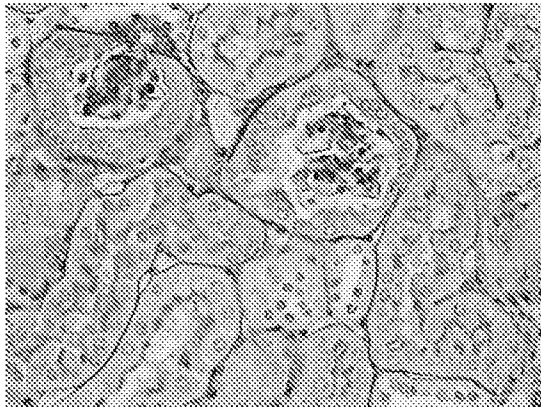


FIG. 7C

Compound 1+RAAS Blocker

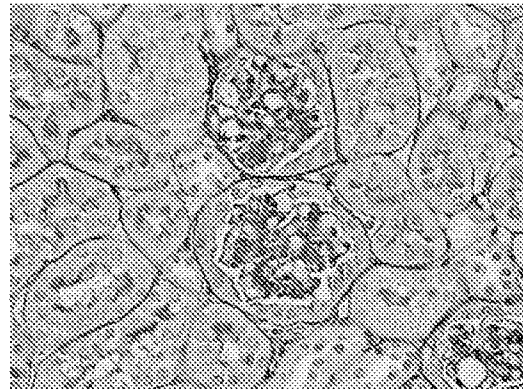


FIG. 7D

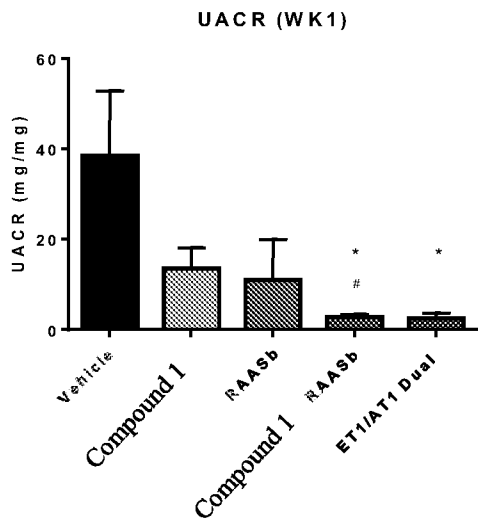


FIG. 8A

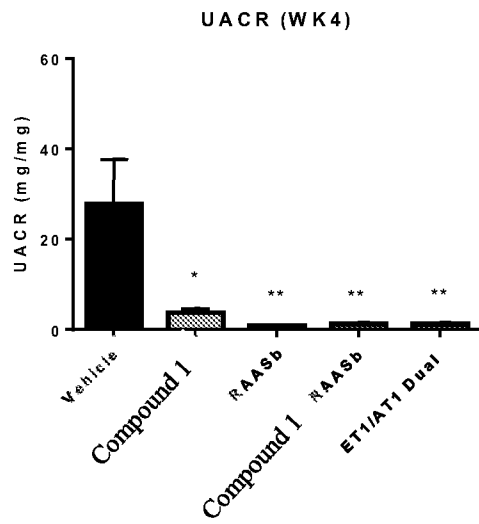


FIG. 8B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/55244

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/18; A61K 31/437; C07D 213/44; A61P 13/12 (2018.01)

CPC - A61K 31/18; A61K 31/437; C07D 213/24; C07D 213/44; C07D 487/04

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)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MIAO et al. 'CCR2 ANTAGONISM REDUCES PROTEINURIA AND GLOMERULAR INJURY IN MURINE MODELS OF FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS)', Nephrology Dialysis Transplantation, Volume 32, Issue suppl-3, 01 May 2017, Pages iii97, https://doi.org/10.1093/ndt/gfx134.T0042 ; [Published: 26 May 2017] pg 1, para 2-para 4	1, (9-15)/1
Y		2-8, (9-15)/(2-8)
Y	WO 2008/008431 A2 (CHEMOCENTRYX, INC.) 17 January 2008 (17.01.2008) para [0068], [0552]-[0553], [1207], [1721], Table 1; claim 29	2-8, (9-15)/(2-8)
X	WO 2016/187393 A1 (CHEMOCENTRYX, INC.) 24 November 2016 (24.11.2016) para [0094], [0099]-[0100]	1
X	US 2013/0252951 A1 (BOYD et al.) 26 September 2013 (26.09.2013) para [0019], [0181], [0183]	1
X,P	WO 2018/098353 A1 (CHEMOCENTRYX, INC.) 31 May 2018 (31.05.2018) Entire Document	1-15
A	US 2013/0210149 A1 (LI et al.) 15 August 2013 (15.08.2013) Entire Document	1-15

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

* Special categories of cited documents:

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

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

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

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

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

"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

25 November 2018

Date of mailing of the international search report

04 JAN 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/55244

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.: 16-30
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.