NEP inhibitor compound as a therapeutic agent, however, it would be desirable to have a solid-state form that can be readily manufactured and that has acceptable chemical and physical stability. For example, it would be highly desirable to have a physical form that is thermally stable at reasonably high temperature, thereby facilitating processing and storage of the material, and a small crystal size that increases dissolution, bioavailability and absorption, thereby allowing favorable drug delivery characteristics. Crystalline solids are generally preferred over amorphous forms, for enhancing purity and stability of the manufactured product. However, the formation of crystalline forms of organic compounds is highly unpredictable. No reliable methods exist for predicting which, if any, form of an organic compound will be crystalline. Moreover, no methods exist for predicting which, if any, crystalline form will have the physically properties desired for use as pharmaceutical agents. Accordingly, a need exists for a stable, crystalline form which has a reasonably high melting point and a small crystal size.

SUMMARY OF THE INVENTION

The present invention provides a novel crystalline form of Compound **I**, which has been found to possess neprilysin (NEP) enzyme inhibition activity. Accordingly, this compound is expected to be useful and advantageous as a therapeutic agent for treating conditions such as hypertension, pulmonary hypertension, heart failure and renal disease.

In one aspect, there is provided a crystalline free acid form of (2S,4R)-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-4-(3-hydroxyisoxazole-5-carboxamido)-2-methylpentanoic acid (I'), wherein the crystalline form is characterized by a powder x-ray diffraction pattern comprising diffraction peaks at 2θ values of 6.51±0.20, 11.62±0.20, 13.05±0.20, 15.07±0.20, and 23.28±0.20.

In another aspect, there is provided a pharmaceutical composition comprising the crystalline form described herein and a pharmaceutically acceptable carrier.

In another aspect, there is provided a pharmaceutical composition comprising the crystalline form described herein and an AT_1 receptor antagonist, a phosphodiesterase inhibitor, a renin inhibitor, a soluble guanylate cyclase inhibitor, a mineralocorticoid-receptor antagonist, a diuretic, or combinations thereof.

In another aspect, there is provided an oral dosage form comprising the crystalline form described herein in a capsule, tablet, or suspension.

In another aspect, there is provided a crystalline form described herein for use in therapy.

In another aspect, there is provided the use of a compound described herein, for the manufacture of a medicament for treating hypertension, heart failure, or renal disease.

In another aspect, there is provided the use of the compound described herein for the manufacture of a medicament for treating a renally-impaired patient once-daily.

In another aspect, there is provided a process for preparing (2S,4R)-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-4-(3-hydroxyisoxazole-5-carboxamido)-2-methylpentanoic acid (I), the process comprising:

(a) coupling benzyl (2S,4R)-4-amino-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-2-methylpentanoate hydrochloride with 3-((4-methoxybenzyl)oxy)isoxazole-5-carboxylic acid in a solvent in approximately a 1:1 molar ratio to give benzyl (2S,4R)-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-4-(3-((4-methoxybenzyl)oxy)isoxazole-5-carboxamido)-2-methylpentanoate; and

(b) deprotecting benzyl (2S,4R)-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-4-(3-((4-methoxybenzyl)oxy)isoxazole-5-carboxamido)-2-methylpentanoate to form (2S,4R)-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-4-(3-hydroxyisoxazole-5-carboxamido)-2-methylpentanoic acid (I).

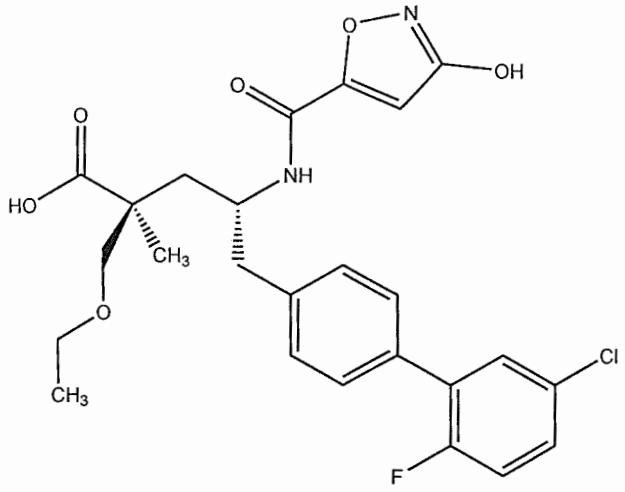
In another aspect, there is provided a process for preparing the crystalline form described herein, the process comprising:

(a) forming a solution comprising (2S,4R)-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-4-(3-hydroxyisoxazole-5-carboxamido)-2-methylpentanoic acid and a solvent, optionally with a metal scavenger, at an elevated temperature;

(b) cooling the solution to a temperature between about -20 ^{o}C and 5 ^{o}C; and

(c) isolating the resulting solids to yield the crystalline form.

The structure of Compound **I** is:



(**I**).

One embodiment of the invention relates to a crystalline free acid form of (*2S,4R*)-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-4-(3-hydroxyisoxazole-5-carboxamido)-2-methylpentanoic acid (Compound **I'**). In one embodiment, Compound **I'** is anhydrous,

non-hygroscopic or both. Compound **I′** may also be referred to as crystalline form **I′**.

Another embodiment of the invention relates to pharmaceutical compositions comprising one or more pharmaceutically acceptable carriers and Compound **I′**. Such compositions may optionally contain other therapeutic agents, including but not limited to, an AT_1 receptor antagonist, an angiotensin-converting enzyme inhibitor, a phosphodiesterase (PDE) inhibitor, a renin inhibitor, a diuretic, or combinations thereof.

Compound **I′** of the invention possesses NEP enzyme inhibition activity, and is therefore expected to be useful as a therapeutic agent for treating patients suffering from a disease or disorder that is treated by inhibiting the NEP enzyme or by increasing the levels of its peptide substrates. Thus, one embodiment of the invention relates to a method of treating patients suffering from a disease or disorder that is treated by inhibiting the NEP enzyme, comprising administering to a patient a therapeutically effective amount of Compound **I′**. Another embodiment of the invention relates to a method of treating hypertension, pulmonary hypertension, heart failure, or renal disease, comprising administering to a subject a therapeutically effective amount of Compound **I′**. Still another embodiment of the invention relates to a method for inhibiting a NEP enzyme in a subject comprising administering to the subject, a NEP enzyme-inhibiting amount of Compound **I′**.

Yet another embodiment of the invention relates to processes useful for preparing Compound **I** and its crystalline form, Compound **I′**.

Yet another embodiment of the invention relates to the use of Compound **I** and its crystalline form, Compound **I′**, for the manufacture of a medicament, especially for the manufacture of a medicament useful for treating hypertension, heart failure, or renal disease. Another embodiment of the invention relates to use of Compound **I** or Compound **I′** for inhibiting a NEP enzyme in a subject. Other aspects and embodiments of the invention are disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Various aspects of the present invention are illustrated by reference to the accompanying drawings.

FIG. 1 shows a powder x-ray diffraction (PXRD) pattern of crystalline (*2S,4R*)-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-4-(3-hydroxyisoxazole-5-carboxamido)-2-methylpentanoic acid (**I′**).

thereof.

8. The pharmaceutical composition of Claim 7, further comprising a pharmaceutical acceptable carrier.

9. An oral dosage form comprising the crystalline form of any one of Claims 1 to 4 in a capsule, tablet, or suspension.

10. The oral dosage form of Claim 9, wherein a release of the crystalline form in a subject is an immediate, controlled or delayed release.

11. The oral dosage form of Claim 9, wherein the capsule material is gelatin, polysaccharide, chitosan or synthetic polymers.

12. The oral dosage form of Claim 9, wherein the hard capsule comprises gelatin, polysaccharides, or synthetic polymers.

13. The oral dosage form of Claim 9, wherein the capsule comprises hydroxypropyl methylcellulose.

14. A crystalline form according to any one of Claims 1 to 4 for use in therapy.

15. The use of a compound according to any one of Claims 1 to 4, for the manufacture of a medicament for treating hypertension, heart failure, or renal disease.

16. The use according to Claim 15, wherein hypertension is primary hypertension, pulmonary arterial hypertension, chronic thromboembolic pulmonary hypertension, or hypertension with renal artery stenosis.

17. The use according to Claim 15, wherein renal disease is diabetic nephropathy, chronic kidney disease, proteinuria, acute kidney injury, nephrotic syndrome, focal segmental glomerulosclerosis or polycystic kidney disease.

18. The use of the compound of any one of claims 1 to 4 for the manufacture of a medicament for treating a renally-impaired patient once-daily.

19. The use according to claim 18, wherein the renally-impaired patient has chronic kidney disease with an estimated glomerular filtration rate (eGFR) between 60 mL/min/1.73 m^2 and 15 mL/min/1.73 m^2.

20. The use according to claim 15, where atrial natriuretic peptide (ANP) or cyclic guanosine monophosphate (cGMP) levels in a human increase for at least 24 hours.

21. The use according to Claim 20, wherein levels of ANP and cGMP are measured in either urine or plasma or both in the human.

22. A process for preparing (*2S,4R*)-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-4-(3-hydroxyisoxazole-5-carboxamido)-2-methylpentanoic acid (**I**), the process comprising:

(a) coupling benzyl (*2S,4R*)-4-amino-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-2-methylpentanoate hydrochloride with 3-((4-methoxybenzyl)oxy)isoxazole-5-carboxylic acid in a solvent in approximately a 1:1 molar ratio to give benzyl (*2S,4R*)-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-4-(3-((4-methoxybenzyl)oxy)isoxazole-5-carboxamido)-2-methylpentanoate; and

(b) deprotecting benzyl (*2S,4R*)-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-4-(3-((4-methoxybenzyl)oxy)isoxazole-5-carboxamido)-2-methylpentanoate to form (*2S,4R*)-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-4-(3-hydroxyisoxazole-5-carboxamido)-2-methylpentanoic acid (**I**).

23. The process of Claim 22, where step (a) further comprises a peptide coupling agent and a base in approximately a 1:3:1:1 molar ratio of coupling agent to base to benzyl (*2S,4R*)-4-amino-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-2-methylpentanoate hydrochloride to 3-((4-methoxybenzyl)oxy)isoxazole-5-carboxylic acid.

24. The process of Claim 23, where the coupling agent is 2-(6-chloro-1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate(V) and the base is *N,N*-diisopropylethylamine.

5

25. The process of Claim 22, where the deprotecting step (b) is performed with a palladium catalyst and hydrogen gas.

26. A process for preparing the crystalline form of Claim 1, the process comprising:

(a) forming a solution comprising (*2S,4R*)-5-(5'-chloro-2'-fluoro-[1,1'-biphenyl]-4-yl)-2-(ethoxymethyl)-4-(3-hydroxyisoxazole-5-carboxamido)-2-methylpentanoic acid and a solvent, optionally with a metal scavenger, at an elevated temperature;

(b) cooling the solution to a temperature between about -20 °C and 5 °C; and

(c) isolating the resulting solids to yield the crystalline form.

27. The process according to Claim 26, where the solvent in step (a) is a polar solvent.

28. The process according to Claim 27, where the solvent is a mixture of ethyl acetate and water, ethyl acetate, or ethanol.

29. The process according to Claim 26, where the temperature in step (a) is between about 60 °C and 95 °C.

30. The process according to Claim 29, where the temperature is between about 70 °C and 85 °C.

31. The process according to Claim 26, where isolating the solids in step (c) involves filtering, washing with one or more solvents, drying in air or under vacuum, or a combination of these steps.

32. The process according to Claim 31, where the solvent is ethyl acetate and water, ethyl acetate or ethanol.

33. The process according to Claim 31, where drying under vacuum is performed at a temperature between 25 °C and 70 °C.