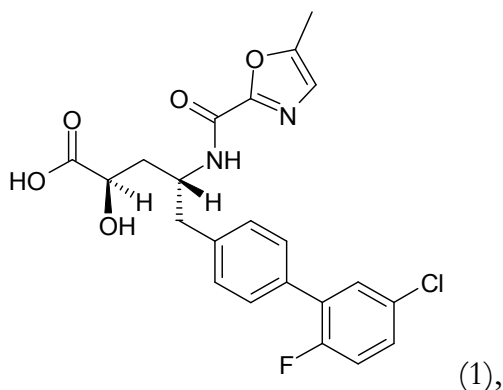


## REIVINDICACIONES

1. Un compuesto CARACTERIZADO porque tiene la estructura:



o una sal farmacéuticamente aceptable del mismo.

2. Ácido (2R,4R)-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxi-4-[(5-metil-oxazol-2-carbonil)amino]pentanoico.
3. Una forma cristalina de ácido (2R,4R)-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxi-4-[(5-metil-oxazol-2-carbonil)amino]pentanoico.
4. La forma cristalina de la reivindicación 3, en donde la forma cristalina está CARACTERIZADA por un patrón de difracción de rayos X en polvo que comprende picos de difracción a valores  $2\theta$  de  $8,48\pm0,20$ ;  $14,19\pm0,20$ ;  $17,03\pm0,20$ ;  $21,15\pm0,20$ ; y  $25,41\pm0,20$ .
5. La forma cristalina de la reivindicación 3, en donde la forma cristalina está CARACTERIZADA por un patrón de difracción de rayos X en polvo que comprende picos de difracción a valores  $2\theta$  de  $7,51\pm0,20$ ;  $8,48\pm0,20$ ;  $14,19\pm0,20$ ;  $17,03\pm0,20$ ;  $17,62\pm0,20$ ;  $17,87\pm0,20$ ;  $20,59\pm0,20$ ;  $21,15\pm0,20$ ;  $21,88\pm0,20$ ;  $24,45\pm0,20$ ;  $24,78\pm0,20$ ;  $25,41\pm0,20$ ;  $25,67\pm0,20$ ;  $27,67\pm0,20$ ; y  $28,22\pm0,20$ .
6. La forma cristalina de la reivindicación 5, en donde la forma cristalina está CARACTERIZADA además por tener uno o más picos de difracción adicionales a valores  $2\theta$  seleccionados entre  $16,09\pm0,20$ ;  $18,70\pm0,20$ ;  $19,21\pm0,20$ ;  $19,40\pm0,20$ ;  $21,64\pm0,20$ ;  $22,25\pm0,20$ ;  $26,43\pm0,20$ ;  $28,55\pm0,20$ ;  $30,73\pm0,20$ ;  $31,10\pm0,20$ ;  $32,64\pm0,20$ ;  $33,14\pm0,20$ ; y  $34,46\pm0,20$ .
7. La forma cristalina de la reivindicación 3, en donde la forma cristalina está CARACTERIZADA por una traza de calorimetría diferencial de barrido registrada a una tasa de calentamiento de  $10\text{ }^{\circ}\text{C}$  por minuto que muestra un valor máximo en el flujo de calor endotérmico a una temperatura de entre  $165\text{ }^{\circ}\text{C}$  y  $169\text{ }^{\circ}\text{C}$ .

8. Una composición farmacéutica CARACTERIZADA porque comprende el compuesto de cualquiera de las reivindicaciones 1 a 7 y uno o más portadores farmacéuticamente aceptables.

9. La composición farmacéutica de la reivindicación 8, CARACTERIZADA porque el portador farmacéuticamente aceptable es estearato de magnesio.

10. Una composición farmacéutica CARACTERIZADA porque comprende el compuesto de cualquiera de las reivindicaciones 1 a 7 y un antagonista de los receptores AT<sub>1</sub>, un inhibidor de la enzima convertidora de angiotensina, un inhibidor de la fosfodiesterasa (PDE), un inhibidor de la renina, un diurético, o combinaciones de los mismos, y opcionalmente uno o más portadores farmacéuticamente aceptables.

11. Una forma farmacéutica oral CARACTERIZADA porque comprende el compuesto de cualquiera de las reivindicaciones 1 a 7 en una cápsula, comprimido, líquido o suspensión.

12. La forma farmacéutica oral de la reivindicación 11, CARACTERIZADA porque la liberación del compuesto es liberación inmediata, controlada o retardada.

13. La forma farmacéutica oral de la reivindicación 11, CARACTERIZADA porque el material de la cápsula es gelatina, polisacárido, quitosano o polímeros sintéticos.

14. La forma farmacéutica oral de la reivindicación 11, CARACTERIZADA porque la cápsula dura comprende gelatina, polisacáridos, o polímeros sintéticos.

15. La forma farmacéutica oral de la reivindicación 11, CARACTERIZADA porque la cápsula comprende hidroxipropil metilcelulosa.

16. Una forma farmacéutica intravenosa CARACTERIZADA porque comprende el compuesto de cualquiera de las reivindicaciones 1 a 7 en una solución tamponada.

17. Un proceso para preparar el compuesto de la reivindicación 1, CARACTERIZADO porque el proceso comprende copular éster etílico del ácido (2R,4R)-4-amino-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxipentanoico con ácido 2-metiloxazol-2-carboxílico para obtener el compuesto de la reivindicación 1.

18. Un proceso para preparar el compuesto de la reivindicación 1, CARACTERIZADO porque el proceso comprende:

- (a) combinar ácido 2-metiloxazol-2-carboxílico y hexafluorofosfato de

*N,N,N',N'*-tetrametil-O-(7-azabenzotriazol-1-il)uronio (HATU) en *N,N*-dimetilformamida (DMF) y agitar a temperatura ambiente;

(b) agregar éster etílico del ácido (2*R*,4*R*)-4-amino-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxipentanoico y *N,N*-diisopropiletilamina y agitar a temperatura ambiente;

(c) aislar y luego disolver los sólidos resultantes en etanol seco y tetrahidrofurano seco;

(d) agregar una solución de hidróxido de litio en agua; y

(e) aislar los sólidos resultantes para obtener el compuesto de la reivindicación 1.

19. El proceso de acuerdo con la reivindicación 18, CARACTERIZADO porque los sólidos resultantes en los pasos (c) y (e) son purificados mediante cromatografía.

20. Un proceso para preparar la forma cristalina de la reivindicación 3, CARACTERIZADO porque el proceso comprende:

(a) disolver ácido (2*R*,4*R*)-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxi-4-[(5-metiloxazol-2-carbonil)amino]pentanoico en etil acetato y hexanos para completar la disolución; y

(b) aislar los sólidos resultantes para formar la forma cristalina.

21. Un proceso para preparar la forma cristalina de la reivindicación 3, CARACTERIZADO porque el proceso comprende:

(a) copular éster etílico del ácido (2*R*,4*R*)-4-amino-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxipentanoico con 5 -metiloxazol-2-carboxilato de sodio para formar ácido (2*R*,4*R*)-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxi-4-[(5-metiloxazol-2-carbonil)amino]pentanoico;

(b) tratar el ácido (2*R*,4*R*)-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxi-4-[(5-metiloxazol-2-carbonil)amino]pentanoico con etil acetato y hexanos para completar la disolución; y

(c) aislar los sólidos resultantes para formar la forma cristalina.

P-2017/14020

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO  
**DIRECCIÓN DE NUEVAS CREACIONES**

E. S. D.

Re: Expediente No. **NC2017/0008426**  
Solicitud de patente de Invención a nombre de **THERAVANCE**  
**BIOPHARMA R&D IP LLC**

Yo, CAROLINA DAZA MONTALVO identificada como aparece al pie de mi firma, domiciliada en la Carrera 11 No. 86-53, piso 3 obrando como apoderada de la sociedad **THERAVANCE BIOPHARMA R&D IP LLC**, en respuesta al oficio No. **12556** notificado por fijación en lista el 28 de agosto de 2017, relacionado con el resultado de examen de forma, me permito presentar la siguiente información:

1. En relación con las objeciones a **las reivindicaciones**, me permito informar lo siguiente:

Con fundamento en la facultad que confiere el artículo 34 de la Decisión 486 al solicitante para modificar la solicitud de patente en cualquier momento del trámite, presento junto con este memorial, un nuevo pliego reivindicatorio que comprende 21 reivindicaciones, en donde únicamente se realizaron modificaciones por concepto de claridad, en un esfuerzo por acelerar el trámite.

**2. Descripción**

De acuerdo con la acción oficial que se contesta, *“la memoria descriptiva no revela la invención de manera lo suficientemente completa para que la persona medianamente versada en el arte pueda reproducir la composición que comprende el compuesto de la Reivindicación 1 y un principio activo seleccionado entre un antagonista de los receptores AT<sub>1</sub>, un inhibidor de la enzima convertidora de angiotensina, un inhibidor de la fosfodiesterasa, un inhibidor de la renina, un diurético, o combinaciones de los mismos”*.

De acuerdo con lo anterior, la solicitante desea manifestar respetuosamente su desacuerdo ya que la memoria descriptiva sí divulga de manera suficiente y completa la materia que se está reclamando. Al respecto, se adjuntan referencias que evidencian estas clases de combinaciones. Por lo cual, sería esperable que las combinaciones de diversas clases de drogas antihipertensivas con los inhibidores de la NEP novedosos de esta invención brindarían un beneficio terapéutico en la hipertensión, insuficiencia cardíaca y enfermedad renal.

Por lo tanto, se solicita amablemente sea retirada dicha objeción.

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### 3. Uso

Con respecto a dicha objeción, se le informa al presente Despacho que se han eliminado las reivindicaciones 24 a 26 que hacían referencia a uso. Por tanto, se ve superada esta objeción.

### 4. Excepción a la patentabilidad

Con respecto a dicha objeción, se le informa al presente Despacho que se han eliminado las reivindicaciones 27 a 31 que hacían referencia a un método para tratar hipertensión, insuficiencia cardíaca, o enfermedad renal, que consiste en administrar a un paciente una cantidad terapéuticamente efectiva del compuesto de la solicitud. Por tanto, se ve superada esta objeción.

### 5. Claridad

El artículo 30 de la Decisión 486 de la Comunidad Andina establece:

***Artículo 30.-** Las reivindicaciones definirán la materia que se desea proteger mediante la patente. Deben ser claras y concisas y estar enteramente sustentadas por la descripción.*

5.1 Se eliminó el término “aproximadamente” de la antigua reivindicación 8 (nueva reivindicación 7) por ser de carácter impreciso y siguiendo las recomendaciones del señor examinador.

5.2 Respecto a la antigua reivindicación 14 (nueva reivindicación 12), la solicitante desea manifestar respetuosamente su desacuerdo ya que dicha reivindicación no corresponde a un método de tratamiento sino más bien a la forma de liberación de un compuesto. De acuerdo con lo anterior, y con el fin de dar mayor claridad a la invención, se reescribió dicha reivindicación eliminando la expresión “*en un sujeto*”, la cual podría interpretarse como un método de tratamiento. Por lo tanto, se ve superada la objeción realizada por el señor examinador.

5.3 Finalmente, se eliminaron las antiguas reivindicaciones 7 y 9 por hacer referencia a una figura de acuerdo con las recomendaciones del señor examinador.

De esta manera, se considera que se ha respondido a las observaciones hechas por el señor Examinador en este respecto, por tanto, se superan las objeciones planteadas y en consecuencia, el nuevo pliego reivindicatorio de la presente solicitud, cumple a cabalidad con este requisito.

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## 6. Cesión

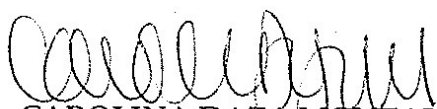
Aunque no fue requerido, se adjunta al presente memorial los documentos en que consta la cesión de derechos de los inventores a favor de la sociedad THERAVANCE BIOPHARMA R&D IP LLC, con el fin de completar la documentación de la presente solicitud.

Habiendo atendido cabalmente las objeciones presentadas por ese Despacho en el Oficio No. 12556 solicito atentamente sea tenida en cuenta la información aquí presentada y continuar con el trámite de la presente solicitud.

## 7. Anexos

- Nuevo capítulo reivindicatorio conformado por 21 reivindicaciones.
- Documento de cesión a favor de la sociedad solicitante.
- Documentos de referencia que evidencian las clases de combinaciones de la presente invención.

Señor Director,



CAROLINA DAZA MONTALVO

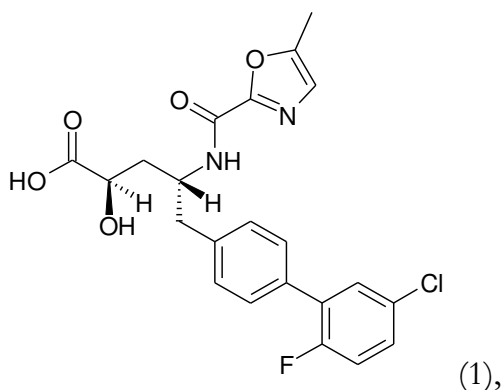
C.C. 66.783.790 de Palmira Valle

TP-141563 CSJ

[notificaciones@clarkemodet.com.co](mailto:notificaciones@clarkemodet.com.co)

## **REIVINDICACIONES**

1. Un compuesto CARACTERIZADO porque tiene la estructura:



o una sal farmacéuticamente aceptable del mismo.

2. Ácido (2R,4R)-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxi-4-[(5-metil-oxazol-2-carbonil)amino]pentanoico.
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6. La forma cristalina de la reivindicación 5, en donde la forma cristalina está CARACTERIZADA además por tener uno o más picos de difracción adicionales a valores  $2\theta$  seleccionados entre  $16,09 \pm 0,20$ ;  $18,70 \pm 0,20$ ;  $19,21 \pm 0,20$ ;  $19,40 \pm 0,20$ ;  $21,64 \pm 0,20$ ;  $22,25 \pm 0,20$ ;  $26,43 \pm 0,20$ ;  $28,55 \pm 0,20$ ;  $30,73 \pm 0,20$ ;  $31,10 \pm 0,20$ ;  $32,64 \pm 0,20$ ;  $33,14 \pm 0,20$ ; y  $34,46 \pm 0,20$ .
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8. Una composición farmacéutica CARACTERIZADA porque comprende el compuesto de cualquiera de las reivindicaciones 1 a 7 y uno o más portadores farmacéuticamente aceptables.

9. La composición farmacéutica de la reivindicación 8, CARACTERIZADA porque el portador farmacéuticamente aceptable es estearato de magnesio.

10. Una composición farmacéutica CARACTERIZADA porque comprende el compuesto de cualquiera de las reivindicaciones 1 a 7 y un antagonista de los receptores AT<sub>1</sub>, un inhibidor de la enzima convertidora de angiotensina, un inhibidor de la fosfodiesterasa (PDE), un inhibidor de la renina, un diurético, o combinaciones de los mismos, y opcionalmente uno o más portadores farmacéuticamente aceptables.

11. Una forma farmacéutica oral CARACTERIZADA porque comprende el compuesto de cualquiera de las reivindicaciones 1 a 7 en una cápsula, comprimido, líquido o suspensión.

12. La forma farmacéutica oral de la reivindicación 11, CARACTERIZADA porque la liberación del compuesto es liberación inmediata, controlada o retardada.

13. La forma farmacéutica oral de la reivindicación 11, CARACTERIZADA porque el material de la cápsula es gelatina, polisacárido, quitosano o polímeros sintéticos.

14. La forma farmacéutica oral de la reivindicación 11, CARACTERIZADA porque la cápsula dura comprende gelatina, polisacáridos, o polímeros sintéticos.

15. La forma farmacéutica oral de la reivindicación 11, CARACTERIZADA porque la cápsula comprende hidroxipropil metilcelulosa.

16. Una forma farmacéutica intravenosa CARACTERIZADA porque comprende el compuesto de cualquiera de las reivindicaciones 1 a 7 en una solución tamponada.

17. Un proceso para preparar el compuesto de la reivindicación 1, CARACTERIZADO porque el proceso comprende copular éster etílico del ácido (2R,4R)-4-amino-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxipentanoico con ácido 2-metiloxazol-2-carboxílico para obtener el compuesto de la reivindicación 1.

18. Un proceso para preparar el compuesto de la reivindicación 1, CARACTERIZADO porque el proceso comprende:

- (a) combinar ácido 2-metiloxazol-2-carboxílico y hexafluorofosfato de

*N,N,N',N'*-tetrametil-O-(7-azabenzotriazol-1-il)uronio (HATU) en *N,N*-dimetilformamida (DMF) y agitar a temperatura ambiente;

(b) agregar éster etílico del ácido (2*R*,4*R*)-4-amino-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxipentanoico y *N,N*-diisopropiletilamina y agitar a temperatura ambiente;

(c) aislar y luego disolver los sólidos resultantes en etanol seco y tetrahidrofurano seco;

(d) agregar una solución de hidróxido de litio en agua; y

(e) aislar los sólidos resultantes para obtener el compuesto de la reivindicación 1.

19. El proceso de acuerdo con la reivindicación 18, CARACTERIZADO porque los sólidos resultantes en los pasos (c) y (e) son purificados mediante cromatografía.

20. Un proceso para preparar la forma cristalina de la reivindicación 3, CARACTERIZADO porque el proceso comprende:

(a) disolver ácido (2*R*,4*R*)-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxi-4-[(5-metiloxazol-2-carbonil)amino]pentanoico en etil acetato y hexanos para completar la disolución; y

(b) aislar los sólidos resultantes para formar la forma cristalina.

21. Un proceso para preparar la forma cristalina de la reivindicación 3, CARACTERIZADO porque el proceso comprende:

(a) copular éster etílico del ácido (2*R*,4*R*)-4-amino-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxipentanoico con 5 -metiloxazol-2-carboxilato de sodio para formar ácido (2*R*,4*R*)-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxi-4-[(5-metiloxazol-2-carbonil)amino]pentanoico;

(b) tratar el ácido (2*R*,4*R*)-5-(5'-cloro-2'-fluorobifenil-4-il)-2-hidroxi-4-[(5-metiloxazol-2-carbonil)amino]pentanoico con etil acetato y hexanos para completar la disolución; y

(c) aislar los sólidos resultantes para formar la forma cristalina.

# Clarke, Modet & C<sup>o</sup>

COLOMBIA

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SANTAFÉ DE BOGOTÁ, A.A. 92397 COLOMBIA

## CESION

El (la) abajo firmante (En adelante EL CEDENTE)

- 1. FLEURY, Melissa
- 2. HUGHES, Adam D.
- 3. BEAUSOLEIL, Anne-Marie
- 4. FENSTER, Erik
- 5. THALLADI, Venkat R.
- 6. RAPTA, Miroslav

en su carácter de inventor /diseñador con domicilios en:

- 1. 47D Joy Avenue Brisbane, California 94005, Estados Unidos de América.
- 2. 2029 Touraine Lane Half Moon Bay, California 94019, Estados Unidos de América.
- 3. 600 Brewster Avenue, Apt. 306, Redwood City, California 94063, Estados Unidos de América.
- 4. 535 Redwood Avenue San Bruno, California 94066, Estados Unidos de América.
- 5. 888 Foster City Boulevard, Apt. E3 Foster City, California 94404, Estados Unidos de América.
- 6. 1240 Highland Court San Carlos, California 94070, Estados Unidos de América.

por el presente documento declara que cede todos y cada uno de los derechos que le corresponden o puedan corresponderle sobre la siguiente invención , modelo de utilidad, diseño industrial No. PCT/US2016/017699

a: THERAVANCE BIOPHARMA R&D IP, LLC

con domicilio en 901 Gateway Boulevard South San Francisco, California 94080, Estados Unidos de América.

En prueba de la mutua aceptación de esta cesión ambas partes CEDENTE y CESIONARIO firman el presente documento.

Dado y firmado en  
A los           Días de           De

1. FLEURY, Melissa

El Cedente

2. HUGHES, Adam D.

El Cedente

3. BEAUSOLEIL, Anne-Marie

El Cedente

4. FENSTER, Erik

El Cedente

## ASSIGNMENT

The undersigned (Hereinafter THE ASSIGNOR)

- 1. FLEURY, Melissa
- 2. HUGHES, Adam D.
- 3. BEAUSOLEIL, Anne-Marie
- 4. FENSTER, Erik
- 5. THALLADI, Venkat R.
- 6. RAPTA, Miroslav

Inventor (designor of domiciled at

- 1. 47D Joy Avenue Brisbane, California 94005, Estados Unidos States of America.
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- 6. 1240 Highland Court San Carlos, California 94070, United States of America.

do hereby declares that it assigns all and each of his rights and privileges on the following invention, industrial design, utility model No. PCT/US2016/017699

to THERAVANCE BIOPHARMA R&D IP, LLC

with domicile in 901 Gateway Boulevard South San Francisco, CA 94080, United States of America

As a proof of the mutual acceptance of this assignment, both parties ASSIGNOR and ASSIGNEE sign the present document.

Granted and signed at South San Francisco, CA on this 28 day of AUGUST of 2017

1. FLEURY, Melissa

The Assignor

2. HUGHES, Adam D.

The Assignor

3. BEAUSOLEIL, Anne-Marie

The Assignor

4. FENSTER, Erik

The Assignor

5. THALLADI, Venkat R.

El Cedente

5. THALLADI, Venkat R.

The Assignor

6. RAPTA, Miroslav

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El Cedente

The Assignor

**By: Theravance Biopharma R&D, Inc., its Managing Member**

El Cesionario

  
The Assignee  
Jeffrey A. Hagenah  
Vice President and Chief Patent Counsel

# Clarke, Modet & C<sup>o</sup>

COLOMBIA

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por el presente documento declara que cede todos y cada uno de los derechos que le corresponden o puedan corresponderle sobre la siguiente invención , modelo de utilidad, diseño industrial No. PCT/US2016/017699

a: THERAVANCE BIOPHARMA R&D IP, LLC

con domicilio en 901 Gateway Boulevard South San Francisco, California 94080, Estados Unidos de América.

En prueba de la mutua aceptación de esta cesión ambas partes CEDENTE y CESIONARIO firman el presente documento.

Dado y firmado en  
A los           Días de           De

- 1. FLEURY, Melissa

El Cedente

- 2. HUGHES, Adam D.

El Cedente

- 3. BEAUSOLEIL, Anne-Marie

El Cedente

- 4. FENSTER, Erik

El Cedente

## ASSIGNMENT

The undersigned (Hereinafter THE ASSIGNOR)

- 1. FLEURY, Melissa
- 2. HUGHES, Adam D.
- 3. BEAUSOLEIL, Anne-Marie
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- 5. THALLADI, Venkat R.
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Inventor /designor of domiciled at

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- 5. 888 Foster City Boulevard, Apt. E3 Foster City, California 94404, United States of America.
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do hereby declares that it assigns all and each of his rights and privileges on the following invention, industrial design, utility model No. PCT/US2016/017699

to THERAVANCE BIOPHARMA R&D IP, LLC

with domicile in 901 Gateway Boulevard South San Francisco, CA 94080, United States of America

As a proof of the mutual acceptance of this assignment, both parties ASSIGNOR and ASSIGNEE sign the present document.

Granted and signed at  
on this 29 day of August of 2017

- 1. FLEURY, Melissa

The Assignor

- 2. HUGHES, Adam D.

The Assignor

- 3. BEAUSOLEIL, Anne-Marie

The Assignor

- 4. FENSTER, Erik

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5. THALLADI, Venkat R.

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6. RAPTA, Miroslav

6. RAPTA, Miroslav

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The Assignor

**By: Theravance Biopharma R&D, Inc., its Managing Member**

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Vice President and Chief Patent Counsel

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## CESION

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- 2. HUGHES, Adam D.
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Granted and signed at SOUTH SAN FRANCISCO, CA on this 8 day of SEPTEMBER of 2017

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Venkat R. Thalladi 08 SEP 2017  
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**12.08.92 Bulletin 92/33**(84) Designated Contracting States:  
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**Patentanwälte Von Kreisler-Selting-Werner,**  
**Deichmannhaus am Hauptbahnhof**  
**W-5000 Köln 1(DE)**(54) **Combination of an angiotensin II antagonist or renin inhibitor with a neutral endopeptidase inhibitor.**

(57) Treatment of hypertension or congestive heart failure with a combination of an angiotensin II antagonist or a renin inhibitor with a neutral endopeptidase inhibitor, pharmaceutical compositions comprising said combinations and kits for administering separate pharmaceutical compositions in combination are disclosed, wherein the angiotensin II antagonists include saralasin, sar 1, ile 8 angiotensin II, Dup 753, EXP 6155, EXP 6803 and PD 123319, the renin inhibitors include enalkrein, RO 42-5892, A 65317, CP 80794, ES 1005, ES 8891, SQ 34017, CGP 29287, CGP 38560, SR 43845, U-71038, A 62198, and A 64662, and the neutral endopeptidase inhibitors include N-[N-[1(S)-carboxyl-3-phenylpropyl]- (S)-phenylalanyl]- (S)-isoserine, N-[N-[[(1S)-carboxy-2-phenyl)ethyl]- (S)-phenylalanyl]- $\beta$ -alanine; N-[2(S)-mercaptomethyl-3-(2-methylphenyl)-propionyl]methionine, SQ 28603, UK 69578, SQ 29072, thiorphan, retro-thiorphan and phosphoramidon.

**EP 0 498 361 A2**

## BACKGROUND OF THE INVENTION

The present invention relates to the treatment of hypertension and congestive heart failure with a combination of an angiotensin II antagonist or a renin inhibitor with a neutral endopeptidase inhibitor.

In a second aspect, this invention relates to a pharmaceutical composition comprising an A II antagonist or a renin inhibitor in combination with an NEP inhibitor and to kits comprising an A II antagonist and an NEP inhibitor or a renin inhibitor and an NEP inhibitor.

The renin angiotensin system is a complex hormonal system comprised of a large molecular weight precursor, angiotensinogen, two processing enzymes, renin and angiotensin converting enzyme (ACE), and a vasoactive mediator, A II. See *J. Cardiovasc. Pharmacol.*, 15(Supp B) (1990) p. S1-S5. The enzyme renin catalyzes the cleavage of angiotensinogen into the decapeptide angiotensin I, which has minimal biological activity on its own and is converted into the active octapeptide A II by ACE. A II has multiple biological actions on the cardiovascular system, including vasoconstriction, activation of the sympathetic nervous system, stimulation of aldosterone production, antinatriuresis, stimulation of vascular growth and stimulation of cardiac growth. A II functions as a pressor hormone and is involved the pathophysiology of several forms of hypertension.

Inhibitors of the renin angiotensin system are well known; such drugs lower blood pressure and exert beneficial actions in hypertension and in congestive heart failure as described, for example, in *N. Eng. J. Med.*, 316, 23 (1987) p. 1429-1435. A large number of peptide and non-peptide inhibitors of the renin angiotensin system are known, the most widely studied being the ACE inhibitors, which class includes the drugs captopril, enalapril, lisinopril and spirapril. Although a major mode of action of ACE inhibitors involves prevention of formation of the vasoconstrictor peptide A II, it has been reported in *Hypertension*, 16, 4 (1990) p. 363-370 that ACE cleaves a variety of peptide substrates, including the vasoactive peptides bradykinin and substance P. Prevention of the degradation of bradykinin by ACE inhibitors has been demonstrated, and the activity of the ACE inhibitors in some conditions has been reported in *Circ. Res.*, 66, 1 (1990) p. 242-248 to be mediated by elevation of bradykinin levels rather than inhibition of A II formation. Consequently, it cannot be presumed that the effect of an ACE inhibitor is due solely to prevention of angiotensin formation and subsequent inhibition of the renin angiotensin system.

Neutral endopeptidase (EC 3.4.24.11; enkephalinase; atriopeptidase; NEP) is a zinc-containing metalloprotease which cleaves a variety of peptide substrates on the amino terminal side of aromatic amino acids. See *Biochem. J.*, 241, (1987) p. 237-247. Substrates for this enzyme include, but are not limited to, atrial natriuretic factors (ANF), brain natriuretic peptide, met and leu enkephalin, bradykinin, neurokinin A, and substance P.

ANF are a family of vasodilator, diuretic and antihypertensive peptides which have been the subject of many recent reports in the literature, for example *Annu. Rev. Pharm. Tox.*, 29, (1989) p. 23-54. One form, ANF 99-126, is a circulating peptide hormone which is released from the heart during conditions of cardiac distension. The function of ANF is to maintain salt and water homeostasis as well as to regulate blood pressure. ANF is rapidly inactivated in the circulation by at least two processes: a receptor-mediated clearance reported in *Am. J. Physiol.*, 256 (1989) p. R469-R475 and an enzymatic inactivation via NEP reported in *Biochem. J.*, 243 (1987) p. 183-187. It has been previously demonstrated that inhibitors of NEP potentiate the hypotensive, diuretic, natriuretic and plasma ANF responses to pharmacological injection of ANF in experimental animals. The potentiation of ANF by two specific NEP inhibitors is reported by Sybertz et al in *J. Pharmacol. Exp. Ther.*, 250, 2 (1989) p. 624-631 and in *Hypertension*, 15, 2 (1990) p. 152-161, while the potentiation of ANF by NEP in general was disclosed in U.S. patent 4,749,688. In U.S. 4,740,499, Olins disclosed the use of thiorphan and kelatorphan to potentiate atrial peptides. Moreover, NEP inhibitors lower blood pressure and exert ANF-like effects such as diuresis and increased cyclic guanosine 3',5'-monophosphate (cGMP) excretion in some forms of experimental hypertension. The antihypertensive action of NEP inhibitors is mediated through ANF because antibodies to ANF will neutralize the reduction in blood pressure.

U.S. 4,749,688 also established the antihypertensive action of NEP inhibitors and that co-administration of an ACE inhibitor and a NEP inhibitor results in a greater reduction of blood pressure than observed with either agent alone. The antihypertensive effect is best manifested under conditions in which the renin angiotensin system is suppressed, as reported by Sybertz et al in the references cited above. For example, NEP inhibitors reduce blood pressure effectively in the Desoxycorticosterone sodium acetate (DOCA NA) hypertensive rat, a volume-dependent, renin-suppressed model of hypertension, but are less effective under conditions in which the renin angiotensin system is activated, such as in the spontaneously hypertensive rat (SHR) and in the two kidney Goldblatt hypertension model. Studies in the SHR and in the two-kidney Goldblatt hypertension model using a prodrug of the NEP inhibitor N-[2(S)-mercaptomethyl-3-(2-methyl-

phenyl)propionyl]methionine in combination with the ACE inhibitor spirapril demonstrated the greater efficacy of the combination compared to either drug alone. However, this interaction was inhibited in SHR which had been nephrectomized, a manipulation which markedly suppresses renin levels.

An explanation of this interactive effect of ACE inhibitors and NEP inhibitors on blood pressure is that suppression of the renin angiotensin system allows for full expression of the ANF-like antihypertensive effect of the NEP inhibitor. A II and ANF exert opposite effects on the cardiovascular system and it has been proposed by Johnston et al in *Am. J. Med.*, 87, (Supp 6) (1990) p. 6B-24S-6B-28S that these two hormonal systems act to counterbalance one another.

An enhanced effect from a combination of an A II receptor antagonist or a renin inhibitor with an NEP inhibitor is, however, unexpected for several reasons. First, as discussed above, ACE inhibitors exert pharmacological effects other than inhibition of formation of A II. ACE degrades numerous substrates, including bradykinin, neurotensin, and substance P. In some instances, e.g. with bradykinin and substance P, both ACE and NEP will degrade the peptide. Since substance P and bradykinin are vasodilators, an alteration of the metabolism of either of these, or more efficient protection from degradation by inhibiting the two enzymes could account for an enhanced effect. Moreover, although nephrectomy, a maneuver which strikingly reduces plasma renin levels, eliminated the enhanced interaction of the ACE inhibitor and NEP inhibitor, the NEP inhibitor alone did not lower blood pressure in this state. Thus, the interactions of ACE inhibitors and NEP inhibitors are complex and the effect of an A II receptor antagonist or a renin inhibitor in combination with an NEP inhibitor cannot be predicted solely from data obtained from the combination of an ACE inhibitor and NEP inhibitor.

#### SUMMARY OF THE INVENTION

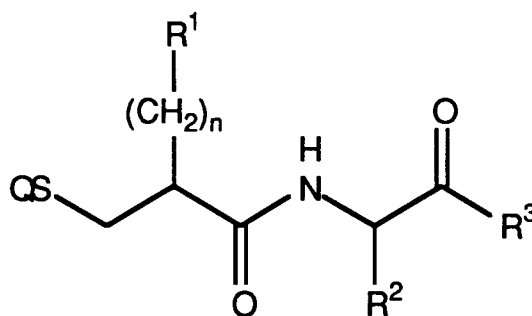
The present invention relates to a method of treating hypertension or congestive heart failure comprising administering an effective amount of a combination of an A II antagonist and a NEP inhibitor to a mammal in need of such treatment. The invention also relates to a method of treating hypertension or congestive heart failure comprising administering an effective amount of a combination of a renin inhibitor and a NEP inhibitor to a mammal in need of such treatment.

Another aspect of the invention relates to pharmaceutical compositions comprising an effective amount of a combination of an A II antagonist and a NEP inhibitor in a pharmaceutically acceptable carrier and to pharmaceutical compositions comprising an effective amount of a combination of a renin inhibitor and a NEP inhibitor in a pharmaceutically acceptable carrier.

Since the present invention relates to a method of treatment comprising a combination of actives wherein the actives may be administered separately, the invention in a third aspect relates to combining separate pharmaceutical compositions in kit form.

#### DETAILED DESCRIPTION

The NEP inhibitors suitable for use in this invention include, but are not limited to compounds disclosed in U.S. 4,610,816, herein incorporated by reference, including in particular N-[N-[1(S)-carboxyl-3-phenylpropyl]-(S)-phenylalanyl]-(S)-isoserine and N-[N-[(1S)-carboxy-2-phenylethyl]-(S)-phenylalanyl]-β-alanine; compounds disclosed in U.S. 4,801,609 and 4,929,641, each herein incorporated by reference, including compounds of the formula



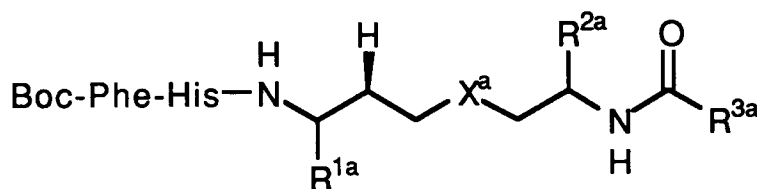
wherein R<sup>1</sup> is phenyl substituted by alkyl, R<sup>2</sup> is alkyl-S(O)<sub>0-2</sub>(CH<sub>2</sub>)<sub>q</sub>, R<sup>3</sup> is OR<sup>7</sup> wherein R<sup>7</sup> is hydrogen or lower alkyl, Q is hydrogen or R<sup>10</sup>CO- wherein R<sup>10</sup> is alkyl, n is 0-2 and q is 1-4, and in particular N-[2(S)-

mercaptomethyl-3-(2-methylphenyl)-propionyl]methionine; SQ 28603 (N-[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]- $\beta$ -alanine), disclosed in South African Patent Application 84/0670; UK 69578 (cis-4-[[[1-2-carboxy-3-(2-methoxyethoxy)propyl]-cyclopentyl]carbonyl]amino]-cyclohexanecarboxylic acid) and its active enantiomer(s); thiorphan and its enantiomers; retro-thiorphan; phosphoramidon; and SQ 29072 (7-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]-heptanoic acid). Also suitable for use are any pro-drug forms of the above-listed NEP inhibitors, e.g., compounds in which one or more carboxylic acid groups are esterified.

The A II antagonists suitable for use in this invention include, but are not limited to saralasin; sar 1 (1-(N-methylglycine-angiotensin II); ile 8 angiotensin II (1-de-L-aspartic acid -8-L-isoleucine-angiotensin II); Dup 753 (2-butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol, monopotassium salt) and active metabolites thereof; EXP 6155 (2-butyl-1-[(4-carboxyphenyl)methyl]-4-chloro-1H-imidazole-5-acetic acid, disodium salt); EXP 6803 (2-butyl-1-[[4-[(2-carboxybenzoyl)amino]phenyl]methyl]-4-chloro-1H-imidazole-5-acetic acid  $\alpha$ -methyl ester, monosodium salt); and PD 123319 (1-(4-dimethylamino-3-methylphenyl)methyl-5-diphenylacetyl-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid). Dup 753, EXP 6155 and EXP 6803 are disclosed in European Patent Applications 253,310 and 324,377; PD 123319 is disclosed in European Patent Application 245,637.

The renin inhibitors suitable for use in the present invention include, but are not limited to, enalkrein, RO 42-5892, A 65317 [(2R)-2-benzyl-3-[(2-methoxyethoxymethoxyethyl)methylaminocarbonyl]-propionyl-L-His(2'S,1'R,5S)-3-ethyl-5(1'-hydroxy-2'-amino-3'-cyclohexylpropyl)oxazolidin-2-one amide]; CP 80794; ES 1005 (N-[4-[[1-[(5-amino-6-hydroxyhexyl)-amino]carbonyl]-3-methyl-butyl]amino]-2-hydroxy-1-(2-methylpropyl)-4-oxobutyl]- $\alpha$ -[[3-(1-naphthalenyl)-2-(1-naphthalenylmethyl)-1-oxopropyl]-amino]-1H-imidazole-4-propanamide dihydrochloride); ES 8891 (5-cyclohexyl-2,4,5-trideoxy-N-hexyl-4-[[N-[3-(1-naphthalenyl)-N-(4-morpholinyl-acetyl)-L-alanyl]-3-(4-thiazolyl)-L-alanyl]amino]-L-threo-pentonamide); SQ 34017; CGP 29287 (carbobenzyloxy-Arg-Arg-Pro-Phe-His-Sta-Ile-His-Lys(BOC)OMe); CGP 38560 (N-[4-[(butylamino)-carbonyl]-1-(cyclohexylmethyl)-2-hydroxy-5-methylhexyl]- $\alpha$ -[[2-[[1,1-dimethylethyl]-sulfonyl]methyl]-1-oxo-3-phenylpropyl]amino]-1H-imidazole-4-propanamide), disclosed in U.S. 4,758,584; SR 43845 (3-Pyr-(CH<sub>2</sub>)-CO-Phe-His-ACHPA-Ile-NH-C(CH<sub>3</sub>-(CH<sub>2</sub>OH)<sub>2</sub>); U-71038 (BOC-Pro-Phe-N-MeHis-Leu $\psi$ (CHOHCH<sub>2</sub>)Val-Ile-Amp); A 62198 ([N-(2-methyl-1-oxopropyl)-L-phenylalanyl]-N-[1S,2R,3S]-4-azido-1-(cyclohexylmethyl)-2,3-dihydroxybutyl-L-histidinamide); A 64662 ([N-(3-amino-3-methyl-1-oxobutyl)-4-methoxy-L-phenylalanyl]-N-[1S,2R,3S]-1-(cyclohexyl-methyl)-2,3-dihydroxy-5-methylhexyl-L-histidinamide) and those disclosed by Watkins et al in U.S. 4,906,613, including those disclosed in the following publications, cited therein:

Compounds of the formula



wherein R<sup>1a</sup> is selected from cyclohexylmethyl, benzyl or butyl; X<sup>a</sup> is S or O; R<sup>2a</sup> is selected from isobutyl, cyclohexylmethyl or benzyl; and R<sup>3a</sup> is phenethyl. A preferred compound within this class is one wherein R<sup>1a</sup> is cyclohexylmethyl, R<sup>2a</sup> is isobutyl, R<sup>3a</sup> is phenethyl and X<sup>a</sup> is S. These compounds are described by Luly et al., *Pharmacologist*, 27 (3), (1985) p. 260, and can be prepared by known techniques from known materials.

Other renin inhibitors are disclosed, for example Szelke et al., U.S. Pat. No. 4,424,207 discloses as having the formula



where

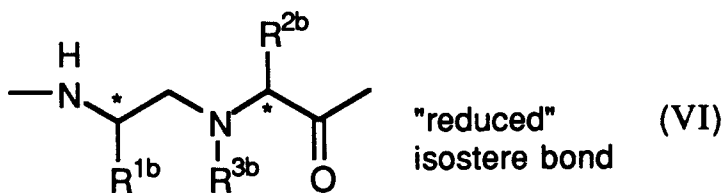
Pro, Phe and His may be in substituted form;

X<sup>b</sup> = H; or an acyl or other N-protecting group e.g. acetyl, pivaloyl, t-butyloxycarbonyl (Boc), benzoyl or lower alkyl (primarily C<sub>1</sub>-C<sub>5</sub>); or an L- or D-amino-acyl residue, which may itself be N-protected similarly;

Y<sup>b</sup> = D- or L-His or other D- or L-basic or aromatic amino-acetyl residue, or is absent;

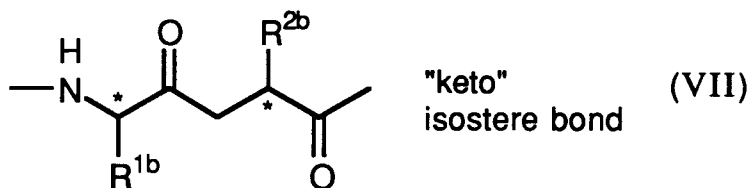
A<sup>b</sup> =

5



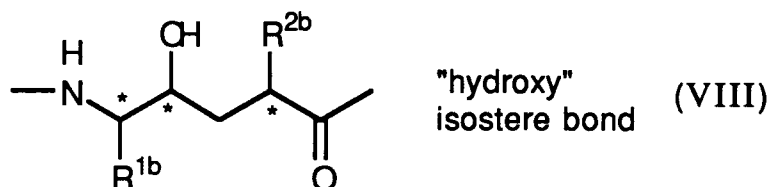
10 or

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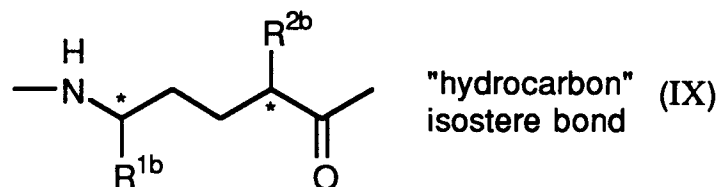
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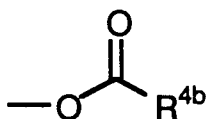
30 or

35



40 where the configuration at asymmetric centers \* is either R or S, where in VIII the hydroxy group may be present as such or protected in ether -OR<sup>4b</sup> or ester

45



50 form where R<sup>4b</sup> is as given under W below and where R<sup>1b</sup> and R<sup>2b</sup>, the same or different = <sup>1</sup>Pro(isopropyl), <sup>1</sup>Bu(isobutyl), Bz(benzyl) or other lipophilic or aromatic amino-acid side chain;

R<sup>3b</sup> = -H; lower alkyl (C<sub>1</sub>-C<sub>5</sub>); or -SO<sub>2</sub>Ph, -SO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>(p), Boc, formyl or other N-protecting group;

B<sup>b</sup> = D- or L-Val or Ile or other D- or L-lipophilic aminoacyl residue;

Z<sup>b</sup> = D- or L-Tyr, Phe, His or other D- or L-aromatic aminoacyl residue; and

W<sup>b</sup> =

55

(a) -OH

(b) -OR<sup>4b</sup> where R<sup>4b</sup> = (1), lower alkyl C<sub>1</sub>-C<sub>5</sub>(n<sup>1</sup>), cycloalkyl C<sub>3</sub>-C<sub>7</sub> or Bzi

(c) -NH<sub>2</sub>

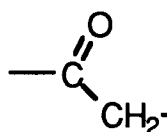
(d) -NHR<sup>3b</sup> or -N(R<sup>5b</sup>)<sub>2</sub> wherein R<sup>5b</sup> is an N-protecting group or R<sup>4b</sup>

- (e) L- or D-Lys  
 (f) L- or D-Arg unprotected or as the ester or amide  
 (g) L- or D-Ser and  
 (h) amino alcohol derived from (e)-(g) as such or protected in ester or ether form

5  $Z^b + W^b =$  alcohol derived from

- (i) L-Tyr  
 (ii) L-Phe  
 (iii) D-Tyr or D-Phe  
 (iv) His

10 such peptide being in the above form or modified by isosteric replacement of one or more remaining peptide bonds by reduced,  $-\text{CH}_2-\text{NH}-$ , keto,



hydroxy,  $-\text{CH}(\text{OH})-\text{CH}_2-$ , or hydrocarbon,  $-\text{CH}_2-\text{CH}_2-$  isosteric links and further being in free form or in  
 20 protected or salt form at one or more remaining peptide, carboxyl, amino, hydroxy or other reactive groups, in particular as their physiologically acceptable acid addition salts at basic centers.

Veber et al., U.S. 4,479,941, discloses renin inhibitor compounds such as

IBU-His-Pro-Phe-His-Sta-Leu-benzylamide;

IBU-His-Pro-Phe-His-Sta-Leu-2-phenylethylamide;

25 IBU-His-Pro-Phe-His-Sta-Leu-3-phenylpropylamide;

IBU-His-Pro-Phe-His-Sta-Leu-1,2-diphenylethylamide;

BOC-Phe-His-Sta-Leu-(+)-1,2-diphenylethylamide;

BOC-Phe-His-Sta-Leu-(-)-1,2-diphenylethylamide;

BOC-Phe-His-Sta-Leu-benzylamide;

30 BOC-Phe-His-Sta-Leu-(+)- $\alpha$ -phenylethylamide;

BOC-Phe-His-Sta-Leu-(-)- $\alpha$ -phenylethylamide;

BOC-Phe-His-Sta-Leu-(+)- $\alpha$ -naphthylethylamide;

BOC-Phe-His-Sta-Leu-(-)- $\alpha$ -naphthylethylamide;

BOC-Phe-His-Sta-Leu-p-chlorobenzylamide;

35 BOC-Phe-His-Sta-Leu-p-methoxybenzylamide;

BOC-Phe-His-Sta-Leu-10,11-dihydro-5H-dibenzo[a,d]-cyclohepteneamide;

BOC-Phe-His-Sta-Leu-D,L-threo-1,2-diphenyl-2-hydroxyethylamide;

BOC-Phe-His-Sta-Leu-Sta;

BOC-Phe-His-AHPPA-Leu-benzylamide;

40 Acetyl-Phe-His-AHPPA-Leu-benzylamide;

BOC-Phe-His-Sta-Leu-(2-amidomethylpyridine);

BOC-Phe-His-Sta-Leu-(4-amidomethylpyridine);

BOC-Phe-His-Sta-Leu-(4-amido-1-benzylpiperidine);

BOC-Phe-His-Sta-Leu-[N-(3-amidopropyl)diethanolamine];

45 BOC-Phe-His-AHPPA-Leu-(2-amidomethylpyridine);

BOC-Phe-His-ACHPA-Ile-(2-amidomethylpyridine);

IVA-His-D-Pro-Phe-His-ACHPA-Ile-(2-amidomethylpyridine);

<sup>1</sup> (+) refers to the optical rotation of the amine.

A preferred compound within this class is BOC-Phe-His-Sta-Leu-(4-amido-1-benzyl-piperidine).

50 Veber et al., U.S. 4,478,826, discloses renin inhibitor compounds such as

tert-Butyloxycarbonyl-His-Pro-Phe-His-Sta-Leu-Leu-Leu-OCH<sub>3</sub>,

tert-Butyloxycarbonyl-His-Pro-Phe-His-Sta-Leu-Tyr-NH<sub>2</sub>,

iso-Butyryl-His-Pro-Phe-His-Sta-Leu-Phe-Lys-NH<sub>2</sub>,

tert-Butyloxycarbonyl-His-Pro-Phe-p-I-Phe-Sta-Leu-Phe-NH<sub>2</sub>,

55 iso-Valeryl-His-Pro-Phe-His-Sta-Leu-Val-Phe-NH<sub>2</sub>,

His-Pro-Phe-His-Sta-Leu-Phe-NH<sub>2</sub>,

iso-Valeryl-His-Pro-Phe-His-Sta-Leu-Phe-NH<sub>2</sub>,

Acetyl-Pro-Phe-His-Sta-Leu-Phe-NH<sub>2</sub>,

Acetyl-Phe-His-Sta-Leu-Phe-NH<sub>2</sub>,  
 tert-Butyloxycarbonyl-Phe-His-Sta-Leu-Phe-NH<sub>2</sub>,  
 tert-Butyloxycarbonyl-His-Pro-Phe-Phe-Sta-Leu-Phe-NH<sub>2</sub>,  
 iso-Butyryl-His-Pro-Phe-His-Sta-Ala-Phe-NH<sub>2</sub>,

5

10

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iso-Butyryl-His-Pro-Phe-His-Sta { Cyclo-  
 hexyl-Phe-NH<sub>2</sub>  
 Ala

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15

A preferred compound within this class is IVA-His-Pro-Phe-His-Sta-Leu-Phe-NH<sub>2</sub>.

Veber et al., U.S. 4,384,994 discloses renin inhibitor compounds such as

N-phenoxyacetyl-L-leucyl-(3S,4S)-statyl-L-valyl-L-phenylalanine;

N-phenoxyacetyl-L-leucyl-(3S,4S)-statyl-L-leucyl-L-phenylalanine

20

N-phenoxyacetyl-L-leucyl-(4S)-amino-(3S)-hydroxy-5-phenylpentanoyl-L-leucyl-L-phenylalanine;

L-leucyl-(3S,4S)-statyl-L-valyl-L-phenylalanine;

L-leucyl-(3S,4S)-statyl-L-leucyl-L-phenylalanine;

L-leucyl-(4S)-amino-(3S)-hydroxy-5-phenylpentanoyl-L-leucyl-L-phenylalanine;

and the amide and C<sub>1-4</sub> alkyl ester forms of the above peptides.

25

Boger et al., U.S. 4,485,099, discloses renin inhibitor compounds such as

30

IBU-His-Pro-Phe-Lys-Sta-Leu-Phe

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IBU-His-Pro-Phe-Orn-Sta-Leu-Phe

40

IBU-His-Pro-Phe-DAB-Sta-Leu-Phe-Gly

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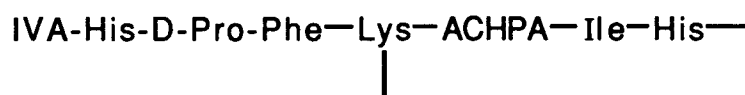
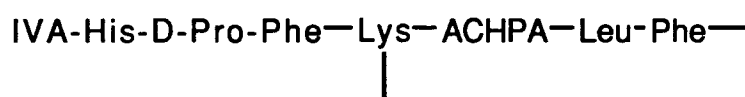
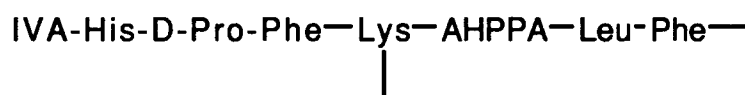
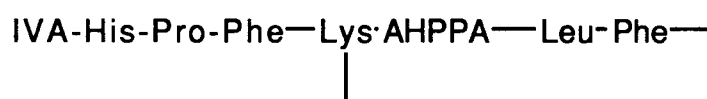
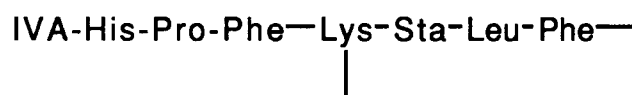
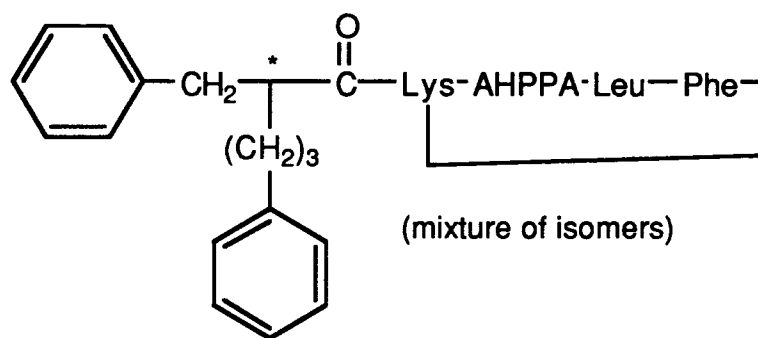
IBU-His-Pro-Phe-Orn-Sta-Leu-Phe-Gly

50

IBU-His-Pro-Phe-Lys-Sta-Leu-Phe-Gly

55

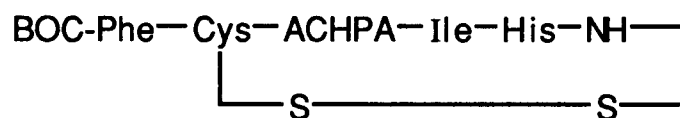
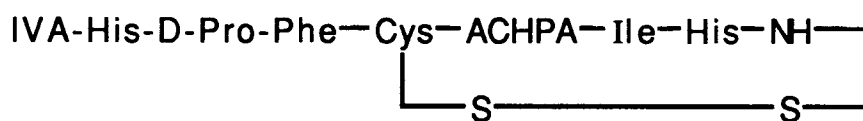
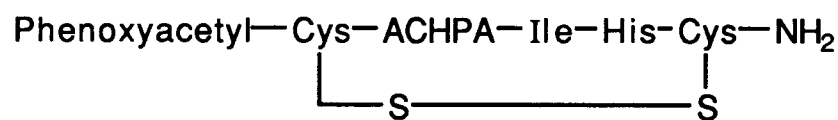
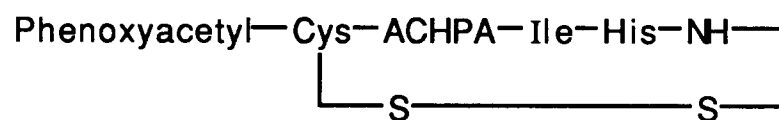
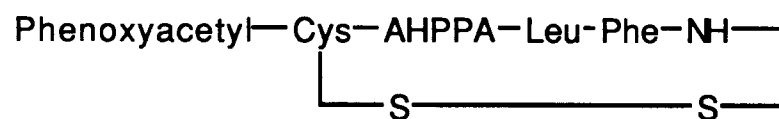
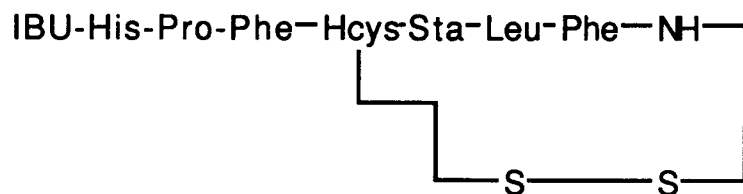
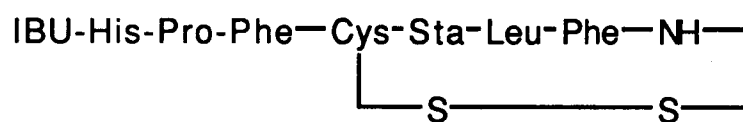
BOC-Phe-Lys-Sta-Leu-Phe



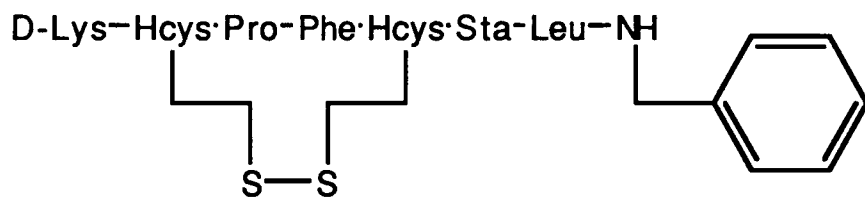
A preferred compound within this class is

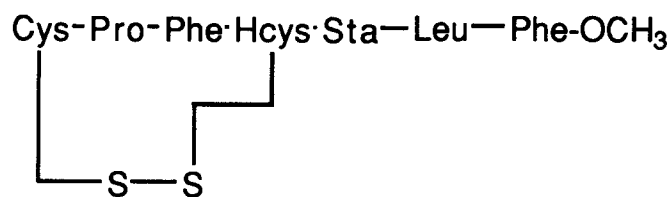
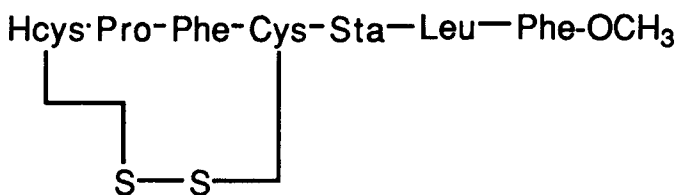
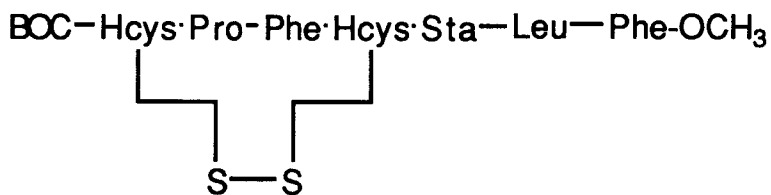
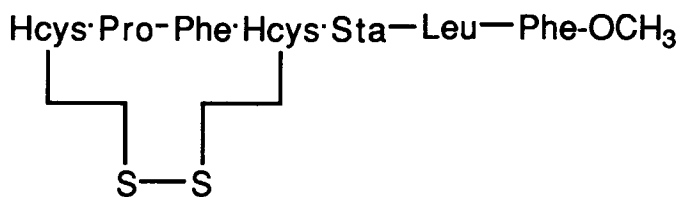
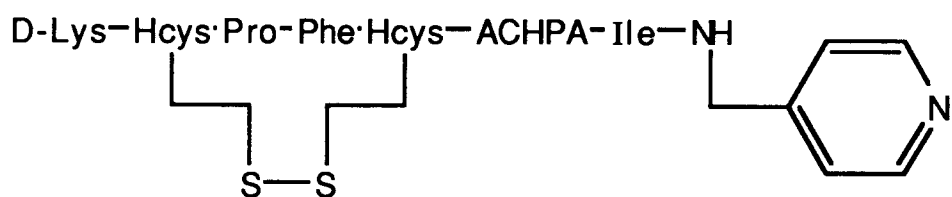
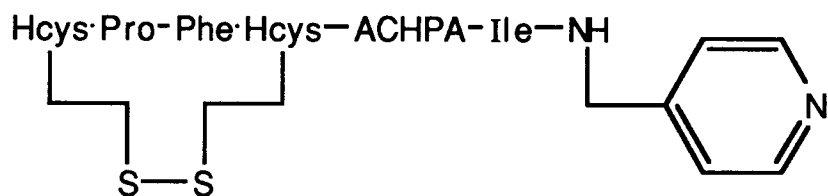
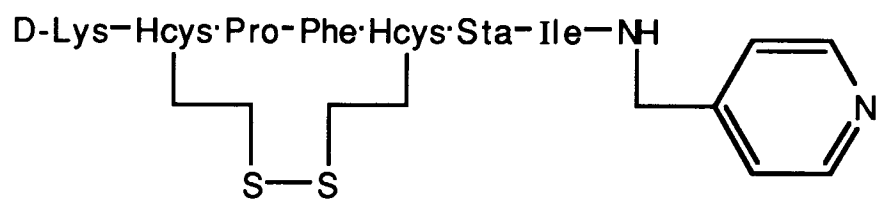


Boger et al., U.S. 4,477,441, discloses renin inhibitor compounds such as



Boger et al., U.S. 4,477,440, discloses renin inhibitor compounds such as





Boger et al., U.S. 4,470,971, discloses renin inhibitor compounds such as

iso-Butyryl-His-Pro-Phe-His-Sta-Val-His-Gly-NH<sub>2</sub>

iso-Butyryl-His-Pro-Phe-His-Sta-Ile-His-NH<sub>2</sub>

tert-Butyloxycarbonyl-Phe-His-Sta-Ile-His-NH<sub>2</sub>

5 Benzyloxycarbonyl-Phe-His-Sta-Ile-His-NH<sub>2</sub>

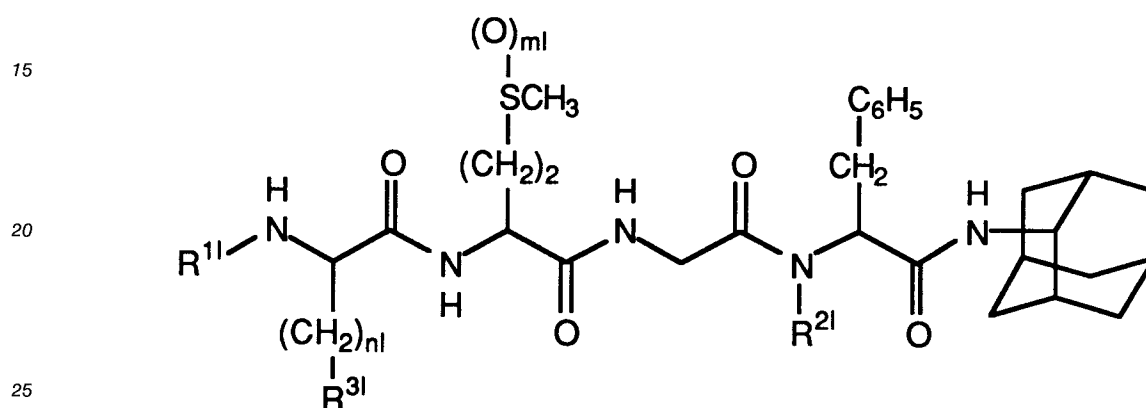
iso-Valeryl-His-Pro-Phe-His-Sta-Ile-His-NH<sub>2</sub>

iso-Valeryl-His-Pro-Phe-His-Sta-Leu-His-NH<sub>2</sub>

A preferred compound within this class is IVA-His-Pro-Phe-His-Sta-Ile-His-NH<sub>2</sub>.

10 Cazaubon et al., U.S. 4,481,192, discloses renin inhibitor compounds such as BOC-Phe-His-Sta-Ala-Sta-OMe.

Hansen, Jr. et al., U.S. 4,510,085, discloses compounds of the formula



as having renin inhibitory activity

wherein R<sup>11</sup> is:

- 30 (a) hydrogen; or  
(b) alkyl of 1 to 6 carbon atoms, inclusive;

wherein R<sup>21</sup> is:

- (a) hydrogen; or  
(b) alkyl of 1 to 6 carbon atoms, inclusive;

35 wherein R<sup>31</sup> is:

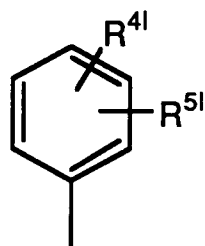
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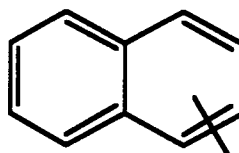
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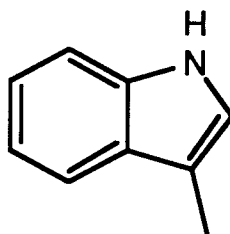
(a)



(b)



(c)



wherein  $R^{4l}$  and  $R^{5l}$  each being the same or different, are:

(a) hydrogen;

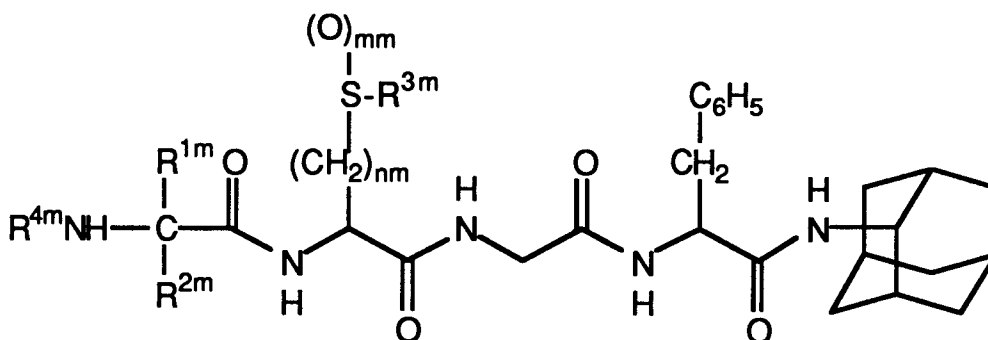
(b) alkyl of 1 to 6 carbon atoms; or

(c) halogen;

wherein  $ml$  is 0, 1 or 2,

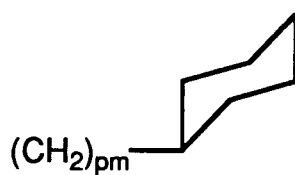
wherein  $nl$  is 0 or an integer of from 1 to 4; and the pharmaceutically acceptable salts thereof.

U.S. 4,514,332 discloses renin inhibitory activity for tetrapeptide adamantyl amides of the formula



wherein  $R^{1m}$  is:

(a)



or

(b) straight or branched chain alkyls of 1 to 6 carbon atoms, inclusive;

wherein  $R^{2m}$  is:

(a) hydrogen; or

(b) alkyl of 1 to 3 carbon atoms;

5 wherein  $R^{3m}$  is:

(a)  $\text{CH}_2\text{C}_6\text{H}_5$ ; or

(b) alkyl of 1 to 3 carbon atoms;

wherein  $R^{4m}$  is:

(a) hydrogen; or

10 (b) alkyl of 1 to 6 carbon atoms;

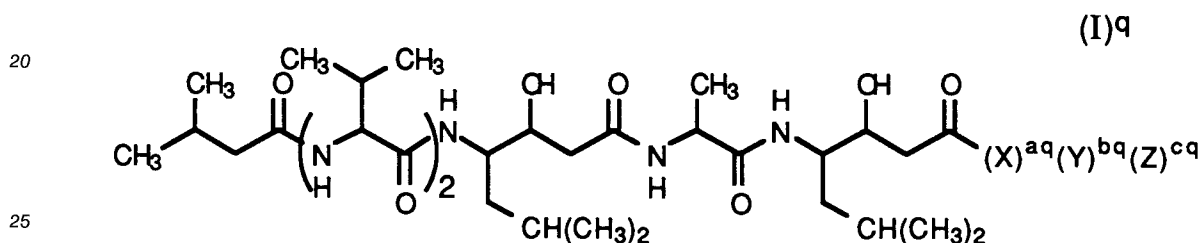
wherein  $m^m$  is 0, 1 or 2;

wherein  $n^m$  is 1 or 2;

wherein  $p^m$  is 0, 1 or 2;

and the stereochemical configuration of each of the optically active amino acid residues may independently  
15 be D, L or DL; and the pharmacologically acceptable salts thereof.

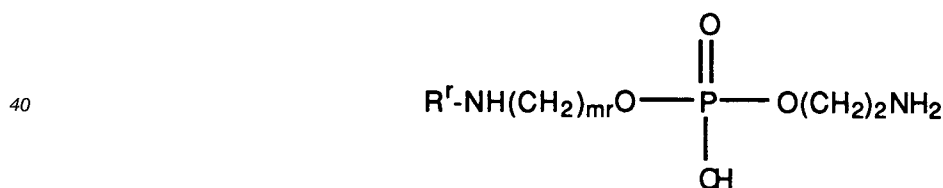
Castro et al., U.S. 4,185,096, discloses renin inhibitor compounds such as



as having renin inhibitory activity.

In the general formula (I)<sup>a</sup> each of the symbols X<sup>aq</sup>, Y<sup>bq</sup> and Z<sup>cq</sup> which are identical or different, 30 represent an amino-acid residue selected from arginine, glutamic acid, aspartic acid, lysine, histidine or valine, forming with the free carboxyl group of the pepstatin or of the adjacent amino-acid a peptide bond -CONH-; the carboxyl function of the terminal amino-acid may exist in free form or in the form of an ester of an aliphatic alcohol containing 1 to 4 carbon atoms, and the indices aq, bq, and cq are each equal to zero or 1, the sum aq + bq + cq being equal to 1, 2 or 3.

35 Hayashi et al., U.S. 3,985,875, discloses renin inhibitors of the formula



45 wherein R<sup>r</sup> is an octadeca-9,12-dienoyl, octadeca-9,12,15-trienoyl, 4-(4'-chlorophenoxy)phenoxyacetyl or α-[4-(4'-chlorophenoxy)phenoxy]-propionyl group, and m<sup>r</sup> is 2 or 3, or their pharmaceutically acceptable acid addition salts.

The following definitions apply throughout the specification: IBU = Iso-butyryl; BOC = Tert-butyloxycarbonyl; AHPPA = (3S,4S)-4-amino-3-hydroxy-5-phenylpentanoic acid; ACHPA = (3S,4S)-4-amino-5-  
50 cyclohexyl-3-hydroxypentanoic acid; IVA = Iso-valeryl; DAB = 2S-amino-4-aminobutyric acid; HLys = homolysine, 2S-amino-6-aminoheptanoic acid; POA = phenoxyacetyl; Hcys = L-homocysteine.

The above descriptions of classes of renin inhibitors for use in the present invention were taken from the noted patents and publications or abstracts thereof. Reference should be made to such patents and publications themselves for their full disclosures of such classes and specific compounds within such 55 classes, and as to any typographical errors or the like which may have occurred in transcription. Also, in describing such renin inhibitors, the superscript letters a,b,l,m,q and r were included to distinguish among the various classes of compounds and the variable substituent groups thereof.

The antihypertensive effects of NEP inhibitors, A II antagonists and renin inhibitors, and of combinations

of NEP inhibitors with A II antagonists or renin inhibitors are determined according to the following procedures.

For the DOCA salt hypertension model, male Sprague Dawley rats weighing 100-150 g are anesthetized with ether and the right kidney is removed. Three pellets containing Doc acetate (desoxycorticosterone acetate, DOCA, 25 mg/pellet) are implanted subcutaneously. Animals recover from surgery, are maintained on normal rat chow and are allowed free access to a fluid of 1% NaCl and 0.2% KCl instead of tap water for a period of 25-30 days. This procedure results in a sustained elevation in blood pressure and is a slight modification of published procedures (e.g. Brock et al, 1982) that have been used to produce DOCA salt hypertension in the rat.

On the day of the study, animals are again anesthetized with ether and the caudal artery is cannulated for blood pressure measurement. Patency of the caudal artery cannula is maintained with a continuous infusion of dextrose in water at a rate of 0.2 ml/hr. Animals are placed into restraining cages where they recover consciousness. Blood pressure is measured from caudal artery catheter using a Statham pressure transducer attached to a Beckman oscillographic recorder. In addition, a cardiovascular monitoring device (Buxco Electronics, Inc.) and a digital computer are used to calculate average blood pressures.

After an equilibration period of at least 1.5 hr., animals are dosed subcutaneously (1 ml/kg) with vehicle (methylcellulose, hereinafter MC), NEP inhibitor, A II antagonist, renin inhibitor, a combination of NEP inhibitor and A II antagonist, or a combination of NEP inhibitor and renin inhibitor and blood pressure is monitored for the next 4 hours. The doses of drug are chosen based on amounts previously determined to be effective for inhibition of the respective enzymes.

Two kidney, 1-clip (2K,1C) Goldblatt hypertension is produced in male Sprague Dawley rats as described by DeForrest et al (1984). Rats weighing 180-200 g are anesthetized with ether or Brevital (50 mg/kg, ip) and the left kidney is exposed through a flank incision. A silver clip with an internal diameter of 0.15 mm is placed around the left renal artery. The contralateral kidney remains untouched. The animal is used 3-4 weeks after surgery when sustained hypertension greater than 150 mm Hg is established.

On the day of the experiment, fasted rats are anesthetized with ether and the abdominal aorta is cannulated via the caudal artery with polyethylene tubing. The rats are placed in plastic restrainers and allowed to regain consciousness for at least 90 min. The rats are dosed by oral gavage with a single drug or with a combination of drugs as suspensions in 0.4% methylcellulose vehicle. Blood pressure is recorded continuously from the caudal artery on an oscillographic recorder.

The antihypertensive effect in SHR is determined as follows. Animals are prepared for blood pressure measurement as described above. After stabilization, animals are dosed subcutaneously with test drugs, combinations thereof or placebo and blood pressure is monitored for the next 4 hours.

ANF has been shown to exert beneficial hemodynamic and renal actions in congestive heart failure (CHF) with the exception of the most severe states, in which its actions may be blunted. ANF and the renin angiotensin system also act as physiological antagonists of one another in CHF. Therefore, it is contemplated that the combination of an ANF-potentiating NEP inhibitor and an inhibitor of the renin angiotensin system will be useful in the treatment of CHF. Measurements of the degree of diuresis and natriuresis, as well as hemodynamics, are used to determine the efficacy of the present combination in the treatment of CHF.

The combinations of this invention comprise an NEP inhibitor and an A II antagonist, and an NEP inhibitor and a renin inhibitor. The components of each combination can be administered in the same pharmaceutical composition or by co-administration of separate pharmaceutical compositions. A variety of pharmaceutical dosage forms are suitable, preferably for oral or parenteral administration, although mechanical delivery systems such as transdermal dosage forms are also contemplated.

The daily dosages of the combinations of this invention for treatment of hypertension or congestive heart failure are as follows: for NEP inhibitors, the typical dosage is about 0.3 mg/kg to about 100 mg/kg of mammalian weight per day administered in single or divided doses; for A II antagonists, the typical dosage is about 0.1 mg/kg to about 50 mg/kg of mammalian weight per day administered in single or divided doses; and for renin inhibitors, the typical daily dosage is about 0.1 mg/kg to about 100 mg/kg mammalian weight, administered in single or divided doses. The exact dose of any component or combination to be administered is determined by the attending clinician and is dependent on the potency of the compound administered, the age, weight, condition and response of the patient.

Generally, in treating humans having hypertension or congestive heart failure, the combinations of this invention can be administered in dosage ranges as follows: for the combination of NEP inhibitor and A II antagonist, about 10 to about 500 mg NEP inhibitor per dose given 1 to 4 times a day, and about 5 to about 100 mg A II antagonist given 1 to 3 times a day; and for the combination of NEP inhibitor and renin inhibitor, about 10 to about 500 mg NEP inhibitor given 1 to 4 times a day, and about 5 to about 600 mg

renin inhibitor given 1 to 3 times a day. Where the components of a combination are administered separately, the number of doses of each component given per day may not necessarily be the same, e.g., where one component may have a greater duration of activity, and will therefore need to be administered less frequently.

Typical oral formulations include tablets, capsules, syrups, elixirs and suspensions. Typical injectable formulations include solutions and suspensions.

The typical pharmaceutically acceptable carriers for use in the formulations described above are exemplified by: sugars such as lactose, sucrose, mannitol and sorbitol; starches such as cornstarch, tapioca starch and potato starch; cellulose and derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and methyl cellulose; calcium phosphates such as dicalcium phosphate and tricalcium phosphate; sodium sulfate; calcium sulfate; polyvinylpyrrolidone; polyvinyl alcohol; stearic acid; alkaline earth metal stearates such as magnesium stearate and calcium stearate; stearic acid; vegetable oils such as peanut oil, cottonseed oil, sesame oil, olive oil and corn oil; non-ionic, cationic and anionic surfactants; ethylene glycol polymers; betacyclodextrin; fatty alcohols; and hydrolyzed cereal solids, as well as other non-toxic compatible fillers, binders, disintegrants, buffers, preservatives, antioxidants, lubricants, flavoring agents, and the like commonly used in pharmaceutical formulations.

Since the present invention relates to treatment of hypertension and congestive heart failure with combinations of active ingredients wherein said active ingredients can be administered separately, the invention also relates to combining separate pharmaceutical compositions in kit form. That is, two kits are contemplated, each combining two separate units: an NEP inhibitor pharmaceutical composition and an A II antagonist pharmaceutical composition in one kit, and an NEP inhibitor pharmaceutical composition and a renin inhibitor pharmaceutical composition in a second kit. The kit form is particularly advantageous when the separate components must be administered in different dosage forms (e.g. oral NEP formulation and parenteral A II antagonist formulation) or are administered at different dosage intervals.

## Claims

1. A pharmaceutical composition for treating hypertension or congestive heart failure comprising an effective amount of a combination of a neutral endopeptidase inhibitor and either a renin inhibitor or an angiotensin II antagonist, in a pharmaceutically acceptable carrier.

2. A composition of claim 1 wherein:

the neutral endopeptidase inhibitor is selected from the group consisting of N-[N-[1(S)-carboxyl-3-phenylpropyl]-(S)-phenylalanyl]-(S)-isoserine; N-[N-[(1S)-carboxy-2-phenylethyl]-(S)-phenylalanyl]-β-alanine; N-[2(S)-mercaptomethyl-3-(2-methylphenyl)propionyl]methionine; SQ 28603; UK 69578; thiorphan; retro-thiorphan; phosphoramidon; SQ 29072; and the pro-drugs thereof;

the angiotensin II antagonist is selected from the group consisting of saralasin; sar 1; ile 8 angiotensin II; Dup 753; EXP 6155; EXP 6803; and PD 123319; and

the renin inhibitor is selected from the group consisting of enalkrein; RO 42-5892; A 65317; CP 80794; ES 1005; ES 8891; SQ 34017; CGP 29287; CGP 38560; SR 43845; U-71038; A 62198; and A 64662.

3. A kit comprising in separate containers in a single package pharmaceutical compositions for use in combination to treat hypertension or congestive heart failure in mammals which comprises in one container a pharmaceutical composition comprising a neutral endopeptidase inhibitor, and in a second container a pharmaceutical composition comprising a renin inhibitor or an angiotensin II antagonist.

4. The use of a neutral endopeptidase (NMEP) inhibitor, in combination with either a renin inhibitor or an angiotensin II antagonist, for the preparation of a pharmaceutical composition useful in the treatment of hypertension or congestive heart failure.

5. The use according to claim 4, wherein:

the neutral endopeptidase inhibitor is selected from the group consisting of N-[N-[1(S)-carboxyl-3-phenylpropyl]-(S)-phenylalanyl]-(S)-isoserine; N-[N-[(1S)-carboxy-2-phenylethyl]-(S)-phenylalanyl]-β-alanine; N-[2(S)-mercaptomethyl-3-(2-methylphenyl)propionyl]methionine; SQ 28603; UK 69578; thiorphan; retro-thiorphan; phosphoramidon; SQ 29072; and the pro-drugs thereof;

the angiotensin II antagonist is selected from the group consisting of saralasin; sar 1; ile 8 angiotensin II; Dup 753; EXP 6155; EXP 6803; and PD 123319; and

the renin inhibitor is selected from the group consisting of enalkrein; RO 42-5892; A 65317; CP 80794; ES 1005; ES 8891; SQ 34017; CGP 29287; CGP 38560; SR 43845; U-71038; A 62198; and A 64662.

- 5 6. A composition according to any of claims 1 or 2, wherein:  
the NMEP inhibitor is administered at a dosage level of 0.3 mg/kg mammalian weight per day;  
the angiotensin II antagonist is administered at a dosage level of 0.1 mg/kg to 50 mg/kg  
mammalian weight per day; and  
the renin inhibitor is administered at a dosage level of 0.1 mg/kg to 100 mg/kg mammalian weight  
10 per day.
7. A process for the preparation of a pharmaceutical composition according to any of claims 1, 2 or 6,  
which comprises mixing a NMEP inhibitor, in combination with either a renin inhibitor or an angiotensin  
II antagonist, with a pharmaceutically acceptable carrier.
- 15 8. A method of treating hypertension or congestive heart failure comprising administering an effective  
amount of a combination of a neutral endopeptidase inhibitor and either a renin inhibitor or an  
angiotensin II antagonist to a mammal in need of such treatment.
- 20 9. A method of claim 8 wherein:  
the neutral endopeptidase inhibitor is selected from the group consisting of N-[N-[1(S)-carboxyl-3-  
phenylpropyl]-(S)-phenylalanyl]-(S)-isoserine; N-[N-[[(1S)-carboxy-2-phenyl)ethyl](S)-phenylalanyl]-β-  
alanine; N-[2(S)-mercaptomethyl-3-(2-methylphenyl)propionyl]methionine; SQ 28603; UK 69578; thior-  
phan; retro-thiorphan; phosphoramidon; SQ 29072; and pro-drugs thereof;  
25 the angiotensin II antagonist is selected from the group consisting of saralasin; sar 1; ile 8  
angiotensin II; Dup 753; EXP 6155; EXP 6803; and PD 123319; and  
the renin inhibitor is selected from the group consisting of enalkrein; RO 42-5892; A 65317; CP  
80794; ES 1005; ES 8891; SQ 34017; CGP 29287; CGP 38560; SR 43845; U-71038; A 62198; and A  
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(54) Title: COMBINATION OF ORGANIC COMPOUNDS

(57) Abstract: The present invention relates to a combination comprising: (a) an angiotensin II receptor blocker (ARB), or a pharmaceutically acceptable salt thereof; (b) a calcium channel blocker (CCB), or a pharmaceutically acceptable salt thereof; and (c) one of the two active agents selected from (i) a renin inhibitor, or a pharmaceutically acceptable salt thereof; and (ii) a neutral endopeptidase (NEP) inhibitor, or a pharmaceutically acceptable salt thereof; for the prevention of, delay the onset of and/or treatment of cardiovascular disorders, which method comprises administering to a warm-blooded animal, in need thereof, a therapeutically effective amount of a combination of the present invention.



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Combination of Organic Compounds

In one aspect, the present invention relates to a combination, such as a pharmaceutical combination, comprising:

- (a) an angiotensin II receptor blocker (ARB), or a pharmaceutically acceptable salt thereof;
- (b) a calcium channel blocker (CCB), or a pharmaceutically acceptable salt thereof; and
- (c) one of the two active agents selected from
  - (i) a renin inhibitor, or a pharmaceutically acceptable salt thereof; and
  - (ii) a neutral endopeptidase (NEP) inhibitor, or a pharmaceutically acceptable salt thereof.

In a further aspect, the present invention provides a method for the prevention of, delay the onset of and/or treatment of cardiovascular disorders which method comprises administering to a warm-blooded animal, including man, in need thereof, a therapeutically effective amount of a combination comprising:

- (a) an angiotensin II receptor blocker (ARB), or a pharmaceutically acceptable salt thereof;
- (b) a calcium channel blocker (CCB), or a pharmaceutically acceptable salt thereof; and
- (c) one of the two active agents selected from
  - (i) a renin inhibitor, or a pharmaceutically acceptable salt thereof; and
  - (ii) a neutral endopeptidase (NEP) inhibitor, or a pharmaceutically acceptable salt thereof.

Cardiovascular disorders include, but are not limited to, hypertension (whether for malignant, essential, reno-vascular, diabetic, isolated systolic, or other secondary type of hypertension), heart failure such as diastolic and congestive heart failure (acute and chronic), left ventricular dysfunction, endothelial dysfunction, diastolic dysfunction, hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias,

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atrial fibrillation (AF), cardiac fibrosis, atrial flutter, detrimental vascular remodeling, plaque stabilization, myocardial infarction (MI) and its sequelae, atherosclerosis including coronary arterial disease (CAD), angina pectoris (whether unstable or stable), renal insufficiency (diabetic and non-diabetic), renal fibrosis, polycystic kidney disease (PKD), type 2 diabetes, metabolic syndrome, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions such as nephrotic syndrome, diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, renal vascular hypertension, diabetic retinopathy and end-stage renal disease (ESRD), the management of other vascular disorders such as migraine, peripheral vascular disease (PVD), Raynaud's disease, luminal hyperplasia, cognitive dysfunction (such as Alzheimer's), glaucoma and cerebrovascular disease such as embolic or thrombotic stroke.

Prolonged and uncontrolled hypertensive vascular disease ultimately leads to a variety of pathological changes in target organs such as the heart and kidney. Furthermore, sustained hypertension may lead to an increased occurrence of stroke. Therefore, there has been a strong need to evaluate the efficacy of anti-hypertensive therapy by an examination of additional cardiovascular endpoints, beyond those of blood pressure lowering, to get further insight into the benefits of the treatment with anti-hypertensive agents.

The nature of hypertensive vascular diseases is multifactorial, and under certain circumstances, therapeutic agents with different mechanism of action have been combined. However, just considering any combination of drugs having different mode of action does not necessarily lead to drug combinations with advantageous effects. Accordingly, there is an urgent need to identify more efficacious therapies, in particular combination therapies, which have less deleterious side effects for the treatment of, e.g., cardiovascular and renal diseases as described herein above.

The natural enzyme renin released from the kidneys cleaves angiotensinogen in the circulation to form the decapeptide called angiotensin I. This in turn is cleaved by angiotensin converting enzyme (ACE) in the lungs, kidneys and other organs to form the octapeptide called angiotensin II. Through its interaction with specific receptors on the surface of the target cells the octapeptide increases blood pressure both directly by arterial vasoconstriction and indirectly by liberating from the adrenal glands the sodium-ion-retaining hormone aldosterone, accompanied by an increase in extracellular fluid volume. It has been possible to identify receptor subtypes that are termed, e.g., AT<sub>1</sub>- and AT<sub>2</sub>-receptors.

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Inhibitors of the enzymatic activity of renin bring about a reduction in the formation of angiotensin I. As a result a smaller amount of angiotensin II is produced. The reduced concentration of that active peptide hormone is the direct cause of, e.g., the antihypertensive effect of renin inhibitors. Accordingly, renin inhibitors, or salts thereof, may be employed, e.g., as antihypertensives or for treating congestive heart failure.

On the other hand, in recent times great efforts have been made to identify substances that antagonize the AT<sub>1</sub>-receptor. Such active ingredients are often called as angiotensin II antagonists or angiotensin II blockers (ARBs). As a result of the inhibition of the AT<sub>1</sub>-receptor activity such antagonists may also be employed, e.g., as antihypertensives or for the treatment of congestive heart failure, among other indications. Angiotensin II blockers are therefore understood to be those active agents which bind to the AT<sub>1</sub>-receptor subtype but do not result in activation of the receptor.

Further evaluations have revealed that renin inhibitors and angiotensin II blockers may also be employed for a much broader range of therapeutic indications.

Neutral endopeptidase (EC 3.4.24.11; enkephalinase; atriopeptidase; NEP; Biochem. J., 241, p. 237-247, 1987) is a zinc-containing metalloprotease that cleaves a variety of peptide substrates on the amino terminal side of aromatic amino acids. Substrates for this enzyme include, but are not limited to, atrial natriuretic factors (ANF, also known as ANP), brain natriuretic peptide (BNP), met and leu enkephalin, bradykinin, neurokinin A, and substance P.

ANPs are a family of vasodilator, diuretic and antihypertensive peptides which have been the subject of many recent reports in the literature, e.g., Annu. Rev. Pharm. Tox., 29, 23-54, 1989. One form, ANF 99-126, is a circulating peptide hormone which is released from the heart during conditions of cardiac distension. The function of ANF is to maintain salt and water homeostasis as well as to regulate blood pressure. ANF is rapidly inactivated in the circulation by at least two processes: by a receptor-mediated clearance as reported in Am. J. Physiol., 256, R469-R475, 1989, and by an enzymatic inactivation via NEP as described in Biochem. J., 243, 183-187, 1987. It has been previously demonstrated that inhibitors of NEP potentiate the hypotensive, diuretic, natriuretic and plasma ANF responses to pharmacological injection of ANF in experimental animals. The potentiation of ANF by two specific NEP inhibitors is reported by Sybertz et al. in J. Pharmacol. Exp. Ther. 250, 2, 624-631, 1989, and in Hypertension, 15, 2, 152-161, 1990, while the potentiation of ANF by NEP

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in general was disclosed in U.S. Patent No. 4,749,688. In U.S. Patent No. 4,740, 499 Olins disclosed the use of thiorphan and kelatorphan to potentiate atrial peptides. Moreover, NEP inhibitors lower blood pressure and exert ANF-like effects such as diuresis and increased cyclic guanosine 3',5'-monophosphate (cGMP) excretion in some forms of experimental hypertension. The antihypertensive action of NEP inhibitors is mediated through ANF because antibodies to ANF will neutralize the reduction in blood pressure.

Listed below are some of the definitions of various additional terms used herein to describe certain aspects of the present invention. However, the definitions used herein are those generally known in the art, e.g., hypertension, heart failure and atherosclerosis, and apply to the terms as they are used throughout the specification unless they are otherwise limited in specific instances.

The term "prevention" refers to prophylactic administration to healthy patients to prevent the development of the conditions mentioned herein. Moreover, the term "prevention" means prophylactic administration to patients being in a pre-stage of the conditions to be treated.

The term "delay the onset of", as used herein, refers to administration to patients being in a pre-stage of the condition to be treated in which patients with a pre-form of the corresponding condition is diagnosed.

The term "treatment" is understood the management and care of a patient for the purpose of combating the disease, condition or disorder.

The term "therapeutically effective amount" refers to an amount of a drug or a therapeutic agent that will elicit the desired biological or medical response of a tissue, system or an animal (including man) that is being sought by a researcher or clinician. Non-limiting examples of the desired effect include but are not limited to, at least partially inhibiting or inactivating the AT1 receptor, or the calcium channel blocker, or the rennin inhibitor, or the neutral endopeptidase; or controlling the blood pressure; or lowering the cholesterol level; or treating the cardiovascular or metabolic conditions or diseases, for example, those diseases or conditions described in this application.

The term "synergistic", as used herein, means that the effect achieved with the methods, combinations and pharmaceutical compositions of the present invention is greater than the sum of the effects that result from individual methods and compositions comprising the active ingredients of this invention separately.

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The term "warm-blooded animal or patient" are used interchangeably herein and include, but are not limited to, humans, dogs, cats, horses, pigs, cows, monkeys, rabbits, mice and laboratory animals. The preferred mammals are humans.

The term "pharmaceutically acceptable salt" refers to a non-toxic salt commonly used in the pharmaceutical industry which may be prepared according to methods well-known in the art.

The term "type 2 diabetes" including type 2 diabetes associated with hypertension refers to a disease in which the pancreas does not secrete sufficient insulin due to an impairment of pancreatic beta-cell function and/or in which there is to insensitivity to produced insulin (insulin resistance). Typically, the fasting plasma glucose is less than 126 mg/dL, while pre-diabetes is, e.g., a condition which is characterized by one of following conditions: impaired fasting glucose (110-125 mg/dL) and impaired glucose tolerance (fasting glucose levels less than 126 mg/dL and post-prandial glucose level between 140 mg/dL and 199 mg/dL). Type 2 diabetes mellitus can be associated with or without hypertension. Diabetes mellitus occurs frequently, e.g., in African American, Latino/Hispanic American, Native American, Native American, Asian American and Pacific Islanders. Markers of insulin resistance include HbA1C, HOMA IR, measuring collagen fragments, TGF- $\beta$  in urine, PAI-1 and prorenin.

The term "hypertension" refers to a condition where the pressure of blood within the blood vessels is higher than normal as it circulates through the body. When the systolic pressure exceeds 150 mmHg or the diastolic pressure exceeds 90 mmHg for a sustained period of time, damage is done to the body. For example, excessive systolic pressure can rupture blood vessels anywhere, and when it occurs within the brain, a stroke results. Hypertension may also cause thickening and narrowing of the blood vessels which ultimately could lead to atherosclerosis.

The term "severe hypertension" refers to hypertension characterized by a systolic blood pressure of  $\geq 180$  mmHg and a diastolic blood pressure of  $\geq 110$  mmHg.

The term "pulmonary hypertension" (PH) refers to a blood vessel disorder of the lung in which the pressure in the pulmonary artery rises above normal level of  $\leq 25/10$  (especially primary and secondary PH), e.g., because the small vessels that supply blood to the lungs constrict or tighten up. According to the WHO, PH may be divided into five categories: pulmonary arterial hypertension (PAH), a PH occurring in the absence of a known cause is referred to as primary pulmonary hypertension, while secondary PH is caused by a condition

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selected, e.g., from emphysema; bronchitis; collagen vascular diseases, such as scleroderma, Crest syndrome or systemic lupus erythematosus (SLE); PH associated with disorders of the respiratory system; PH due to chronic thrombotic or embolic disease; PH due to disorders directly affecting the pulmonary blood vessels; and pulmonary venous hypertension (PVH).

The term "malignant hypertension" is usually defined as very high blood pressure with swelling of the optic nerve behind the eye, called papilledema (grade IV Keith-Wagner hypertensive retinopathy). This also includes malignant HTN of childhood.

The term "isolated systolic hypertension" refers to hypertension characterized by a systolic blood pressure of  $\geq 140$  mmHg and a diastolic blood pressure of  $< 90$  mmHg.

The term "familial dyslipidemic hypertension" is characterized by mixed dyslipidemic disorders. Biomarkers include oxidized LDL, HDL, glutathione and homocysteine LPA.

The term "renovascular hypertension" (renal artery stenosis) refers to a condition where the narrowing of the renal artery is significant which leads to an increase of the blood pressure resulting from signals sent out by the kidneys. Biomarkers include renin, PRA and prorenin.

The term "endothelial dysfunction" with or without hypertension refers to a condition in which normal dilation of blood vessels is impaired due to lack of endothelium-derived vasodilators. Biomarkers include CRP, IL6, ET1, BIG-ET1, VCAM and ICAM. Survival post-MI biomarkers include BNP and procollagen factors.

The term "diastolic dysfunction" refers to abnormal mechanical properties of the heart muscle (myocardium) and includes abnormal left ventricle (LV) diastolic distensibility, impaired filling, and slow or delayed relaxation regardless of whether the ejection fraction is normal or depressed and whether the patient is asymptomatic or symptomatic. Asymptomatic diastolic dysfunction is used to refer to an asymptomatic patient with a normal ejection fraction and an abnormal echo-Doppler pattern of LV filling which is often seen, for example, in patients with hypertensive heart disease. Thus, an asymptomatic patient with hypertensive left ventricular hypertrophy and an echocardiogram showing a normal ejection fraction and abnormal left ventricular filling can be said to have diastolic dysfunction. If such a patient were to exhibit symptoms of effort intolerance and dyspnea, especially if there were evidence of venous congestion and pulmonary edema, it would be more appropriate to use the term diastolic heart failure. This terminology parallels that used in asymptomatic and

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symptomatic patients with LV systolic dysfunction, and it facilitates the use of a pathophysiologic, diagnostic, and therapeutic framework that includes all patients with LV dysfunction whether or not they have symptoms (William H. Gaasch and Michael R. Zile, *Annu. Rev. Med.* 55: 373–94, 2004; Gerard P. Aurigemma, William H. Gaasch, *N. Engl. J. Med.* 351:1097-105, 2004).

The term "cardiac fibrosis" is defined as abnormally high accumulation of collagen and other extracellular matrix proteins due to the enhanced production or decreased degradation of these proteins. Biomarkers include BNP, procollagen factors, LVH, AGE RAGE and CAGE.

The term "peripheral vascular disease" (PVD) refers to the damage or dysfunction of peripheral blood vessels. There are two types of peripheral vascular diseases: peripheral arterial disease (PAD) which refers to diseased peripheral arteries and peripheral venous disorders, which can be measured by an ankle brachial index. PAD is a condition that progressively hardens and narrows arteries due to a gradual buildup of plaque and refers to conditions that effect the blood vessels, such as arteries, veins and capillaries, of the body outside the heart. This is also known as peripheral venous disorder.

The term "atherosclerosis" comes from the Greek words athero (meaning gruel or paste) and sclerosis (hardness). It's the name of the process in which deposits of fatty substances, cholesterol, cellular waste products, calcium and other substances build up in the inner lining of an artery. This buildup is called plaque. It usually affects large and medium-sized arteries. Some hardening of arteries often occurs when people grow older. Plaques can grow large enough to significantly reduce the blood's flow through an artery. But most of the damage occurs when they become fragile and rupture. Plaques that rupture cause blood clots to form that can block blood flow or break off and travel to another part of the body. If either happens and blocks a blood vessel that feeds the heart, it causes a heart attack. If it blocks a blood vessel that feeds the brain, it causes a stroke. And if blood supply to the arms or legs is reduced, it can cause difficulty walking and eventually gangrene.

The term "coronary arterial disease" (CAD) also refers to a condition that progressively hardens and narrows arteries due to a gradual buildup of plaque and refers to conditions that effect the blood vessels such as arteries within the heart. CAD is peculiar form of atherosclerosis that occurs in the three small arteries supplying the heart muscle with oxygen-rich blood. Biomarkers include CPK and Troponin.

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The term "cerebrovascular diseases" comprise stroke conditions, such as embolic and thrombotic stroke; large vessel thrombosis and small vessel disease; and hemorrhagic stroke.

The term "embolic stroke" refers to a condition characterized by the formation of blood clots, e.g., in the heart, when clots travel down through the bloodstream in the brain. This may lead to a blockade of small blood vessels and causing a stroke.

The term "thrombotic stroke" refers to a condition where the blood flow is impaired because of a blockade to one or more of the arteries supplying blood to the brain. This process normally leads to thrombosis causing thrombotic strokes. Biomarkers include PAI 1, TPA and platelet function.

The term "metabolic syndrome" (Syndrome X) refers to an overall condition characterized by three or more of the following criteria:

1. abdominal obesity: waist circumference > 102 cm in men, and > 88 cm in women;
2. hypertriglyceridemia: > 150 mg/dL (1.695 mmol/L);
3. low HDL cholesterol: < 40 mg/dL (1.036 mmol/L) in men, and < 50 mg/dL (1.295 mmol/L) in women;
4. high blood pressure: > 130/85 mmHg; and
5. high-fasting glucose: > 110 mg/dL (> 6.1 mmol/L).

Metabolic syndrome may also be characterized by three or more of the following criteria: triglycerides > 150 mg/dL, systolic blood pressure (BP)  $\geq$  130 mmHg or diastolic BP  $\geq$  85 mmHg, or on anti-hypertensive treatment, high-density lipoprotein cholesterol < 40 mg/dL, fasting blood sugar (FBS) > 110 mg/dL, and a body mass index (BMI) > 28.8 kg/m<sup>2</sup>.

Metabolic syndrome may also be characterized by diabetes, impaired glucose tolerance, impaired fasting glucose, or insulin resistance plus two or more of the following abnormalities:

1. high blood pressure:  $\geq$  160/90 mmHg;
2. hyperlipidemia: triglyceride concentration  $\geq$  150 mg/dL (1.695 mmol/L) and/or HDL cholesterol < 35 mg/dL (0.9 mmol/L) in men, and < 39 mg/dL (1.0 mmol/L) in women;
3. central obesity: waist-to-hip ratio of > 0.90 in men, and > 0.85 in women and/or BMI > 30 kg/m<sup>2</sup>; and

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4. microalbuminuria: urinary albumin excretion rate  $\geq 20 \mu\text{g}/\text{min}$  or an albumin-to-creatinine ratio  $\geq 20 \text{ mg}/\text{g}$ . Biomarkers include proteinuria, TGF- $\beta$ , TNF- $\alpha$  and adiponectin.

Biomarkers include LDL, HDL and all the endothelial dysfunction markers.

The term "atrial fibrillation" (AF) refers to a type of irregular or racing heartbeat that may cause blood to collect in the heart and potentially form a clot which may travel to the brain and can cause a stroke.

The term "renal failure", e.g., chronic renal failure; is characterized, e.g., by proteinuria and/or slight elevation of plasma creatinine concentration (106-177 mmol/L corresponding to 1.2-2.0 mg/dL).

The term "glomerulonephritis" refers to a condition which may be associated with the nephrotic syndrome, a high blood pressure and a decreased renal function, focal, segmental glomerulonephritis, minimal change nephropathy, Lupus nephritis, post-streptococcal GN and IgA nephropathy.

The term "nephrotic syndrome" refers to a compilation of conditions including massive proteinuria, edema and central nervous system (CNS) irregularities. Biomarkers include urinary protein excretion.

The term "plaque stabilization" means rendering a plaque less dangerous by preventing, fibrous cap thinning/rupture, smooth muscle cell loss and inflammatory cell accumulation.

The term "renal fibrosis" refers to an abnormal accumulation of collagen and other extracellular matrix proteins, leading to loss of renal function. Biomarkers include collagen fragments and TGF- $\beta$  in urine.

The term "end-stage renal disease" (ESRD) refers to loss of renal function to the extent that dialysis or renal replacement is needed. Biomarkers include glomerular filtration rate and creatinine clearance.

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The term "polycystic kidney disease" (PKD) refers to a genetic disorder characterized by the growth of numerous cysts in the kidney. PKD cysts can slowly reduce much of the mass of kidneys reducing kidney function and leading to kidney failure. PKD may be classified as two major inherited forms of PKD which are autosomal dominant PKD and autosomal recessive PKD, while the non-inherited PKD may be called acquired cystic kidney disease. Biomarkers include reduction of renal cysts by non-invasive imaging.

The term "combination" of an angiotensin II receptor blocker (ARB), or a pharmaceutically acceptable salt thereof; a calcium channel blocker (CCB), or a pharmaceutically acceptable salt thereof; and one of the two active agents selected from a renin inhibitor, or a pharmaceutically acceptable salt thereof; and a neutral endopeptidase (NEP) inhibitor, or a pharmaceutically acceptable salt thereof; means that the components can be administered together as a pharmaceutical composition or as part of the same, unitary dosage form. A combination also includes administering an angiotensin II receptor blocker (ARB), or a pharmaceutically acceptable salt thereof; a calcium channel blocker (CCB), or a pharmaceutically acceptable salt thereof; and one of the two active agents selected from a renin inhibitor, or a pharmaceutically acceptable salt thereof; and a neutral endopeptidase (NEP) inhibitor, or a pharmaceutically acceptable salt thereof; each separately but as part of the same therapeutic regimen. The components, if administered separately, need not necessarily be administered at essentially the same time, although they can if so desired. Thus, a combination also refers, for example, administering an angiotensin II receptor blocker (ARB), or a pharmaceutically acceptable salt thereof; a calcium channel blocker (CCB), or a pharmaceutically acceptable salt thereof; and one of the two active agents selected from a renin inhibitor, or a pharmaceutically acceptable salt thereof; and a neutral endopeptidase (NEP) inhibitor, or a pharmaceutically acceptable salt thereof; as separate dosages or dosage forms, but at the same time. A combination also includes separate administration at different times and in any order.

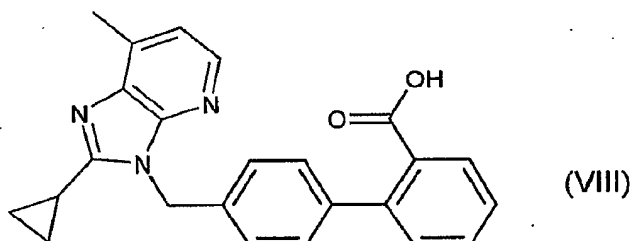
The renin inhibitors to which the present invention applies are any of those having renin inhibitory activity *in vivo* and, therefore, pharmaceutical utility, e.g., as therapeutic agents for the prevention of, delay the onset of and/or treatment of hypertension (whether for malignant, essential, reno-vascular, diabetic, isolated systolic, or other secondary type of hypertension), heart failure such as diastolic and congestive heart failure (acute and chronic), left ventricular dysfunction, endothelial dysfunction, diastolic dysfunction, hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular

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arrhythmias, atrial fibrillation (AF), cardiac fibrosis, atrial flutter, detrimental vascular remodeling, plaque stabilization, myocardial infarction (MI) and its sequelae, atherosclerosis including coronary arterial disease (CAD), angina pectoris (whether unstable or stable), renal insufficiency (diabetic and non-diabetic), renal fibrosis, polycystic kidney disease (PKD), type 2 diabetes, metabolic syndrome, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions such as nephrotic syndrome, diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, renal vascular hypertension, diabetic retinopathy and end-stage renal disease (ESRD), the management of other vascular disorders such as migraine, peripheral vascular disease (PVD), Raynaud's disease, luminal hyperplasia, cognitive dysfunction (such as Alzheimer's), glaucoma and cerebrovascular disease such as embolic or thrombotic stroke.

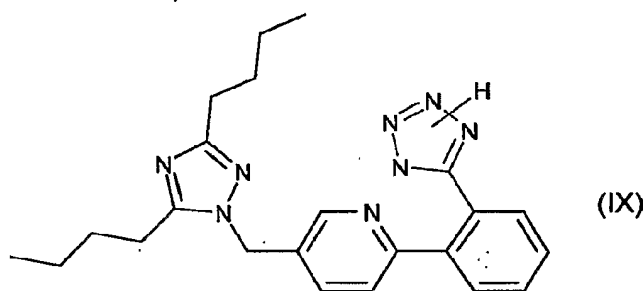
In particular, the present invention relates to renin inhibitors disclosed in U.S. Patents No. 5,559,111; No. 6,197,959 and No. 6,376,672, the entire contents of which are incorporated herein by reference.

Suitable angiotensin II receptor blockers which may be employed in the combination of the present invention include AT<sub>1</sub>-receptor antagonists having differing structural features, preferred are those with the non-peptidic structures. For example, mention may be made of the compounds that are selected from the group consisting of valsartan (EP 443983), losartan (EP 253310), candesartan (EP 459136), eprosartan (EP 403159), irbesartan (EP 454511), olmesartan (EP 503785), tasosartan (EP 539086), telmisartan (EP 522314), the compound with the designation E-4177 of the formula

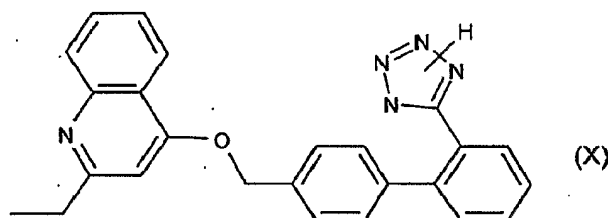


the compound with the designation SC-52458 of the following formula

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and the compound with the designation the compound ZD-8731 of the formula



or, in each case, a pharmaceutically acceptable salt thereof.

Preferred AT<sub>1</sub>-receptor antagonists are those agents that have reach the market, most preferred is valsartan, or a pharmaceutically acceptable salt thereof.

Suitable Calcium channel which may be employed in the combination of the present invention include the following. The class of CCBs essentially comprises dihydropyridines (DHPs) and non-DHPs such as diltiazem-type and verapamil-type CCBs.

A CCB useful in the combination of the present invention is preferably a DHP representative selected from the group consisting of amlodipine, felodipine, ryosidine, isradipine, lacidipine, nicardipine, nifedipine, niguldipine, niludipine, nimodipine, nisoldipine, nitrendipine, and nivaldipine, and is preferably a non-DHP representative selected from the group consisting of flunarizine, prenylamine, diltiazem, fendiline, gallopamil, mibefradil, anipamil, tiapamil and verapamil, and in each case, a pharmaceutically acceptable salt thereof. All these CCBs are therapeutically used, e.g. as anti-hypertensive, anti-angina pectoris or anti-arrhythmic drugs. Preferred CCBs comprise amlodipine, diltiazem, isradipine, nicardipine, nifedipine, nimodipine, nisoldipine, nitrendipine, and verapamil, or, e.g. dependent on the specific CCB, a pharmaceutically acceptable salt thereof. Especially preferred as DHP is amlodipine or a pharmaceutically acceptable salt, especially the besylate, thereof. An especially preferred

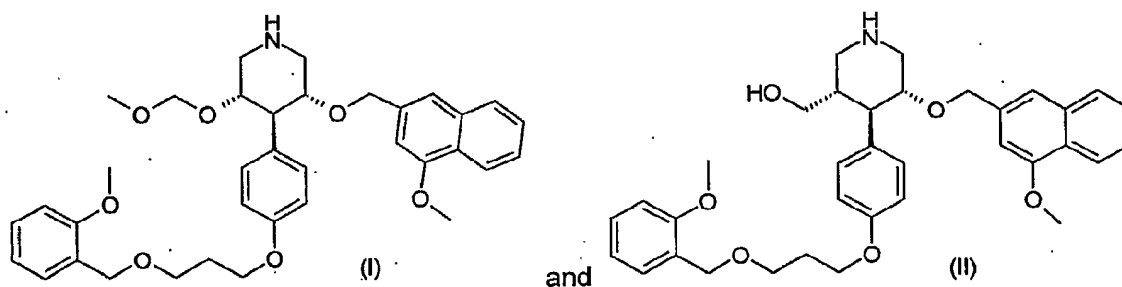
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representative of non-DHPs is verapamil or a pharmaceutically acceptable salt, especially the hydrochloride, thereof.

The most preferred CCB is amlodipine besylate.

Suitable renin inhibitors useful in the combination of the present invention include compounds having different structural features. For example, mention may be made of compounds which are selected from the group consisting of ditekiren (chemical name: [1S-[1R\*,2R\*,4R\*(1R\*,2R\*)]]-1-[(1,1-dimethylethoxy)carbonyl]-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-N- $\alpha$ -methyl-L-histidinamide); terlakiren (chemical name: [R-(R\*,S\*)]-N-(4-morpholinylcarbonyl)-L-phenylalanyl-N-[1-(cyclohexylmethyl)-2-hydroxy-3-(1-methylethoxy)-3-oxopropyl]-S-methyl-L-cysteineamide); and zankiren (chemical name: [1S-[1R\*[R\*(R\*)],2S\*,3R\*]]-N-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]- $\alpha$ -[[2-[[[4-methyl-1-piperazinyl)sulfonyl]methyl]-1-oxo-3-phenylpropyl]-amino]-4-thiazolepropanamide), preferably, in each case, the hydrochloride salt thereof.

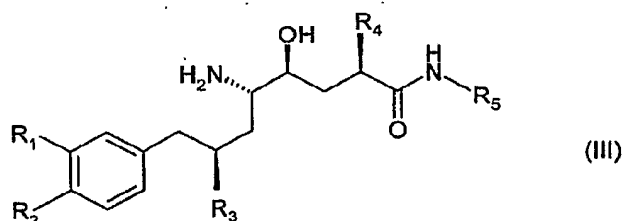
Preferred renin inhibitor of the present invention include RO 66-1132 and RO 66-1168 of formulae (I) and (II)



respectively, or a pharmaceutically acceptable salt thereof.

In particular, the present invention relates to a renin inhibitor which is a  $\delta$ -amino- $\gamma$ -hydroxy- $\omega$ -aryl-alkanoic acid amide derivative of the formula

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wherein  $R_1$  is halogen,  $C_{1-6}$ halogenalkyl,  $C_{1-6}$ alkoxy- $C_{1-6}$ alkyloxy or  $C_{1-6}$ alkoxy- $C_{1-6}$ alkyl;  $R_2$  is halogen,  $C_{1-4}$ alkyl or  $C_{1-4}$ alkoxy;  $R_3$  and  $R_4$  are independently branched  $C_{3-6}$ alkyl; and  $R_5$  is cycloalkyl,  $C_{1-6}$ alkyl,  $C_{1-6}$ hydroxyalkyl,  $C_{1-6}$ alkoxy- $C_{1-6}$ alkyl,  $C_{1-6}$ alkanoyloxy- $C_{1-6}$ alkyl,  $C_{1-6}$ aminoalkyl,  $C_{1-6}$ alkylamino- $C_{1-6}$ alkyl,  $C_{1-6}$ dialkylamino- $C_{1-6}$ alkyl,  $C_{1-6}$ alkanoylamino- $C_{1-6}$ alkyl,  $HO(O)C-C_{1-6}$ alkyl,  $C_{1-6}$ alkyl- $O-(O)C-C_{1-6}$ alkyl,  $H_2N-C(O)-C_{1-6}$ alkyl,  $C_{1-6}$ alkyl-HN- $C(O)-C_{1-6}$ alkyl or  $(C_{1-6}alkyl)_2N-C(O)-C_{1-6}alkyl$ ; or a pharmaceutically acceptable salt thereof.

As an alkyl,  $R_1$  may be linear or branched and preferably comprise 1 to 6 C atoms, especially 1 or 4 C atoms. Examples are methyl, ethyl, n- and i-propyl, n-, i- and t-butyl, pentyl and hexyl.

As a halogenalkyl,  $R_1$  may be linear or branched and preferably comprise 1 to 4 C atoms, especially 1 or 2 C atoms. Examples are fluoromethyl, difluoromethyl, trifluoromethyl, chloromethyl, dichloromethyl, trichloromethyl, 2-chloroethyl and 2,2,2-trifluoroethyl.

As an alkoxy,  $R_1$  and  $R_2$  may be linear or branched and preferably comprise 1 to 4 C atoms. Examples are methoxy, ethoxy, n- and i-propyloxy, n-, i- and t-butyloxy, pentyloxy and hexyloxy.

As an alkoxyalkyl,  $R_1$  may be linear or branched. The alkoxy group preferably comprises 1 to 4 and especially 1 or 2 C atoms, and the alkyl group preferably comprises 1 to 4 C atoms. Examples are methoxymethyl, 2-methoxyethyl, 3-methoxypropyl, 4-methoxybutyl, 5-methoxypentyl, 6-methoxyhexyl, ethoxymethyl, 2-ethoxyethyl, 3-ethoxypropyl, 4-ethoxybutyl, 5-ethoxypentyl, 6-ethoxyhexyl, propyloxymethyl, butyloxymethyl, 2-propyloxyethyl and 2-butyloxyethyl.

As a  $C_{1-6}$ alkoxy- $C_{1-6}$ alkyloxy,  $R_1$  may be linear or branched. The alkoxy group preferably comprises 1 to 4 and especially 1 or 2 C atoms, and the alkyloxy group preferably comprises 1 to 4 C atoms. Examples are methoxymethyloxy, 2-methoxyethyloxy, 3-methoxypropyloxy, 4-methoxybutyloxy, 5-methoxypentyloxy, 6-methoxyhexyloxy, ethoxymethyloxy, 2-

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ethoxyethyloxy, 3-ethoxypropyloxy, 4-ethoxybutyloxy, 5-ethoxypentyloxy, 6-ethoxyhexyloxy, propyloxymethyloxy, butyloxymethyloxy, 2-propyloxyethyloxy and 2-butyloxyethyloxy.

In a preferred embodiment,  $R_1$  is methoxy- or ethoxy- $C_{1-4}$ alkyloxy, and  $R_2$  is preferably methoxy or ethoxy. Particularly preferred are compounds of formula (III), wherein  $R_1$  is 3-methoxypropyloxy and  $R_2$  is methoxy.

As a branched alkyl,  $R_3$  and  $R_4$  preferably comprise 3 to 6 C atoms. Examples are i-propyl, i- and t-butyl, and branched isomers of pentyl and hexyl. In a preferred embodiment,  $R_3$  and  $R_4$  in compounds of formula (III) are in each case i-propyl.

As a cycloalkyl,  $R_5$  may preferably comprise 3 to 8 ring-carbon atoms, 3 or 5 being especially preferred. Some examples are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cyclooctyl. The cycloalkyl may optionally be substituted by one or more substituents, such as alkyl, halo, oxo, hydroxy, alkoxy, amino, alkylamino, dialkylamino, thiol, alkylthio, nitro, cyano, heterocyclyl and the like.

As an alkyl,  $R_5$  may be linear or branched in the form of alkyl and preferably comprise 1 to 6 C atoms. Examples of alkyl are listed herein above. Methyl, ethyl, n- and i-propyl, n-, i- and t-butyl are preferred.

As a  $C_{1-6}$ hydroxyalkyl,  $R_5$  may be linear or branched and preferably comprise 2 to 6 C atoms. Some examples are 2-hydroxyethyl, 2-hydroxypropyl, 3-hydroxypropyl, 2-, 3- or 4-hydroxybutyl, hydroxypentyl and hydroxyhexyl.

As a  $C_{1-6}$ alkoxy- $C_{1-6}$ alkyl,  $R_5$  may be linear or branched. The alkoxy group preferably comprises 1 to 4 C atoms and the alkyl group preferably 2 to 4 C atoms. Some examples are 2-methoxyethyl, 2-methoxypropyl, 3-methoxypropyl, 2-, 3- or 4-methoxybutyl, 2-ethoxyethyl, 2-ethoxypropyl, 3-ethoxypropyl, and 2-, 3- or 4-ethoxybutyl.

As a  $C_{1-6}$ alkanoyloxy- $C_{1-6}$ alkyl,  $R_5$  may be linear or branched. The alkanoyloxy group preferably comprises 1 to 4 C atoms and the alkyl group preferably 2 to 4 C atoms. Some examples are formyloxymethyl, formyloxyethyl, acetyloxyethyl, propionyloxyethyl and butyroyloxyethyl.

As a  $C_{1-6}$ aminoalkyl,  $R_5$  may be linear or branched and preferably comprise 2 to 4 C atoms. Some examples are 2-aminoethyl, 2- or 3-aminopropyl and 2-, 3- or 4-aminobutyl.

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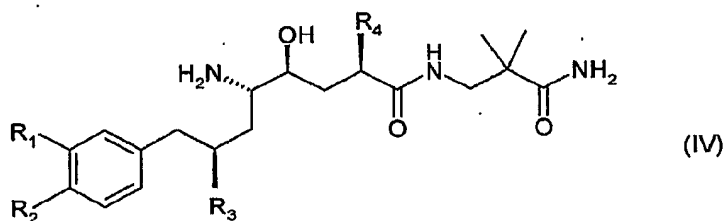
As  $C_{1-6}$ alkylamino- $C_{1-6}$ alkyl and  $C_{1-6}$ dialkylamino- $C_{1-6}$ alkyl,  $R_5$  may be linear or branched. The alkylamino group preferably comprises  $C_{1-4}$ alkyl groups and the alkyl group has preferably 2 to 4 C atoms. Some examples are 2-methylaminoethyl, 2-dimethylaminoethyl, 2-ethylaminoethyl, 2-ethylaminoethyl, 3-methylaminopropyl, 3-dimethylaminopropyl, 4-methylaminobutyl and 4-dimethylaminobutyl.

As a  $HO(O)C-C_{1-6}$ alkyl,  $R_5$  may be linear or branched and the alkyl group preferably comprises 2 to 4 C atoms. Some examples are carboxymethyl, carboxyethyl, carboxypropyl and carboxybutyl.

As a  $C_{1-6}$ alkyl-O-(O) $C-C_{1-6}$ alkyl,  $R_5$  may be linear or branched, and the alkyl groups preferably comprise independently of one another 1 to 4 C atoms. Some examples are methoxycarbonylmethyl, 2-methoxycarbonylethyl, 3-methoxycarbonylpropyl, 4-methoxycarbonylbutyl, ethoxycarbonylmethyl, 2-ethoxycarbonylethyl, 3-ethoxycarbonylpropyl, and 4-ethoxycarbonylbutyl.

As a  $H_2N-C(O)-C_{1-6}$ alkyl,  $R_5$  may be linear or branched, and the alkyl group preferably comprises 2 to 6 C atoms. Some examples are carbamidomethyl, 2-carbamidoethyl, 2-carbamido-2,2-dimethylethyl, 2- or 3-carbamidopropyl, 2-, 3- or 4-carbamidobutyl, 3-carbamido-2-methylpropyl, 3-carbamido-1,2-dimethylpropyl, 3-carbamido-3-ethylpropyl, 3-carbamido-2,2-dimethylpropyl, 2-, 3-, 4- or 5-carbamidopentyl, 4-carbamido-3,3- or -2,2-dimethylbutyl. Preferably,  $R_5$  is 2-carbamido-2,2-dimethylethyl.

Accordingly, preferred are  $\delta$ -amino- $\gamma$ -hydroxy- $\omega$ -aryl-alkanoic acid amide derivatives of formula (III) having the formula

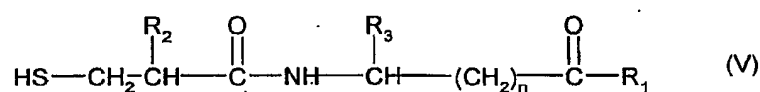


wherein  $R_1$  is 3-methoxypropyloxy;  $R_2$  is methoxy; and  $R_3$  and  $R_4$  are isopropyl; or a pharmaceutically acceptable salt thereof; chemically defined as 2(S),4(S),5(S),7(S)-N-(3-amino-2,2-dimethyl-3-oxopropyl)-2,7-di(1-methylethyl)-4-hydroxy-5-amino-8-[4-methoxy-3-(3-methoxy-propoxy)phenyl]-octanamide, also known as aliskiren.

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The term "aliskiren", if not defined specifically, is to be understood both as the free base and as a salt thereof, especially a pharmaceutically acceptable salt thereof, most preferably a hemi-fumarate salt thereof.

A suitable NEP inhibitor which may be employed in the combination of the present invention is, e.g., a compound of the formula



wherein

R<sub>2</sub> is C<sub>1</sub>-C<sub>7</sub> alkyl, trifluoromethyl, optionally substituted phenyl or -(CH<sub>2</sub>)<sub>1-4</sub>-(optionally substituted phenyl);

R<sub>3</sub> is hydrogen, C<sub>1</sub>-C<sub>7</sub>alkyl, optionally substituted phenyl, -(CH<sub>2</sub>)<sub>1-4</sub>-(optionally substituted phenyl);

R<sub>1</sub> is hydroxy, C<sub>1</sub>-C<sub>7</sub> alkoxy or NH<sub>2</sub>;

n is an integer from 1 to 15;

or pharmaceutically acceptable salt thereof.

The term "optionally substituted phenyl" refers to a phenyl group which may optionally be substituted with s substituent selected from C<sub>1</sub>-C<sub>4</sub> alkyl, C<sub>1</sub>-C<sub>4</sub> alkoxy, C<sub>1</sub>-C<sub>4</sub> alkylthio, hydroxy, Cl, Br, or F.

Preferred selective NEP inhibitors of formula (V) include compounds wherein:

R<sub>2</sub> is benzyl;

R<sub>3</sub> is hydrogen;

n is an integer from 1 to 9;

R<sub>1</sub> is hydroxy;

or pharmaceutically acceptable salt thereof.

Further preferred is a selective NEP inhibitor of formula (V) which is reported in the literature as SQ 28,603 wherein:

R<sub>2</sub> is benzyl;

R<sub>3</sub> is hydrogen;

n is one; and

R<sub>1</sub> is hydroxy.

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The preparation of selective NEP inhibitors of formula (V) wherein R<sub>2</sub> is other than trifluoromethyl is disclosed by Delaney et al. in U.S. Patent No. 4,722,810. The preparation of selective NEP inhibitors of formula (VI) wherein R<sub>2</sub> is trifluoromethyl is disclosed by Delaney et al. in U.S. Patent No. 5,223,516.

Further NEP inhibitors within the scope of the present invention include compounds disclosed in U.S. Patent No. 4,610,816, herein incorporated by reference, including in particular N-[N-[1(S)-carboxyl-3-phenylpropyl]-(S)-phenylalanyl]-(S)-isoserine and N-[N-[[[(1S)-carboxy-2-phenyl]ethyl]-(S)-phenylalanyl]-β-alanine; compounds disclosed in U.S. Patent No. 4,929,641, in particular N-[2(S)-mercaptomethyl-3-(2-methylphenyl)-propionyl]-methionine; SQ 28,603 (N-[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]-β-alanine), disclosed in South African Patent Application 84/0670; UK 69578 (cis-4-[[[1-[2-carboxy-3-(2-methoxyethoxy)-propyl]-cyclopentyl]carbonyl]amino]-cyclohexanecarboxylic acid) and its active enantiomer(s); thiorphan and its enantiomers; retro-thiorphan; phosphoramidon; and SQ 29,072 (7-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]-heptanoic acid). Also suitable for use are any pro-drug forms of the above-listed NEP inhibitors, e.g., compounds in which one or more carboxylic acid groups are esterified.

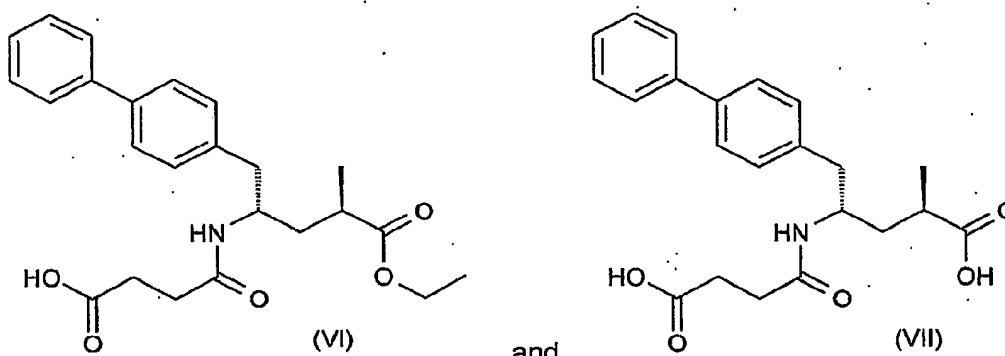
NEP inhibitors within the scope of the present invention also include the compounds disclosed in U.S. Patent No. 5,217,996, particularly, N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid ethyl ester and N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid, or in each case, a pharmaceutically acceptable salt thereof; the compounds disclosed in EP 00342850, particularly (S)-cis-4-[1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido]-1-cyclohexanecarboxylic acid; the compounds disclosed in GB 02218983, particularly 3-(1-[6-endo-hydroxymethylbicyclo[2,2,1]heptane-2-exo-carbamoyl]-cyclopentyl)-2-(2-methoxyethyl)propanoic acid; the compounds disclosed in WO 92/14706, particularly N-(1-(3-(N-t-butoxycarbonyl-(S)-prolylamino)-2(S)-t-butoxy-carbonylpropyl)-cyclopentanecarbonyl)-O-benzyl-(S)-serine methyl ester; the compounds disclosed in EP 00343911; the compounds disclosed in JP 06234754; the compounds disclosed in EP 00361365, particularly 4-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid; the compounds disclosed in WO 90/09374, particularly 3-[1-(cis-4-carboxycarbonyl-cis-3-butylcyclohexyl-r-1-carboamoyl)cyclopentyl]-2S-(2-methoxyethoxymethyl)propanoic acid; the compounds disclosed in JP 07157459, particularly N-((2S)-2-(4-biphenylmethyl)-4-carboxy-5-phenoxyvaleryl)glycine; the compounds disclosed in WO 94/15908 particularly N-(1-(N-

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hydroxycarbamoylmethyl)-1-cyclopentanecarbonyl)-L-phenylalanine; the compounds disclosed in U.S. Patent No. 5,273,990 particularly (S)-(2-biphenyl-4-yl)-1-(1H-tetrazol-5-yl)ethylamino) methylphosphonic acid; the compounds disclosed in U.S. Patent No. 5,294,632 particularly (S)-5-(N-(2-(phosphonomethylamino)-3-(4-biphenyl)propionyl)-2-aminoethyl)tetrazole; the compounds disclosed in U.S. Patent No. 5,250,522, particularly  $\beta$ -alanine, 3-[1,1'-biphenyl]-4-yl-N-[diphenoxyposphinyl)methyl]-L-alanyl; the compounds disclosed in EP 00636621, particularly N-(2-carboxy-4-thienyl)-3-mercapto-2-benzylpropanamide; the compounds disclosed in WO 93/09101, particularly 2-(2-mercaptomethyl-3-phenylpropionamido)thiazol-4-ylcarboxylic acid; the compounds disclosed in EP 00590442 particularly ((L)-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy)carbonyl)-2-phenylethyl)-L-phenylalanyl)- $\beta$ -alanine, N-[N-[(L)-[1-[(2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy]carbonyl]-2-phenylethyl]-L-phenylalanyl]-(R)-alanine, N-[N-[(L)-1-carboxy-2-phenylethyl]-L-phenylalanyl]-(R)-alanine, N-[2-acetylthiomethyl-3-(2-methyl-phenyl)-propionyl]-methionine ethyl ester, N-[2-mercaptomethyl-3-(2-methylphenyl)propionyl]-methionine, N-[2(S)-mercaptomethyl-3-(2-methylphenyl)propanoyl]-(S)-isoserine, N-(S)-[3-mercapto-2-(2-methylphenyl)propionyl]-(S)-2-methoxy-(R)-alanine, N-[1-[[1(S)-benzyloxycarbonyl-3-phenylpropyl]amino]cyclopentylcarbonyl]-(S)-isoserine, N-[1-[[1(S)-carbonyl-3-phenylpropyl]amino]-cyclopentylcarbonyl]-(S)-isoserine, 1,1'-[dithiobis-[2(S)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]-bis-(S)-isoserine, 1,1'-[dithiobis-[2(S)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]-bis-(S)-methionine, N-(3-phenyl-2-(mercaptomethyl)-propionyl)-(S)-4-(methylmercapto)methionine, N-[2-acetylthiomethyl-3-phenyl-propionyl]-3-aminobenzoic acid, N-[2-mercaptomethyl-3-phenyl-propionyl]-3-aminobenzoic acid, N-[1-(2-carboxy-4-phenylbutyl)-cyclopentanecarbonyl]-(S)-isoserine, N-[1-(acetylthiomethyl)-cyclopentane-carbonyl]-(S)-methionine ethyl ester, 3(S)-[2-(acetylthiomethyl)-3-phenyl-propionyl]amino- $\epsilon$ -caprolactam; and the compounds disclosed in WO 93/10773, particularly, N-(2-acetylthiomethyl-3-(2-methylphenyl)propionyl)-methionine ethyl ester.

Especially suitable NEP inhibitors include N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-(2R)-methylbutanoic acid ethyl ester and N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-(2R)-methylbutanoic acid of the formulae.

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respectively, or in each case, a pharmaceutically acceptable salt thereof. Preferred salts of the compound of formula (VI) include, but are not limited to, a sodium salt disclosed in U.S. Patent No. 5,217,996; and a triethanolamine or a tris(hydroxymethyl)aminomethane salt disclosed in WO 03/059345.

The subject matter relating to NEP inhibitors referred herein above, e.g., in U.S. patents and EP, GB, JP or WO patent applications, is herewith incorporated by reference, especially the subject matter corresponding to NEP inhibitors, and pharmaceutically acceptable salts and pharmaceutical compositions thereof, that are disclosed herein.

The combination of the present invention may comprise in addition a diuretic. A diuretic is, for example, a thiazide derivative selected from the group consisting of chlorothiazide, hydrochlorothiazide, methylclothiazide, and chlorothalidon. The most preferred diuretic is hydrochlorothiazide. A diuretic furthermore is a potassium sparing diuretic such as amiloride or triameterine, or a pharmaceutically acceptable salt thereof.

Preferred is a combination according to the present invention comprising an angiotensin II blocker, e.g., valsartan, or a pharmaceutically acceptable salt thereof; a calcium channel blocker, e.g., amlodipine, especially in the form of the besylate salt thereof; and a renin inhibitor, e.g., aliskiren, especially in the form of the hemi-fumarate salt thereof.

Preferred is also a combination according to the present invention comprising an angiotensin II blocker, e.g., valsartan, or a pharmaceutically acceptable salt thereof; a calcium channel blocker, e.g., amlodipine, especially in the form of the besylate salt thereof; and a NEP inhibitor, e.g., N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid ethyl ester, or a pharmaceutically acceptable salt thereof; or N-(3-

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carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-(2R)-methylbutanoic acid, or a pharmaceutically acceptable salt thereof.

Preferred is also a combination according to the present invention comprising an angiotensin II blocker, e.g., valsartan, or a pharmaceutically acceptable salt thereof; a calcium channel blocker, e.g., amlodipine, especially in the form of the besylate salt thereof; a renin inhibitor, e.g., aliskiren, especially in the form of the hemi-fumarate salt thereof; and a diuretic, e.g., hydrochlorothiazide.

Preferred is also a combination according to the present invention comprising an angiotensin II blocker, e.g., valsartan, or a pharmaceutically acceptable salt thereof; a calcium channel blocker, e.g., amlodipine, especially in the form of the besylate salt thereof; a NEP inhibitor, e.g., N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-(2R)-methylbutanoic acid ethyl ester, or a pharmaceutically acceptable salt thereof; or N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-(2R)-methylbutanoic acid, or a pharmaceutically acceptable salt thereof; and a diuretic, e.g., hydrochlorothiazide.

As indicated herein above, the compounds to be combined may be present as their pharmaceutically acceptable salts. If these compounds have, e.g., at least one basic center such as an amino group, they can form acid addition salts thereof. Similarly, the compounds having at least one acid group (for example COOH) can form salts with bases. Corresponding internal salts may furthermore be formed, if a compound comprises, e.g., both a carboxy and an amino group.

The corresponding active ingredients or a pharmaceutically acceptable salts may also be used in form of a solvate, such as a hydrate or including other solvents used, e.g., in their crystallization.

Furthermore, the present invention provides pharmaceutical compositions comprising:

- (a) an angiotensin II receptor blocker (ARB), or a pharmaceutically acceptable salt thereof;
- (b) a calcium channel blocker (CCB), or a pharmaceutically acceptable salt thereof; and
- (c) one of the two active agents selected from

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- (i) a renin inhibitor, or a pharmaceutically acceptable salt thereof; and
- (ii) a neutral endopeptidase (NEP) inhibitor, or a pharmaceutically acceptable salt thereof

and a pharmaceutically acceptable carrier.

As disclosed herein above, an angiotensin II receptor blocker (ARB), e.g., valsartan, or a pharmaceutically acceptable salt thereof; a calcium channel blocker (CCB), e.g., amlodipine, preferably in the form of the besylate salt thereof; and one of the two active agents selected from a renin inhibitor, in particular, aliskiren, preferably in the form of the hemi-fumarate salt thereof; and a neutral endopeptidase (NEP) inhibitor, e.g., N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid ethyl ester, or a pharmaceutically acceptable salt thereof; or N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid, or a pharmaceutically acceptable salt thereof; and optionally a diuretic, e.g., hydrochlorothiazide, may be co-administered as a pharmaceutical composition. The components may be administered together in any conventional dosage form, usually also together with a pharmaceutically acceptable carrier or diluent.

The pharmaceutical compositions according to the invention are those suitable for enteral, such as oral or rectal, transdermal and parenteral administration to mammals, including man. For oral administration the pharmaceutical composition comprising an angiotensin II receptor blocker (ARB), e.g., valsartan, or a pharmaceutically acceptable salt thereof; a calcium channel blocker (CCB), e.g., amlodipine, preferably in the form of the besylate salt thereof; and one of the two active agents selected from a renin inhibitor, in particular, aliskiren, preferably in the form of the hemi-fumarate salt thereof; and a neutral endopeptidase (NEP) inhibitor, e.g., N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid ethyl ester, or a pharmaceutically acceptable salt thereof; or N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid, or a pharmaceutically acceptable salt thereof; and optionally a diuretic, e.g., hydrochlorothiazide, can take the form of solutions, suspensions, tablets, pills, capsules, powders, microemulsions, unit dose packets and the like. Preferred are tablets and gelatin capsules comprising the active ingredient together with: a) diluents, e.g., lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycine; b) lubricants, e.g., silica, talcum, stearic acid, its magnesium or calcium salt and/or polyethyleneglycol; for tablets also c) binders, e.g., magnesium aluminum silicate, starch paste, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose and or polyvinylpyrrolidone; if desired d) disintegrants, e.g.,

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starches, agar, alginic acid or its sodium salt, or effervescent mixtures; and/or e) absorbants, colorants, flavors and sweeteners. Injectable compositions are preferably aqueous isotonic solutions or suspensions, and suppositories are advantageously prepared from fatty emulsions or suspensions.

Said compositions may be sterilized and/or contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure and/or buffers. In addition, they may also contain other therapeutically valuable substances. Said compositions are prepared according to conventional mixing, granulating or coating methods, respectively, and contain about 0.1-90%, preferably about 1-80%, of the active ingredient.

The dosage of the active ingredients can depend on a variety of factors, such as mode of administration, homeothermic species, age and/or individual condition.

Preferred dosages for the active ingredients of the pharmaceutical combinations according to the present invention are therapeutically effective dosages, especially those which are commercially available.

Normally, in the case of oral administration, an approximate daily dose of from about 1 mg to about 360 mg is to be estimated, e.g., for a patient of approximately 75 kg in weight.

For example, angiotensin II receptor blockers, e.g., valsartan, are supplied in the form of a suitable dosage unit form, e.g., a capsule or tablet, and comprising a therapeutically effective amount of an angiotensin II receptor blocker, e.g., from about 20 to about 320 mg, of valsartan, which may be applied to patients. The application of the active ingredient may occur up to three times a day, starting, e.g., with a daily dose of 20 mg or 40 mg of an angiotensin II receptor blocker, e.g., valsartan, increasing via 80 mg daily and further to 160 mg daily, and finally up to 320 mg daily. Preferably, an angiotensin II receptor blocker, e.g., valsartan is applied once a day or twice a day with a dose of preferably 80 mg or 160 mg, respectively, each. Corresponding doses may be taken, e.g., in the morning, at mid-day or in the evening.

In the case of calcium channel blockers, preferred dosage unit forms are, e.g., tablets or capsules, comprising about 1.0 mg to about 180 mg, preferably about 2.5 mg to about 50 mg, more preferably 2.5 to 10 mg, of the CCB, e.g. amlodipine, depending on the specific CCB. Preferred dosage unit forms are, for example, tablets or capsules comprising e.g. from

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about 1 mg to about 40 mg, preferably 2.5 to 20 mg, more preferably 2.5 to 10 mg, daily especially when the CCB, in particular amlodipine, is administered orally.

The doses of renin inhibitors, e.g. aliskiren, to be administered to warm-blooded animals, including man, of approximately 75 kg body weight, especially the doses effective for the inhibition of renin activity, e.g., in lowering blood pressure, are from preferably about 3 mg to about 3 g, more preferably from about 10 mg to about 1 g, e.g., from 20 to 200 mg/person/day, divided preferably into 1 to 4 single doses which may, e.g., be of the same size. Usually, children receive about half of the adult dose. The dose necessary for each individual can be monitored, e.g., by measuring the serum concentration of the active ingredient, and adjusted to an optimum level. Single doses comprise, e.g., 75 mg, 150 mg or 300 mg per adult patient.

In the case of NEP inhibitors, preferred dosage unit forms are, e.g., tablets or capsules comprising, e.g., from about 20 mg to about 800 mg, preferably from about 50 mg to about 700 mg, even more preferably from about 100 mg to about 600 mg, and most preferably from about 100 mg to about 300 mg, of the NEP inhibitor administered preferably once a day.

In case of diuretics, preferred dosage unit forms are, e.g., tablets or capsules comprising, e.g., from about 5 mg to about 50 mg, preferably from about 6.25 mg to about 25 mg. A daily dose of 6.25 mg, 12.5 mg or 25 mg of e.g. hydrochlorothiazide is preferably administered once a day.

The above doses encompass a therapeutically effective amount of the active ingredients of the present invention

An example of a preferred composition, comprises an amount of Valsartan between 60 and 100 mg e.g. 80 mg, an amount of amlodipine between 2 and 12 mg e.g. 2.5 or 5 mg, an amount of aliskiren of 20 to 200 mg, e.g., 75 mg, 150 mg or 300 mg, and an amount of HCTZ between 8 and 16 mg e.g. 12.5 mg.

Another example of a preferred composition, comprises an amount of Valsartan between 140 and 180 mg e.g. 160 mg, an amount of amlodipine between 2 and 12 mg e.g. 2.5 or 5 or

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10 mg, amount of aliskiren of 20 to 200 mg, e.g., 75 mg, 150 mg or 300 mg, and an amount of HCTZ between 8 and 16 mg e.g. 12.5 mg.

Another example of a preferred composition comprises an amount of Valsartan between 140 and 180 mg e.g. 160 mg, an amount of amlodipine between 4 and 12 mg e.g. 5 mg or 10 mg, amount of aliskiren of 20 to 200 mg, e.g., 75 mg, 150 mg or 300 mg, and an amount of HCTZ between 20 and 30 mg e.g. 25 mg.

Since the present invention has an aspect that relates to methods for the prevention of, delay the onset of and/or treatment with a combination of compounds which may be administered separately, the invention also relates to combining separate pharmaceutical compositions in a kit form. The kit may comprise, e.g., three to four separate pharmaceutical compositions: (1) a composition comprising an angiotensin II blocker, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent; (2) a composition comprising a CCB, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent; (3) a composition comprising one of the two active agents selected from a renin inhibitor, or a pharmaceutically acceptable salt thereof, and a NEP inhibitor, or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier or diluent; and (4) optionally a diuretic, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent. The amounts of (1), (2), (3) and (4) are such that, when co-administered separately a beneficial therapeutic effect(s) is achieved. The kit comprises a container for containing the separate compositions such as a divided bottle or a divided foil packet, wherein each compartment contains a plurality of dosage forms (e.g., tablets) comprising, e.g., (1), (2) or (3). Alternatively, rather than separating the active ingredient-containing dosage forms, the kit may contain separate compartments each of which contains a whole dosage which in turn comprises separate dosage forms. An example of this type of kit is a blister pack wherein each individual blister contains three or four (or more) tablets, one (or more) tablet(s) comprising a pharmaceutical composition (1), the second (or more) tablet(s) comprising a pharmaceutical composition (2), the third (or more) tablet(s) comprising a pharmaceutical composition (3) and optionally the fourth (or more) tablet(s) comprising a pharmaceutical composition (4). Typically the kit comprises directions for the administration of the separate components. The kit form is particularly advantageous when the separate components are

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preferably administered in different dosage forms (e.g., oral and parenteral), are administered at different dosage intervals, or when titration of the individual components of the combination is desired by the prescribing physician. In the case of the instant invention a kit may, e.g., comprise:

(1) a therapeutically effective amount of a composition comprising an angiotensin II blocker, e.g., valsartan, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent, in a first dosage form;

(2) a therapeutically effective amount of a composition comprising a CCB, in particular, amlodipine, preferably in the form of the besylate salt thereof, and a pharmaceutically acceptable carrier or diluent, in a second dosage form;

(3) a therapeutically effective amount of a composition comprising one of the two active agents selected from

a renin inhibitor, in particular, aliskiren, preferably in the form of the hemi-fumarate salt thereof, and a pharmaceutically acceptable carrier or diluent, and

a neutral endopeptidase (NEP) inhibitor, e.g., N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid ethyl ester, or a pharmaceutically acceptable salt thereof; or N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid; or a pharmaceutically acceptable salt thereof; in an amount such that, following administration, a beneficial therapeutic effect(s) is achieved, and a pharmaceutically acceptable carrier or diluent, in a third dosage form;

(4) optionally a diuretic, e.g., hydrochlorothiazide, or a pharmaceutically acceptable salt thereof, in a fourth dosage form; and

(5) a container for containing said first, second, third and optionally fourth dosage forms.

The present invention further relates to a method for the prevention of, delay the onset of and/or treatment of a disease or a condition mediated by angiotensin II and/or to NEP activity, which method comprises administering to a warm-blooded animal, including man, in need thereof, a therapeutically effective amount of a pharmaceutical composition comprising:

- (a) an angiotensin II receptor blocker (ARB), or a pharmaceutically acceptable salt thereof;

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- (b) a calcium channel blocker (CCB), or a pharmaceutically acceptable salt thereof;  
and
  - (c) one of the two active agents selected from
    - (i) a renin inhibitor, or a pharmaceutically acceptable salt thereof; and
    - (ii) a neutral endopeptidase (NEP) inhibitor, or a pharmaceutically acceptable salt thereof;
- and a pharmaceutically acceptable carrier.

It has surprisingly been found that, a combination of an angiotensin II receptor blocker (ARB), e.g., valsartan, or a pharmaceutically acceptable salt thereof; a calcium channel blocker (CCB), e.g., amlodipine, preferably in the form of the besylate salt thereof; and one of the two active agents selected from a renin inhibitor, in particular, aliskiren, preferably in the form of the hemi-fumarate salt thereof; and a neutral endopeptidase (NEP) inhibitor, e.g., N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid ethyl ester, or a pharmaceutically acceptable salt thereof; or N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid, or a pharmaceutically acceptable salt thereof; and optionally a diuretic, e.g., hydrochlorothiazide, achieves greater therapeutic effect than the administration of an angiotensin II blocker, a CCB, a renin inhibitor, a NEP inhibitor or a diuretic alone. Greater efficacy can also be documented as a prolonged duration of action. The duration of action can be monitored as either the time to return to baseline prior to the next dose or as the area under the curve (AUC) and is expressed as the product of the change in blood pressure in millimeters of mercury (change in mmHg) and the duration of the effect (min, hours or days).

Further benefits are that lower doses of the individual drugs to be combined according to the present invention can be used to reduce the dosage, e.g., that the dosages need not only often be smaller but are also applied less frequently, or can be used to diminish the incidence of side effects. The combined administration of an angiotensin II receptor blocker (ARB), e.g., valsartan, or a pharmaceutically acceptable salt thereof; a calcium channel blocker (CCB), e.g., amlodipine, preferably in the form of the besylate salt thereof; and one of the two active agents selected from a renin inhibitor, in particular, aliskiren, preferably in the form of the hemi-fumarate salt thereof; and a neutral endopeptidase (NEP) inhibitor, e.g., N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid ethyl ester, or a pharmaceutically acceptable salt thereof; or N-(3-carboxy-1-oxopropyl)-(4S)-

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p-phenylphenylmethyl)-4-amino-(2R)-methylbutanoic acid, or a pharmaceutically acceptable salt thereof; and optionally a diuretic, e.g., hydrochlorothiazide, results in a significant response in a greater percentage of treated patients, i.e., a greater responder rate results, regardless of the underlying etiology of the condition. This is in accordance with the desires and requirements of the patients to be treated.

It can be shown that combination therapy with an angiotensin II receptor blocker (ARB), e.g., valsartan, or a pharmaceutically acceptable salt thereof; a calcium channel blocker (CCB), e.g., amlodipine, preferably in the form of the besylate salt thereof; and one of the two active agents selected from a renin inhibitor, in particular, aliskiren, preferably in the form of the hemi-fumarate salt thereof; and a neutral endopeptidase (NEP) inhibitor, e.g., N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-(2R)-methylbutanoic acid ethyl ester, or a pharmaceutically acceptable salt thereof; or N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-(2R)-methylbutanoic acid, or a pharmaceutically acceptable salt thereof; and optionally a diuretic, e.g., hydrochlorothiazide, results in a more effective antihypertensive therapy (whether for malignant, essential, reno-vascular, diabetic, isolated systolic, or other secondary type of hypertension) through improved efficacy as well as a greater responder rate. The combination is also useful in the prevention of, delay the onset of and/or treatment of heart failure such as (acute and chronic) congestive heart failure, left ventricular dysfunction, diastolic dysfunction, hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation (AF), atrial flutter, detrimental vascular remodeling or plaque stabilization. It can further be shown that a therapy with an angiotensin II receptor blocker (ARB) and a calcium channel blocker (CCB), and one of the two active agents selected from a renin inhibitor and a neutral endopeptidase (NEP) inhibitor and optionally a diuretic proves to be beneficial in the treatment and prevention of myocardial infarction and its sequelae. A combination of the present invention is also useful in treating atherosclerosis including coronary arterial disease (CAD), angina pectoris (whether unstable or stable), renal insufficiency (diabetic and non-diabetic), renal fibrosis, polycystic kidney disease (PKD) and metabolic syndrome. In addition, combination therapy using a combination of the present invention can improve endothelial dysfunction, thereby providing benefit in diseases in which normal endothelial function is disrupted such as heart failure, angina pectoris and type 2 diabetes. Furthermore, a combination of the present invention may be used for the prevention of, delay the onset of and/or treatment of secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions such as nephrotic syndrome, diabetic nephropathy, glomerulonephritis,

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scleroderma, glomerular sclerosis, proteinuria of primary renal disease, renal vascular hypertension, diabetic retinopathy and end-stage renal disease (ESRD), the management of other vascular disorders such as migraine, peripheral vascular disease (PVD), Raynaud's disease, luminal hyperplasia, cognitive dysfunction (such as Alzheimer's), glaucoma and cerebrovascular disease such as embolic or thrombotic stroke.

The structure of the active agents identified by generic or tradenames or code numbers may be taken from the actual edition of the standard compendium "The Merck Index" or from databases, e.g., Life Cycle Patents International (e.g. IMS' World Publications). The corresponding content thereof is hereby incorporated by reference. Any person skilled in the art is fully enabled to identify the active agents and, based on these references, likewise enabled to manufacture and test the pharmaceutical indications and properties in standard test models, both *in vitro* and *in vivo*.

The invention furthermore relates to the use of a combination according to the present invention for the manufacture of a medicament for the prevention of, delay the onset of and/or treatment of cardiovascular disorders.

Accordingly, another embodiment of the present invention relates to the use of a combination according to the invention for the manufacture of a medicament for the prevention of, delay the onset of and/or treatment of cardiovascular disorders, especially a disease or a condition selected from the group consisting of hypertension (whether for malignant, essential, reno-vascular, diabetic, isolated systolic, or other secondary type of hypertension), heart failure such as diastolic and congestive heart failure (acute and chronic), left ventricular or endothelial dysfunction, hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation (AF), cardiac fibrosis, atrial flutter, detrimental vascular remodeling, plaque stabilization, myocardial infarction (MI) and its sequelae, atherosclerosis including coronary arterial disease (CAD), angina pectoris (whether unstable or stable), renal insufficiency (diabetic and non-diabetic), renal fibrosis, polycystic kidney disease (PKD), type 2 diabetes, metabolic syndrome, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions such as nephrotic syndrome, diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, renal vascular hypertension, diabetic retinopathy and end-stage renal disease (ESRD), the management of other vascular disorders such as migraine, peripheral vascular disease (PVD), Raynaud's

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disease, luminal hyperplasia, cognitive dysfunction (such as Alzheimer's), glaucoma and cerebrovascular disease such as embolic or thrombotic stroke.

Especially, a combination according to the present invention may be employed, e.g., for the prevention of, delay the onset of and/or treatment of diseases and conditions selected from the group as specified above, and also diseases, illnesses, conditions or symptoms related to, or encountered or associated therewith.

Preferably, a combination according to the present invention may be employed for the treatment of hypertension, congestive heart failure, atherosclerosis, endothelial dysfunction, and renal insufficiency.

In particular, all the more surprising is the experimental finding that a combination of the present invention results in a beneficial, especially a synergistic, therapeutic effect but also in benefits resulting from combined treatment such as a surprising prolongation of efficacy, a broader variety of therapeutic treatment and surprising beneficial effects on diseases and conditions as specified hereinbefore or hereinafter.

The pharmaceutical activities as effected by administration of a an angiotensin II receptor blocker (ARB), e.g., valsartan, or a pharmaceutically acceptable salt thereof; a calcium channel blocker (CCB), e.g., amlodipine, preferably in the form of the besylate salt thereof; and one of the two active agents selected from a renin inhibitor, in particular, aliskiren, preferably in the form of the hemi-fumarate salt thereof; and a neutral endopeptidase (NEP) inhibitor, e.g., N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid ethyl ester, or a pharmaceutically acceptable salt thereof; or N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid, or a pharmaceutically acceptable salt thereof; and optionally a diuretic, e.g., hydrochlorothiazide, respectively, or by administration of a combination of therapeutic agents used according to the present invention may be demonstrated, e.g., by using corresponding pharmacological models well-known in the pertinent art. A person skilled in the art is fully enabled to select a relevant test model to prove the hereinbefore and hereinafter indicated therapeutic indications and beneficial effects.

A combination according to the present invention can be administered by various routes of administration. Each agent can be tested over a wide-range of dosages to determine the optimal drug level for each therapeutic agent in the specific combination to elicit the maximal

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response. For these studies, it is preferred to use treatment groups consisting of at least 6 animals per group. Each study is best performed in a way wherein the effects of the combination treatment group are determined at the same time as the individual components are evaluated. Although drug effects may be observed with acute administration, it is preferable to observe responses in a chronic setting. The long-term study is of sufficient duration to allow for the full development of compensatory responses to occur and, therefore, the observed effect will most likely depict the actual responses of the test system representing sustained or persistent effects.

Representative studies may be carried out with a combination of an angiotensin II receptor blocker (ARB), e.g., valsartan, or a pharmaceutically acceptable salt thereof; a calcium channel blocker (CCB), e.g., amlodipine, preferably in the form of the besylate salt thereof; and one of the two active agents selected from a renin-inhibitor, in particular, aliskiren, preferably in the form of the hemi-fumarate salt thereof; and a neutral endopeptidase (NEP) inhibitor, e.g., N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid ethyl ester, or a pharmaceutically acceptable salt thereof; or N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid, or a pharmaceutically acceptable salt thereof; and optionally a diuretic, e.g., hydrochlorothiazide, e.g., applying the following methodology:

Drug efficacy is assessed in various animal models including the deoxycorticosterone acetate-salt rat (DOCA-salt), the Dahl salt-sensitive (DS) and salt-resistant (DR) rat, and the spontaneously hypertensive rat (SHR), either maintained on a normal salt diet or with salt loading (4-8% salt in rat chow or 1% NaCl as drinking water).

The DOCA-salt test model utilizes either an acute or chronic study protocol. An acute study procedure involves assessment of the effects of various test substances over a six-hour experimental period using rats with indwelling femoral arterial and venous catheters. The Acute Study Procedure evaluates test substances for their ability to reduce blood pressure during the established phase of DOCA-salt hypertension. In contrast, the Chronic Study Procedure assesses the ability of test substances to prevent or delay the rise in blood pressure during the development phase of DOCA-salt hypertension. Therefore, blood pressure will be monitored in the chronic study procedure by means of a radiotransmitter. The radiotransmitter is surgically implanted into the abdominal aorta of rats, prior to the initiation of DOCA-salt treatment and thus, prior to the induction of hypertension. Blood

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pressure is chronically monitored for periods of up to 6 weeks (approximately one week prior to DOCA-salt administration and for 5 weeks thereafter).

Rats are anesthetized with 2-3% isoflurane in oxygen inhalant followed by Amytal sodium (amobarbital) 100 mg/kg, ip. The level of anesthesia is assessed by a steady rhythmic breathing pattern.

Acute study procedure:

Rats undergo a unilateral nephrectomy at the time of DOCA implantation. Hair is clipped on the left flank and the back of the neck and scrubbed with sterile alcohol swabs and povidone/iodine. During surgery rats are placed on a heating pad to maintain body temperature at 37°C.

A 20 mm incision is made through the skin and underlying muscle to expose the left kidney. The kidney is freed of surrounding tissue, exteriorized and two ligatures (3-0 silk) are tied securely around the renal artery and vein proximal to their juncture with the aorta. The renal artery and vein are then severed and the kidney removed. The muscle and skin wounds are closed with 4-0 silk suture and stainless steel wound clips, respectively. At the same time, a 15 mm incision is made on the back of the neck and a 3-week-release pellet (Innovative Research of America, Sarasota, Florida) containing deoxycorticosterone acetate (100 mg/kg) is implanted subcutaneously. The wound is then closed with stainless-steel clips and both wounds are treated with povidone/iodine; the rats are given a post-surgical intramuscular injection of procaine penicillin G (100,000 U) and buprenorphine (0.05 – 0.1 mg/kg) s.c. The rats are immediately placed on 1% NaCl + 0.2% KCl drinking water; this treatment continues for at least 3 weeks at which time the animals have become hypertensive and available for experimentation.

Forty-eight hours prior to experimentation, animals are anesthetized with isoflurane and catheters are implanted in the femoral artery and vein for measuring arterial pressure, collection of blood, and administration of test compounds. Rats are allowed to recover for 48 hours while tethered in a Plexiglas home cage, which also serves as the experimental chamber.

Chronic study procedure:

This procedure is the same as above except that rats are implanted with a radiotransmitter, 7-10 days prior to the unilateral nephrectomy and initiation of DOCA and salt. In addition,

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rats do not undergo surgery for placement of femoral arterial and venous catheters.

Radiotransmitters are implanted as described by M.K. Bazil, C. Krulan and R.L. Webb. in J. Cardiovasc. Pharmacol. 22: 897-905, 1993.

Protocols are then set-up on the computer for measurement of blood pressure, heart rate, etc, at predetermined time points. Baseline data is collected at various time points and over various time intervals. For example, baseline or pre-dose values usually consist of data collection and averaging over 3 consecutive, 24-hour time periods prior to drug administration.

Blood pressure, heart rate and activity are determined at various pre-selected time points before, during, and after drug administration. All measurements are performed in unrestrained and undisturbed animals. The maximum study time, determined by battery life, could be as long as nine months. For studies of this duration, rats are dosed orally (1-3 ml/kg vehicle), no more than twice daily or drug is administered via the drinking water or mixed with food. For studies of a shorter duration, that is, up to 8 weeks, drugs are given via subcutaneously implanted osmotic minipumps. Osmotic minipumps are selected based on drug delivery rate and time.

The Dahl salt-sensitive (DSS) and salt-resistant (DSR) rat may also be utilized for the study of the combinations according to the present invention. The DSR rat is commonly used as a normotensive control for these studies. For the study of various combinations, a typical protocol is defined as follows:

Dahl salt-sensitive (DSS) rats are 6 weeks of age upon arrival to our animal facilities. Radiotransmitters are implanted into Dahl salt-sensitive rats at 7 weeks of age. All animals are placed on a high salt diet (8%) between 7 and 8 weeks of age. Drug treatment is initiated at 9 weeks of age and is continued for 3 weeks. Drugs are administered once daily by oral gavage but may also be given by other routes (e.g., intra-peritoneal, intra-venous, or subcutaneous). Dahl salt-sensitive rats are randomized to receive one of the various treatments specified above. Drugs are administered by oral gavage, once daily in the morning for 3 weeks. Blood pressure (mean, systolic, and diastolic) and heart rate are continuously monitored, 24 hours per day for the full duration of the study using radiotelemetric procedures. All values depict 24 hour average responses for each animal but data summarization may also be performed using other time intervals, for example, hourly averaging. Body weights are recorded at weekly intervals. Upon completion of the

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study, all rats are sacrificed and hearts are removed, sectioned and weighed. Cardiac mass is determined as the left ventricular weight to body weight ratio for each animal within a treatment group. Other tissues, including but not restricted to the kidney, may be removed at sacrifice for determination of biochemical markers, to assess the extent of tissue damage (histology, immunohistochemistry, etc), and for gene expression profiling.

Additionally, SHR are utilized to study the effects of the claimed combination. The hypertensive background of the SHR is modified either by chronic salt loading in an effort to suppress the renin angiotensin system (RAS) or chronic salt depletion to activate the RAS in the SHR. These manipulations will be carried out to more extensively evaluate the efficacy of the various test substances. Experiments performed in spontaneously hypertensive rats (SHR) are supplied by Taconic Farms, Germantown, New York (Tac:N(SHR)fBR). A radiotelemetric device (Data Sciences International, Inc., St. Paul, Minnesota) is implanted into the lower abdominal aorta of all test animals between the ages of 14 to 16 weeks of age. All SHR are allowed to recover from the surgical implantation procedure for at least 2 weeks prior to the initiation of the experiments. Cardiovascular parameters are continuously monitored via the radiotransmitter and transmitted to a receiver where the digitized signal is then collected and stored using a computerized data acquisition system. Blood pressure (mean arterial, systolic and diastolic pressure) and heart rate are monitored in conscious, freely moving and undisturbed SHR in their home cages. The arterial blood pressure and heart rate are measured every 10 min for 10 seconds and recorded. Data reported for each rat represent the mean values averaged over a 24 hour period and are made up of the 144-10 min samples collected each day. The baseline values for blood pressure and heart rate consist of the average of three consecutive 24 hour readings taken prior to initiating the drug treatments. All rats are individually housed in a temperature and humidity controlled room and are maintained on a 12 hour light dark cycle.

In addition to the cardiovascular parameters, weekly determinations of body weight also are recorded in all rats. Treatments are administered in the drinking water, via daily oral gavage or in osmotic minipumps as stated above. If given in drinking water, water consumption is measured five times per week. Doses of the active agents for individual rats are then calculated based on water consumption for each rat, the concentration of drug substance in the drinking water, and individual body weights. All drug solutions in the drinking water are made up fresh every three to four days.

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Upon completion of the chronic studies, SHR or DOCA-salt rats are anesthetized and the heart rapidly removed. After separation and removal of the atrial appendages, left ventricle and left plus right ventricle (total) are weighed and recorded. Left ventricular and total ventricular mass are then normalized to body weight and reported. All values reported for blood pressure and cardiac mass represent the group mean  $\pm$  sem.

Vascular function and structure are evaluated after treatment to assess the beneficial effects of the combination. SHR are studied according to the methods described by Intengan HD, Thibault G, Li JS, Schiffrin EL, Circulation 100 (22): 2267-2275, 1999. Similarly, the methodology for assessing vascular function in DOCA-salt rats is described in Intengan HD, Park JB, Schiffrin, EL, Hypertension 34 (4 Part 2): 907-913, 1999.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. Therefore, the Examples herein are to be construed as merely illustrative of certain aspects of the present invention and are not a limitation of the scope of the present invention in any way.

### Examples

The products or the combinations described in the examples below provide medicaments with unexpected therapeutic benefits, or superior or more efficient properties to those of individual monotherapies.

#### Example 1:

Composition of aliskiren 150 mg (free base) uncoated tablets in mg/unit.

|                            | Roller<br>compacted<br>tablet | Dosage form 1 | Dosage form 2 | Dosage form 3 |
|----------------------------|-------------------------------|---------------|---------------|---------------|
| Component                  |                               |               |               |               |
| Aliskiren hemi-fumarate    | 165.750                       | 165.750       | 165.750       | 165.750       |
| Microcrystalline cellulose | 220.650                       | 84.750        | 72.250        | 107.250       |
| Polyvinylpyrrolidone K 30  | -                             | -             | 12.000        | 12.000        |
| Crospovidone               | 84.000                        | 45.000        | 44.000        | 48.200        |

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|                     |                |                |                |                |
|---------------------|----------------|----------------|----------------|----------------|
| Aerosil 200         | 4.800          | 1.500          | 1.500          | 1.800          |
| Magnesium stearate  | 4.800          | 3.000          | 4.500          | 5.000          |
| <b>Total weight</b> | <b>480.000</b> | <b>300.000</b> | <b>300.000</b> | <b>340.000</b> |

Composition of aliskiren 150 mg (free base) uncoated tablets in % by weight.

|                            |               | Roller<br>compacted<br>tablet | Dosage form 1 | Dosage form 2 | Dosage form 3 |
|----------------------------|---------------|-------------------------------|---------------|---------------|---------------|
| Component                  |               |                               |               |               |               |
| Aliskiren hemi-fumarate    | 34.53         |                               | 55.25         | 55.25         | 48.75         |
| Microcrystalline cellulose | 45.97         |                               | 28.25         | 24.08         | 31.545        |
| Polyvinylpyrrolidon K 30   | -             |                               | -             | 4             | 3.53          |
| Crospovidone               | 17.5          |                               | 15            | 14.67         | 14.175        |
| Aerosil 200                | 1             |                               | 0.5           | 0.5           | 0.53          |
| Magnesium stearate         | 1             |                               | 1             | 1.5           | 1.47          |
| <b>Total %</b>             | <b>100.00</b> |                               | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> |

Composition of aliskiren 150 mg (free base) uncoated tablets in mg/unit (divided into inner/outer phase).

|                    |                            |        | Roller<br>compacted<br>tablet | Dosage form 1 | Dosage form 2 | Dosage form 3 |
|--------------------|----------------------------|--------|-------------------------------|---------------|---------------|---------------|
| Component          |                            |        |                               |               |               |               |
| <b>Inner Phase</b> | Aliskiren hemi-fumarate    | 165.75 |                               | 165.75        | 165.75        | 165.75        |
|                    | Microcrystalline cellulose | 220.65 |                               | 84.75         | 72.25         | 90.25         |
|                    | Polyvinylpyrrolidon K 30   | -      |                               | -             | 12.00         | 12.00         |
|                    | Crospovidone               | 36.00  |                               | -             | -             | 14.20         |
|                    | Aerosil 200                | -      |                               | -             | -             | -             |
|                    | Magnesium stearate         | 2.40   |                               | -             | -             | -             |
| <b>Outer phase</b> | Crospovidone               | 48.00  |                               | 45.00         | 44.00         | 34.00         |
|                    | Microcrystalline cellulose | -      |                               | -             | -             | 17.00         |

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|                     |               |               |               |               |
|---------------------|---------------|---------------|---------------|---------------|
| Aerosil 200         | 4.80          | 1.50          | 1.50          | 1.80          |
| Magnesium stearate  | 2.40          | 3.00          | 4.50          | 5.00          |
| <b>Total weight</b> | <b>480.00</b> | <b>300.00</b> | <b>300.00</b> | <b>340.00</b> |

Composition of aliskiren 150 mg (free base) uncoated tablets in % by weight (divided into inner/outer phase).

|                    |                            |       | Roller compacted tablet | Dosage form 1 | Dosage form 2 | Dosage form 3 |
|--------------------|----------------------------|-------|-------------------------|---------------|---------------|---------------|
| Component          |                            |       |                         |               |               |               |
| <b>Inner Phase</b> | Aliskiren hemi-fumarate    | 34.53 |                         | 55.25         | 55.25         | 48.75         |
|                    | Microcrystalline cellulose | 45.97 |                         | 28.25         | 24.08         | 26.545        |
|                    | Polyvinylpyrrolidon K 30   | -     |                         | -             | 4             | 3.530         |
|                    | Crospovidone               | 7.5   |                         | -             | -             | 4.175         |
|                    | Aerosil 200                | -     |                         | -             | -             | -             |
|                    | Magnesium stearate         | 0.5   |                         | -             | -             | -             |
| <b>Outer phase</b> | Crospovidone               | 10    |                         | 15            | 14.67         | 10            |
|                    | Microcrystalline cellulose | -     |                         | -             | -             | 5             |
|                    | Aerosil 200                | 1     |                         | 0.5           | 0.5           | 0.53          |
|                    | Magnesium stearate         | 0.5   |                         | 1             | 1.5           | 1.47          |
| <b>Total %</b>     |                            |       | <b>100.00</b>           | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> |

**Example 2:**

Composition of aliskiren (dosage form 3) film-coated tablets in mg/unit.

| Dosage form 3 / Strength   | 75 mg (free base) | 150 mg (free base) | 300 mg (free base) |
|----------------------------|-------------------|--------------------|--------------------|
| <b>Component</b>           |                   |                    |                    |
| Aliskiren hemi-fumarate    | 82.875            | 165.750            | 331.500            |
| Microcrystalline cellulose | 53.625            | 107.250            | 214.500            |
| Polyvinylpyrrolidon K 30   | 6.000             | 12.000             | 24.000             |
| Crospovidone               | 24.100            | 48.200             | 96.400             |
| Aerosil 200                | 0.900             | 1.800              | 3.600              |
| Magnesium stearate         | 2.500             | 5.000              | 10.000             |
| <b>Total tablet weight</b> | <b>170.000</b>    | <b>340.000</b>     | <b>680.000</b>     |

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|                                        |                |                |                |
|----------------------------------------|----------------|----------------|----------------|
| Opadry premix white                    | 9.946          | 16.711         | 23.9616        |
| Opadry premix red                      | 0.024          | 0.238          | 1.8382         |
| Opadry premix black                    | 0.030          | 0.051          | 0.2002         |
| <b>Total film-coated tablet weight</b> | <b>180.000</b> | <b>357.000</b> | <b>706.000</b> |

The dosage forms 1, 2 and 3 may be prepared, e.g., as follows:

- 1) mixing the active ingredient and additives and granulating said components with a granulation liquid;
- 2) drying a resulting granulate;
- 3) mixing the dried granulate with outer phase excipients;
- 4) compressing a resulting mixture to form a solid oral dosage as a core tablet; and
- 5) optionally coating a resulting core tablet to give a film-coated tablet.

The granulation liquid can be ethanol, a mixture of ethanol and water, a mixture of ethanol, water and isopropanol, or a solution of polyvinylpyrrolidones (PVP) in the before mentioned mixtures. A preferred mixture of ethanol and water ranges from about 50/50 to about 99/1 (% w/w), most preferably it is about 94/6 (% w/w). A preferred mixture of ethanol, water and isopropanol ranges from about 45/45/5 to about 98/1/1 (% w/w/w), most preferably from about 88.5/5.5/6.0 to about 91.5/4.5/4.0 (% w/w/w). A preferred concentration of PVP in the above named mixtures ranges from about 5 to about 30% by weight, preferably from about 15 to about 25%, more preferably from about 16 to about 22%.

Attention is drawn to the numerous known methods of granulating, drying and mixing employed in the art, e.g., spray granulation in a fluidized bed, wet granulation in a high-shear mixer, melt granulation, drying in a fluidized-bed dryer, mixing in a free-fall or tumble blender, compressing into tablets on a single-punch or rotary tablet press.

The manufacturing of the granulate can be performed on standard equipment suitable for organic granulation processes. The manufacturing of the final blend and the compression of tablets can also be performed on standard equipment.

For example, step (1) may be carried out by a high-shear granulator, e.g., Collette Gral; step (2) may be conducted in a fluid-bed dryer; step (3) may be carried out by a free-fall mixer

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(e.g. container blender, tumble blender); and step (4) may be carried out using a dry compression method, e.g., a rotary tablet press.

**Example 3** (film-coated tablets):

| Components                                                              | Composition Per Unit (mg) | Standards   |
|-------------------------------------------------------------------------|---------------------------|-------------|
| <b>Granulation</b>                                                      |                           |             |
| Valsartan [= active ingredient]                                         | 80.00                     |             |
| Microcrystalline cellulose/<br>Avicel PH 102                            | 54.00                     | NF, Ph. Eur |
| Crospovidone                                                            | 20.00                     | NF, Ph. Eur |
| Colloidal anhydrous silica /<br>colloidal silicon dioxide / Aerosil 200 | 0.75                      | Ph. Eur/NF  |
| Magnesium stearate                                                      | 2.5                       | NF, Ph. Eur |
| <b>Blending</b>                                                         |                           |             |
| Colloidal anhydrous silica /<br>colloidal silicon dioxide / Aerosil 200 | 0.75                      | Ph. Eur/NF  |
| Magnesium stearate                                                      | 2.00                      | NF, Ph. Eur |
| <b>Coating</b>                                                          |                           |             |
| Purified water <sup>*)</sup>                                            | -                         |             |
| DIOLACK pale red 00F34899                                               | 7.00                      |             |
| Total tablet mass                                                       | 167.00                    |             |

<sup>\*)</sup> Removed during processing.

The film-coated tablets may be manufactured, e.g., as follows:

A mixture of valsartan, microcrystalline cellulose, crospovidone, part of the colloidal anhydrous silica/colloidal silicon dioxide/Aerosile 200, silicon dioxide and magnesium stearate is premixed in a diffusion mixer and then sieve through a screening mill. The resulting mixture is again pre-mixed in a diffusion mixer, compacted in a roller compactor and then sieve through a screening mill. To the resulting mixture, the rest of the colloidal anhydrous silica/colloidal silicon dioxide/Aerosile 200 are added and the final blend is made in a diffusion mixer. The whole mixture is compressed in a rotary tabletting machine and the tablets are coated with a film by using Diolack pale red in a perforated pan.

**Example 4** (film-coated tablets):

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| Components                                                              | Composition Per Unit (mg) | Standards   |
|-------------------------------------------------------------------------|---------------------------|-------------|
| <b>Granulation</b>                                                      |                           |             |
| Valsartan [= active ingredient]                                         | 160.00                    |             |
| Microcrystalline cellulose/<br>Avicel PH 102                            | 108.00                    | NF, Ph. Eur |
| Crospovidone                                                            | 40.00                     | NF, Ph. Eur |
| Colloidal anhydrous silica /<br>colloidal silicon dioxide / Aerosil 200 | 1.50                      | Ph. Eur/NF  |
| Magnesium stearate                                                      | 5.00                      | NF, Ph. Eur |
| <b>Blending</b>                                                         |                           |             |
| Colloidal anhydrous silica /<br>colloidal silicon dioxide / Aerosil 200 | 1.50                      | Ph. Eur/NF  |
| Magnesium stearate                                                      | 4.00                      | NF, Ph. Eur |
| <b>Coating</b>                                                          |                           |             |
| Opadry Light Brown 00F33172                                             | 10.00                     |             |
| <b>Total tablet mass</b>                                                | <b>330.00</b>             |             |

The film-coated tablets are manufactured, e.g., as described in Example 3.

**Example 5** (film-coated tablets):

| Components                                                                 | Composition Per Unit (mg) | Standards       |
|----------------------------------------------------------------------------|---------------------------|-----------------|
| <b>Core / Internal phase</b>                                               |                           |                 |
| Valsartan<br>[= active ingredient]                                         | 40.00                     |                 |
| Silica, colloidal anhydrous<br>(Colloidal silicon dioxide)<br>[= Glidant]  | 1.00                      | Ph. Eur, USP/NF |
| Magnesium stearate<br>[= Lubricant]                                        | 2.00                      | USP/NF          |
| Crospovidone<br>[Disintegrant]                                             | 20.00                     | Ph. Eur         |
| Microcrystalline cellulose<br>[= Binding agent]                            | 124.00                    | USP/NF          |
| <b>External phase</b>                                                      |                           |                 |
| Silica, colloidal anhydrous,<br>(Colloidal silicon dioxide)<br>[= Glidant] | 1.00                      | Ph. Eur, USP/NF |

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|                                       |        |        |
|---------------------------------------|--------|--------|
| Magnesium stearate<br>[Lubricant]     | 2.00   | USP/NF |
| Film coating                          |        |        |
| Opadry® brown OOF 16711 <sup>*)</sup> | 9.40   |        |
| Purified Water <sup>**)</sup>         | -      |        |
| Total tablet mass                     | 199.44 |        |

<sup>\*)</sup> The composition of the Opadry® brown OOF16711 coloring agent is tabulated below.

<sup>\*\*)</sup> Removed during processing.

**Opadry® Composition:**

| Ingredient                                 | Approximate % Composition |
|--------------------------------------------|---------------------------|
| Iron oxide, black (C.I. No. 77499, E 172)  | 0.50                      |
| Iron oxide, brown (C.I. No. 77499, E 172)  | 0.50                      |
| Iron oxide, red (C.I. No. 77491, E 172)    | 0.50                      |
| Iron oxide, yellow (C.I. No. 77492, E 172) | 0.50                      |
| Macrogolum (Ph. Eur)                       | 4.00                      |
| Titanium dioxide (C.I. No. 77891, E 171)   | 14.00                     |
| Hypromellose (Ph. Eur)                     | 80.00                     |

The film-coated tablets are manufactured, e.g., as described in Example 3.

**Example 6 (capsules):**

| Components                                           | Composition Per Unit (mg) |
|------------------------------------------------------|---------------------------|
| Valsartan [= active ingredient]                      | 80.00                     |
| Microcrystalline cellulose                           | 25.10                     |
| Crospovidone                                         | 13.00                     |
| Povidone                                             | 12.50                     |
| Magnesium stearate                                   | 1.30                      |
| Sodium lauryl sulphate                               | 0.60                      |
| Shell                                                |                           |
| Iron oxide, red<br>(C.I. No. 77491, EC No. E 172)    | 0.123                     |
| Iron oxide, yellow<br>(C.I. No. 77492, EC No. E 172) | 0.123                     |
| Iron oxide, black                                    | 0.245                     |

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|                                |        |
|--------------------------------|--------|
| (C.I. No. 77499, EC No. E 172) |        |
| Titanium dioxide               | 1.540  |
| Gelatin                        | 74.969 |
| Total mass                     | 209.50 |

The capsules may be manufactured, e.g., as follows:

**Granulation/Drying:**

Valsartan and microcrystalline cellulose are spray-granulated in a fluidized bed granulator with a granulating solution consisting of povidone and sodium lauryl sulphate dissolved in purified water. The granulate obtained is dried in a fluidized bed dryer.

**Milling/Blending:**

The dried granulate is milled together with crospovidone and magnesium stearate. The mass is then blended in a conical screw type mixer for approximately 10 minutes.

**Encapsulation:**

The empty hard gelatin capsules are filled with the blended bulk granules under controlled temperature and humidity conditions. The filled capsules are dedusted, visually inspected, weightchecked and quarantined until by Quality assurance department.

**Example 7 (capsules):**

| Components                                           | Composition Per Unit (mg) |
|------------------------------------------------------|---------------------------|
| Valsartan [= active ingredient]                      | 160.00                    |
| Microcrystalline cellulose                           | 50.20                     |
| Crospovidone                                         | 26.00                     |
| Povidone                                             | 25.00                     |
| Magnesium stearate                                   | 2.60                      |
| Sodium lauryl sulphate                               | 1.20                      |
| Shell                                                |                           |
| Iron oxide, red<br>(C.I. No. 77491, EC No. E 172)    | 0.123                     |
| Iron oxide, yellow<br>(C.I. No. 77492, EC No. E 172) | 0.123                     |

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|                                                     |        |
|-----------------------------------------------------|--------|
| Iron oxide, black<br>(C.I. No. 77499, EC No. E 172) | 0.245  |
| Titanium dioxide                                    | 1.540  |
| Gelatin                                             | 74.969 |
| Total mass                                          | 342.00 |

The capsules are manufactured, e.g., as described in Example 6.

**Example 8** (hard gelatine capsules):

| Components                      | Composition Per Unit (mg) |
|---------------------------------|---------------------------|
| Valsartan [= active ingredient] | 80.00                     |
| Sodium laurylsulphate           | 0.60                      |
| Magnesium stearate              | 1.30                      |
| Povidone                        | 12.50                     |
| Crospovidone                    | 13.00                     |
| Microcrystalline cellulose      | 21.10                     |
| Total mass                      | 130.00                    |

**Example 9** (hard gelatin capsules):

| Components                      | Composition Per Unit (mg) |
|---------------------------------|---------------------------|
| Valsartan [= active ingredient] | 80.00                     |
| Microcrystalline cellulose      | 110.00                    |
| Povidone K30                    | 45.20                     |
| Sodium laurylsulphate           | 1.20                      |
| Magnesium stearate              | 2.60                      |
| Crospovidone                    | 26.00                     |
| Total mass                      | 265.00                    |

Components (1) and (2) are granulated with a solution of components (3) and (4) in water. The components (5) and (6) are added to the dry granulate and the mixture is filled into size 1 hard gelatin capsules.

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**Example 10**

Composition and quantities for a combination of valsartan and amlodipine

| Components                   | COMPOSITION PER<br>UNIT (mg) | COMPOSITION (%) |
|------------------------------|------------------------------|-----------------|
| Diovan Drug Substance        | 80.00                        | 43.02           |
| Amlodipine Drug<br>Substance | 6.94                         | 3.73            |
| Avicel 102 (I)               | 54.00                        | 29.04           |
| Avicel 102 (II)              | 20.00                        | 10.76           |
| Crospovidone (I)             | 15.00                        | 8.07            |
| Crospovidone (II)            | 4.0                          | 2.15            |
| Cab-O-Sil                    | 1.50                         | 0.81            |
| Magnesium Stearate (I)       | 3.00                         | 1.61            |
| Magnesium Stearate (II)      | 1.50                         | 0.81            |
|                              | 185.94                       | 100.00          |

The tablet is manufactured e.g essentially as described in Formulation Example 1.

All publications and patents mentioned herein are incorporate by reference in their entirety as if set forth in full herein.

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What is claimed is:

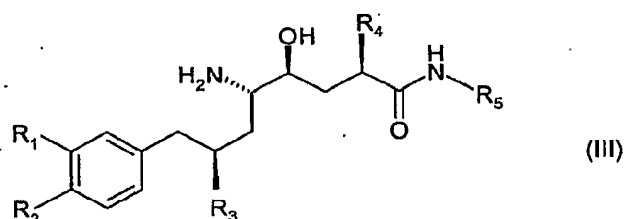
## 1. A combination comprising:

- (a). an angiotensin II receptor blocker (ARB), or a pharmaceutically acceptable salt thereof;
- (b). a calcium channel blocker (CCB), or a pharmaceutically acceptable salt thereof; and
- (c). one of the two active agents selected from
  - (i). a renin inhibitor, or a pharmaceutically acceptable salt thereof;
  - (ii). a neutral endopeptidase (NEP) inhibitor, or a pharmaceutically acceptable salt thereof.

2. A combination according to Claim 1, wherein the angiotensin II receptor is valsartan, or a pharmaceutically acceptable salt thereof.

3. A combination according to Claim 1 or 2 wherein the calcium channel blocker is amlodipine, or a pharmaceutically acceptable salt thereof.

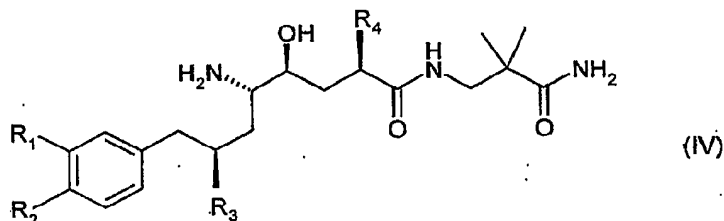
4. A combination according to any of the preceding claims, wherein a renin inhibitor is selected from the group consisting of RO 66-1132, RO 66-1168 and a compound of the formula



wherein  $R_1$  is halogen,  $C_{1-6}$ halogenalkyl,  $C_{1-6}$ alkoxy- $C_{1-6}$ alkyloxy or  $C_{1-6}$ alkoxy- $C_{1-6}$ alkyl;  $R_2$  is halogen,  $C_{1-4}$ alkyl or  $C_{1-4}$ alkoxy;  $R_3$  and  $R_4$  are independently branched  $C_{3-6}$ alkyl; and  $R_5$  is cycloalkyl,  $C_{1-6}$ alkyl,  $C_{1-6}$ hydroxyalkyl,  $C_{1-6}$ alkoxy- $C_{1-6}$ alkyl,  $C_{1-6}$ alkanoyloxy- $C_{1-6}$ alkyl,  $C_{1-6}$ aminoalkyl,  $C_{1-6}$ alkylamino- $C_{1-6}$ alkyl,  $C_{1-6}$ dialkylamino- $C_{1-6}$ alkyl,  $C_{1-6}$ alkanoylamino- $C_{1-6}$ alkyl,  $HO(O)C-C_{1-6}$ alkyl,  $C_{1-6}$ alkyl- $O-(O)C-C_{1-6}$ alkyl,  $H_2N-C(O)-C_{1-6}$ alkyl,  $C_{1-6}$ alkyl- $HN-C(O)-C_{1-6}$ alkyl or  $(C_{1-6}alkyl)_2N-C(O)-C_{1-6}alkyl$ ; or a pharmaceutically acceptable salt thereof.

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5. A combination according to Claim 4, wherein a renin inhibitor is a compound of formula (III) having the formula



wherein R<sub>1</sub> is 3-methoxypropyloxy; R<sub>2</sub> is methoxy; and R<sub>3</sub> and R<sub>4</sub> are isopropyl; or a pharmaceutically acceptable salt thereof.

6. A combination according to Claim 5, wherein the compound of formula (IV) is in the form of the hemi-fumarate salt thereof.

7. A combination according to any of the preceding claims, wherein a neutral endopeptidase inhibitor is selected from the group consisting of SQ 28,603, N-[N-[1(S)-carboxyl-3-phenylpropyl]-(S)-phenylalanyl]-(S)-isoserine, N-[N-[(1S)-carboxy-2-phenyl)ethyl]-(S)-phenylalanyl]-β-alanine, N-[2(S)-mercaptopomethyl-3-(2-methylphenyl)-propionyl]methionine, (cis-4-[[[1-[2-carboxy-3-(2-methoxyethoxy)propyl]-cyclopentyl]carbonyl]amino]-cyclohexane-carboxylic acid), thiorphan, retro-thiorphan, phosphoramidon, SQ 29,072, N-(3-carboxy-1-oxopropyl)-(4S)-p-phenyl-phenylmethyl-4-amino-(2R)-methylbutanoic acid ethyl ester, (S)-cis-4-[1-[2-(5-indanyloxy-carbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido]-1-cyclohexanecarboxylic acid, 3-(1-[6-endo-hydroxymethylbicyclo[2,2,1]heptane-2-exo-carbamoyl]cyclopentyl)-2-(2-methoxyethyl)propanoic acid, N-(1-(3-(N-t-butoxycarbonyl-(S)-prolylamino)-2(S)-t-butoxycarbonylpropyl)cyclopentanecarbonyl)-O-benzyl-(S)-serine methyl ester, 4-[2-(mercaptopomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid, 3-[1-(cis-4-carboxycarbonyl-cis-3-butylcyclohexyl-r-1-carboamoyl)cyclopentyl]-2S-(2-methoxyethoxy-methyl)propanoic acid, N-((2S)-2-(4-biphenylmethyl)-4-carboxy-5-phenoxyvaleryl)glycine, N-(1-(N-hydroxycarbamoyl-methyl)-1-cyclopentanecarbonyl)-L-phenylalanine, (S)-(2-biphenyl-4-yl)-1-(1H-tetrazol-5-yl)ethylamino)methylphosphonic acid, (S)-5-(N-(2-(phosphonomethyl-amino)-3-(4-biphenyl)-propionyl)-2-aminoethyl)tetrazole, β-Alanine, 3-[1,1'-biphenyl]-4-yl-N-[diphenoxyphosphinyl]-methyl]-L-alanyl, N-(2-carboxy-4-thienyl)-3-mercapto-2-benzylpropanamide, 2-(2-mercapto-methyl-3-phenylpropionamido)thiazol-4-ylcarboxylic acid, (L)-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy)carbonyl)-2-phenylethyl]-L-phenylalanyl]-β-alanine, N-[N-[(L)-[1-[(2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy]carbonyl]-2-phenylethyl]-L-phenylalanyl]-(R)-alanine, N-[-

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N-[(L)-1-carboxy-2-phenylethyl]-L-phenylalanyl-(R)- alanine, N-[2-acetylthiomethyl-3-(2-methyl-phenyl)propionyl]-methionine ethyl ester, N-[2-mercapto-methyl-3-(2-methylphenyl)-propionyl]-methionine, N-[2(S)-mercaptomethyl-3-(2-methylphenyl)propanoyl]-(S)-isoserine, N-(S)-[3-mercapto-2-(2-methylphenyl)propionyl]-(S)-2-methoxy-(R)-alanine, N-[1-[[1(S)-benzyloxycarbonyl-3-phenylpropyl]amino]-cyclopentylcarbonyl]-(S)-isoserine, N-[1-[[1(S)-carbonyl-3-phenylpropyl]amino]-cyclopentylcarbonyl]-(S)-isoserine, 1,1'-[dithiobis-[2(S)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]-bis-(S)-isoserine, 1,1'-[dithiobis-[2(S)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]-bis-(S)-methionine, N-(3-phenyl-2-(mercaptomethyl)-propionyl)-(S)-4-(methylmercapto)-methionine, N-[2-acetylthiomethyl-3-phenyl-propionyl]-3-aminobenzoic acid, N-[2-mercapto-methyl-3-phenyl-propionyl]-3-aminobenzoic acid, N-[1-(2-carboxy-4-phenylbutyl)-cyclopentanecarbonyl]-(S)-isoserine, N-[1-(acetylthiomethyl)-cyclopentane-carbonyl]-(S)-methionine ethyl ester, 3(S)-[2-(acetylthiomethyl)-3-phenyl-propionyl]amino-ε-caprolactam and N-(2-acetylthiomethyl-3-(2-methylphenyl)propionyl)-methionine ethyl ester, or in each case, a pharmaceutically acceptable salt thereof.

8. A combination according to any of the preceding claims, wherein a neutral endopeptidase inhibitor is N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid ethyl ester, or a pharmaceutically acceptable salt thereof; or N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid, or a pharmaceutically acceptable salt thereof.

9. A combination according to any of the preceding claims, further containing a diuretic.

10. A combination according to claim 9, wherein the diuretic is hydrochlorothiazide, or a pharmaceutically acceptable salt thereof.

11. A pharmaceutical composition comprising the combination according to any of claims 1 to 10 and a pharmaceutically acceptable carrier.

12. A pharmaceutical composition according to claim 11 for the prevention of, delay the onset of and/or treatment of cardiovascular disorders.

13. A pharmaceutical composition according to Claim 12, wherein the of cardiovascular disorder is selected from the group consisting of hypertension, heart failure, left ventricular dysfunction, endothelial dysfunction, diastolic dysfunction, hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation,

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cardiac fibrosis, atrial flutter, detrimental vascular remodeling, plaque stabilization, myocardial infarction and its sequelae, atherosclerosis, angina pectoris, renal insufficiency, renal fibrosis, polycystic kidney disease, type 2 diabetes, metabolic syndrome, secondary aldosteronism, primary and secondary pulmonary hypertension, nephrotic syndrome, diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, renal vascular hypertension, diabetic retinopathy, end-stage renal disease, migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction, glaucoma and cerebrovascular disease.

14. A method for the prevention of, delay the onset of and/or treatment of cardiovascular disorders, which method comprises administering to a patient, in need thereof, a therapeutically effective amount of the combination according to any of claims 1 to 10 and a pharmaceutically acceptable carrier.

15. A method according to Claim 14, wherein a cardiovascular disorder is selected from the group consisting of hypertension, heart failure, left ventricular dysfunction, endothelial dysfunction, diastolic dysfunction, hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, cardiac fibrosis, atrial flutter, detrimental vascular remodeling, plaque stabilization, myocardial infarction and its sequelae, atherosclerosis, angina pectoris, renal insufficiency, renal fibrosis, polycystic kidney disease, type 2 diabetes, metabolic syndrome, secondary aldosteronism, primary and secondary pulmonary hypertension, nephrotic syndrome, diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, renal vascular hypertension, diabetic retinopathy, end-stage renal disease, migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction, glaucoma and cerebrovascular disease.

16. Use of a combination according to any of claims 1 to 10 for the manufacture of a medicament for the prevention of, delay the onset of and/or treatment of cardiovascular disorders.

17. A use according to Claim 16, wherein a cardiovascular disorder is selected from the group consisting of hypertension, heart failure, left ventricular dysfunction, endothelial dysfunction, diastolic dysfunction, hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, cardiac fibrosis, atrial flutter,

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detrimental vascular remodeling, plaque stabilization, myocardial infarction and its sequelae, atherosclerosis, angina pectoris, renal insufficiency, renal fibrosis, polycystic kidney disease, type 2 diabetes, metabolic syndrome, secondary aldosteronism, primary and secondary pulmonary hypertension, nephrotic syndrome, diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, renal vascular hypertension, diabetic retinopathy, end-stage renal disease, migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction, glaucoma and cerebrovascular disease.

# Synergy between Natriuretic Peptides and Phosphodiesterase 5 Inhibitors Ameliorates Pulmonary Arterial Hypertension

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**Rationale:** Phosphodiesterase 5 (PDE5) inhibitors (e.g., sildenafil) are selective pulmonary vasodilators in patients with pulmonary arterial hypertension. The mechanism(s) underlying this specificity remains unclear, but studies in genetically modified animals suggest it might be dependent on natriuretic peptide bioactivity.

**Objectives:** We explored the interaction between PDE5 inhibitors and the natriuretic peptide system to elucidate the (patho)physiological relationship between these two cyclic GMP (cGMP)-regulating systems and potential of a combination therapy exploiting these cooperative pathways.

**Methods:** Pharmacological evaluation of vascular reactivity was conducted in rat isolated conduit and resistance vessels from the pulmonary and systemic circulation *in vitro*, and in anesthetized mice *in vivo*. Parallel studies were undertaken in an animal model of hypoxia-induced pulmonary hypertension (PH).

**Measurements and Main Results:** Sildenafil augments vasodilatation to nitric oxide (NO) in pulmonary and systemic conduit and resistance arteries, whereas identical vasorelaxant responses to atrial natriuretic peptide (ANP) are enhanced only in pulmonary vessels. This differential activity is mirrored *in vivo* where sildenafil increases the hypotensive actions of ANP in the pulmonary, but not systemic, vasculature. In hypoxia-induced PH, combination of sildenafil plus the neutral endopeptidase (NEP) inhibitor ecadotril (which increases endogenous natriuretic peptide levels) acts synergistically, in a cGMP-dependent manner, to reduce many indices of disease severity without significantly affecting systemic blood pressure.

**Conclusions:** These data demonstrate that PDE5 is a key regulator of cGMP-mediated vasodilation by ANP in the pulmonary, but not systemic, vasculature, thereby explaining the pulmonary selectivity of PDE5 inhibitors. Exploitation of this mechanism (i.e., PDE5 and neutral endopeptidase inhibition) represents a novel, orally active combination therapy for pulmonary arterial hypertension.

**Keywords:** guanylyl cyclase; cyclic GMP; nitric oxide; natriuretic peptides; neutral endopeptidase

Pulmonary arterial hypertension (PAH) is characterized by increased pulmonary arterial blood pressure, vascular remodeling of the pulmonary small arteries, right ventricular hypertrophy, and ultimately right ventricular failure (1, 2). Whether idiopathic PAH, familial PAH, or PAH associated with other diseases (e.g., congenital heart disease, HIV infection), the

## AT A GLANCE COMMENTARY

### Scientific Knowledge on the Subject

The morbidity and mortality associated with pulmonary arterial hypertension remains high; targeted therapy to exclusively dilate the pulmonary vasculature would improve current therapeutic options.

### What This Study Adds to the Field

Atrial natriuretic peptide (ANP) mediated vasodilation is augmented by phosphodiesterase 5 (PDE5) inhibition in the pulmonary vasculature, suggesting that combined use of PDE5 inhibitors and natriuretic peptide receptor activation may act synergistically to alleviate pulmonary arterial hypertension.

disease leads to premature death, in large part due to the paucity of satisfactory treatments, particularly vasodilators that selectively oppose the excessive vasoconstriction observed in the pulmonary vasculature, and agents able to reverse vascular wall remodeling. This therapeutic insufficiency is also a consequence of uncertainty regarding disease etiology. Current treatment options include prostacyclin analogs (3, 4) endothelin receptor antagonists (5, 6), and phosphodiesterase 5 (PDE5) inhibitors (i.e., sildenafil) (7), but despite these advances, 2-year mortality remains around 15% (8). As a consequence, treatments are being combined with the goal of achieving synergy in the pulmonary vascular bed without augmenting systemic effects.

The biological activity of cyclic guanosine-3',5'-monophosphate (cGMP; the intracellular second messenger that mediates the vasodilator activity of nitric oxide [NO] and natriuretic peptides) is regulated by a family of PDEs that rapidly hydrolyze this cyclic nucleotide. In the vasculature, PDE5 is believed to be the predominant PDE isoform involved in degrading cGMP; PDE5 inhibition with sildenafil increases vascular smooth muscle cGMP levels and lowers systemic and pulmonary arterial pressure ( $\overline{Ppa}$ ) under physiologic conditions in animals and humans (9, 10). In contrast, in animal models and in patients with PAH, PDE5 inhibition causes larger reductions in pulmonary compared with systemic vascular resistance, thereby exhibiting relative selectivity for the pulmonary vasculature (11–13).

The mechanism(s) underlying the pulmonary selectivity of PDE5 inhibition remain unclear. Although PDE5 inhibition abrogates functional and structural changes in many animal models of PH, this effect is blunted in the natriuretic peptide receptor (NPR)-A knockout mouse (14). This suggests that the mechanism of pulmonary selectivity of PDE5 inhibition is dependent, at least in part, on the bioactivity of natriuretic peptides (i.e., atrial natriuretic peptide (ANP) or brain natriuretic peptide [BNP]). This thesis is supported by data demonstrating

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that inhibition of natriuretic peptide activity augments the development of pulmonary hypertension (PH), whereas increasing natriuretic peptide activity attenuates PH development (14–16). Indeed, we have shown previously that inhibitors of neutral endopeptidase (NEP; an enzyme that hydrolyzes and terminates the biological activity of endogenous natriuretic peptides [17]) reduce the severity of hypoxia-induced PH (18). Such observations suggest that, in PH, release of natriuretic peptides from the heart represents a cytoprotective mechanism that reduces Ppa.

In the present study, we have explored the interaction between PDE5 inhibition and the natriuretic peptide system *in vitro* and *in vivo*, including an animal model of PH, to more fully elucidate the (patho)physiologic relationship between these two cGMP-regulating systems and the therapeutic potential of a combination drug treatment exploiting these cooperative pathways.

Some of the results of these studies have been previously reported in the form of abstracts (19, 20).

## METHODS

### Materials

N-[4-[1-(3-aminopropyl)-2-hydroxy-2-nitrosohydrazino]butyl]-1,3-propanediamine (SPER-NO) was purchased from Calbiochem (Nottingham, UK). *N*<sup>G</sup>-nitro-L-arginine methyl ester, sodium nitroprusside (SNP), acetylcholine (ACh), and rat ANP were purchased from Sigma Chemical Co (Dorset, UK). The stable prostaglandin endoperoxide analog 9,11-dideoxy-11 $\alpha$ ,9 $\alpha$ -epoxymethano-prostaglandin F<sub>2 $\alpha$</sub>  (U46619) was purchased from Affiniti (Exeter, UK). Sildenafil was extracted from proprietary tablets obtained from the pharmacy at University College London Hospital. The NEP inhibitor ecdotril was the kind gift of Dr. Johannes-Peter Stasch (Bayer AG, Wuppertal, Germany).

### Functional Pharmacologic Studies

These studies were conducted on animals that had not been exposed to hypoxia.

**Thoracic aorta and pulmonary artery.** Male rats (Sprague-Dawley; 200–250 g) were killed by cervical dislocation. The thoracic aorta and pulmonary artery (including first-order branches) were carefully removed and set up for isometric tension recordings, as described previously (21). Tissues were contracted with an approximate EC<sub>80</sub> concentration (80% of the maximal response) of U46619 and cumulative concentration–response curves were constructed to SPER-NO (1 nM–1  $\mu$ M) and ANP (1 nM–1  $\mu$ M). To investigate the functional activity of PDE5, concentration–response curves to SPER-NO and ANP were obtained in the absence (control) and presence of sildenafil (3  $\mu$ M; 30 min preincubation).

**Mesenteric and pulmonary small arteries.** Rat (Sprague-Dawley; 200–250 g; male) pulmonary (third/fourth order; diameter,  $\sim$ 100  $\mu$ m) and mesenteric (third/fourth order; diameter,  $\sim$ 180  $\mu$ m) small arteries were dissected and mounted in Mulvany-type myographs, as previously described (21). The vessels were stretched to 90% of the diameter achieved when under a transmural pressure of 15 mm Hg (pulmonary) and 100 mm Hg (systemic), respectively. Vessels were precontracted with U46619 and endothelial integrity assessed using ACh (1  $\mu$ M), as above. Concentration–response curves to SPER-NO (10 nM–10  $\mu$ M) and ANP (1 nM–1  $\mu$ M) were constructed in the absence and presence of sildenafil (3  $\mu$ M; 30 min preincubation).

**In vivo measurement of mean arterial blood pressure and right ventricular pressure (RVP).** Male C57/BL6 mice (20–25 g) were anesthetized with 1.5% isoflurane and placed supine on a thermostatically controlled heating blanket (37°C). To measure mean arterial blood pressure (MABP), the left common carotid artery was isolated and a fluid-filled (heparin; 100 U/ml diluted in 0.9% saline), 0.28-mm-internal-diameter cannula (Crichtley Electrical Products Pty Ltd, Castle Hill, Australia) introduced into the artery. To measure RVP, the right jugular vein was isolated and a fluid-filled cannula introduced into the superior vena cava and then advanced into the right ventricle. Both MABP and RVP were measured using an in-line P23 XL transducer (Viggo-Spectramed, Oxnard, CA) and recorded onto a precalibrated PowerLab system (ADInstruments, Castle Hill, Australia). The right femoral vein was cannulated for drug administration. After 10 minutes

of stabilization, mice were given 50  $\mu$ l intravenous bolus injection of ANP (10 or 100  $\mu$ g/kg) or SNP (3 or 10  $\mu$ g/kg). To examine the effects of PDE5 inhibitor on MABP and RVP responses, 50  $\mu$ l intravenous bolus injections of ANP (10 or 100  $\mu$ g/kg) or SNP (3 or 10  $\mu$ g/kg) were administered to mice that had received sildenafil (1 mg/kg; 50  $\mu$ l intravenous bolus) 30 minutes previously. Mice were used for the *in vivo* studies to provide evidence that synergy between sildenafil and ANP occurs in the pulmonary vasculature of a second species.

### Hypoxia-induced Pulmonary Hypertension

Male Sprague-Dawley rats (220 g at start of study) were divided into five groups: (1) normoxia control rats that received daily gavage with vehicle, (2) hypoxia control rats that received daily gavage with vehicle, (3) hypoxic rats that received sildenafil (30 mg/kg/d) administered in drinking water, (4) hypoxic rats that received ecdotril (60 mg/kg/d) delivered by gavage, and (5) hypoxic rats that received combination therapy of sildenafil (30 mg/kg/d) + ecdotril (60 mg/kg/d). Treatment with the appropriate drugs/vehicles was started 2 days before placement in a hypoxic chamber (10% O<sub>2</sub>). The sildenafil dose was monitored by weighing drinking bottles daily, and adjusting the concentration to maintain dose if necessary.

In further experiments, to establish a dose–response relationship for the beneficial effects of the combination therapy in PH, essentially identical studies to those described above were conducted with lower doses of sildenafil and/or higher doses of ecdotril. These were (1) sildenafil (10 mg/kg/d) plus ecdotril (60 mg/kg/d) and (2) sildenafil (30 mg/kg/d) plus ecdotril (90 mg/kg/d).

**Hemodynamic measurements.** After 2 weeks of exposure to hypoxia, right ventricular systolic pressure (RVSP) and Ppa were recorded in anesthetized animals by catheterization of the jugular vein (as above; for Ppa, the catheter was advanced from the right ventricle [RV] into the pulmonary artery). MABP was determined via catheterization of the carotid artery (as above). Animals were removed from the chamber individually and anesthetized immediately to minimize the time spent outside the hypoxic environment before hemodynamic measurement. The animal was then killed by anesthetic overdose, plasma was collected, the heart was removed, and individual chamber weights were measured to evaluate right ventricular hypertrophy (right ventricle:left ventricle plus septum [RV/LV+S] ratio). The left lung was fixed by inflation with 10% formalin in phosphate-buffered saline before paraffin embedding and sectioning. Plasma was collected by centrifugation (220  $\times$  g; 20 min) of whole blood and stored at  $-80^{\circ}$ C for measurement of cGMP levels.

**cGMP measurements.** Plasma samples were diluted in ice-cold buffer (50 mM Tris-HCl, pH 7.5, 4 mM ethylenediaminetetraacetic acid) containing 3-isobutyl-1-methyl xanthine (IBMX) (2.5 mM) and cGMP was measured by radioimmunoassay (TRK500; Amersham Little Chalfont, UK) according to the manufacturer's instructions, as we have described previously (22). Tissue samples (lung biopsies) were homogenized on ice in buffer containing IBMX (2.5 mM, as above). Homogenates were assayed to determine protein content (bicinchoninic acid) [BCA] assay; Pierce Biotechnology, Rockford, IL), deproteinized by heating, and the supernatant assayed for cGMP as above.

**ANP measurements.** Plasma ANP concentrations were determined using a specific <sup>125</sup>I-ANP radioimmunoassay (Peninsula Laboratories, Belmont, CA), after extraction with SEP-PAK C<sub>18</sub> columns (Peninsula Laboratories), according to the manufacturer's instructions.

**Morphologic analysis.** Transverse formalin-fixed lung sections were stained with van Gieson's elastic method. Pulmonary arterial muscularization was then assessed as previously described (23). Briefly, vessels of less than 100  $\mu$ m diameter were counted in each lung section, and defined according to degree of muscularization: fully muscularized (two distinct and continuous elastic lamina), partially muscularized (second elastic lamina not continuous [ $<50\%$ ]), and nonmuscularized (single elastic lamina). At least 150 vessels were counted per section and the proportion of vessels in each category was expressed as a percentage of total vessels counted. Morphometric analysis was performed by two independent blinded examiners, with an interperson variability of less than 10%. Twenty-five muscularized arteries ranging from 25–100  $\mu$ m diameter from different fields were then imaged at  $\times 400$  magnification by light microscopy from representative animals in each group.

**Data analysis.** For *in vitro* studies, relaxations are expressed as percentage of reversal of U46619-induced tone (mean  $\pm$  SEM of "n"

animals). Curves were fitted to all the data using nonlinear regression (GraphPad Software, San Diego, CA) and the  $-\log [M]$  of each drug giving a half-maximal response ( $pEC_{50}$ ) was used to compare potency. Curves were analyzed using two-way analysis of variance (ANOVA) and  $P < 0.05$  was taken as statistically significant. For *in vivo* studies, changes in MABP, RVP,  $\overline{Ppa}$ , RVSP, RV/LV ratio, and muscularization of arteries were analyzed by one-way ANOVA. Results are expressed as mean  $\pm$  SEM, and  $P < 0.05$  denotes significance.

## RESULTS

### Effect of PDE5 Inhibition on Responses to SPER-NO and ANP in Conduit Arteries

**Thoracic aorta.** Incubation of rat isolated aortic vessels with sildenafil (3  $\mu$ M) increased the potency of SPER-NO ( $pEC_{50}$ ,  $5.90 \pm 0.09$  and  $6.61 \pm 0.10$  in the absence and presence of sildenafil, respectively;  $P < 0.05$ ; Figure 1). In marked contrast, ANP was equipotent in the thoracic aorta in the absence ( $pEC_{50}$ ,  $7.33 \pm 0.16$ ) and presence of sildenafil (3  $\mu$ M;  $pEC_{50}$ ,  $7.51 \pm 0.17$ ;  $P > 0.05$  vs. control; Figure 1).

**Pulmonary artery.** After incubation of rat isolated pulmonary artery with sildenafil (3  $\mu$ M), responses to SPER-NO were significantly enhanced ( $pEC_{50}$ ,  $6.30 \pm 0.07$  and  $7.12 \pm 0.10$  in the absence and presence of sildenafil, respectively;  $P < 0.05$ ; Figure 1). Sildenafil (3  $\mu$ M) also increased the relaxant potency of ANP in isolated pulmonary artery preparations ( $pEC_{50}$ ,  $8.74 \pm 0.14$  and  $9.71 \pm 0.09$  in the absence and presence of sildenafil, respectively;  $P < 0.05$ ; Figure 1).

### Effect of PDE5 Inhibition on Responses to SPER-NO and ANP in Resistance Arteries

**Mesenteric small arteries.** After treatment of isolated mesenteric small arteries with sildenafil (3  $\mu$ M), responses to SPER-NO were significantly enhanced ( $pEC_{50}$ ,  $6.69 \pm 0.08$  and  $7.26 \pm 0.09$  in the absence and presence of sildenafil, respectively;  $P < 0.05$ ; Figure 2). In marked contrast, ANP had an equivalent relaxant potency in mesenteric small arteries in the absence and presence of sildenafil (3  $\mu$ M;  $EC_{50}$ ,  $8.71 \pm 0.18$  and  $8.46 \pm 0.18$ , respectively;  $P > 0.05$ ; Figure 2).

**Pulmonary small arteries.** SPER-NO and ANP produced concentration-dependent relaxations of U46619 precontracted pulmonary small arteries ( $pEC_{50}$ ,  $6.20 \pm 0.05$  and  $9.72 \pm 0.29$ , respectively). Responses to both vasodilators were significantly enhanced in the presence of sildenafil (3  $\mu$ M;  $pEC_{50}$ ,  $6.86 \pm 0.06$  and  $9.81 \pm 0.18$ , respectively;  $P < 0.05$  vs. control; Figure 2).

### Effect of PDE5 Inhibition on MABP and RVP Changes in Response to ANP and NO

**MABP.** As shown in Figure 3, both SNP and ANP caused dose-dependent decreases in MABP. After bolus administration of sildenafil (1 mg/kg), the hypotensive responses to SNP were significantly enhanced. In contrast, reductions in MABP in responses to ANP were not altered in the presence of sildenafil (Figure 3).

**RVP.** ANP and SNP decreased RVP in a dose-dependent manner (Figure 3). However, in the presence of sildenafil (1 mg/kg), RVP responses to both and SNP were significantly enhanced (Figure 3).

### Hypoxia-induced PH *In Vivo*

Daily weighing of the animals revealed that, after an initial weight loss on initiation of gavage and hypoxia, all animals maintained or increased body weight throughout the study, suggesting good tolerance to the hypoxia, drugs, and handling (data not shown).

### Effect of Combination Therapy on Pulmonary Artery, Right Ventricular, and Mean Arterial Pressures

In untreated control rats, 2 weeks of 10% hypoxia produced markedly elevated  $\overline{Ppa}$  ( $29.58 \pm 1.3$  mm Hg) compared with normoxia control animals ( $12.13 \pm 1.7$  mm Hg). Both sildenafil-treated ( $24.26 \pm 0.7$  mm Hg) and ecadotril-treated ( $25.45 \pm 1.1$  mm Hg) animals showed statistically significant reduction in  $\overline{Ppa}$  compared with untreated hypoxic animals (Figure 4). The combination sildenafil + ecadotril group showed a further reduction ( $20.68 \pm 0.9$  mm Hg) in  $\overline{Ppa}$  that was statistically different from both the sildenafil ( $P < 0.05$ ) and ecadotril groups

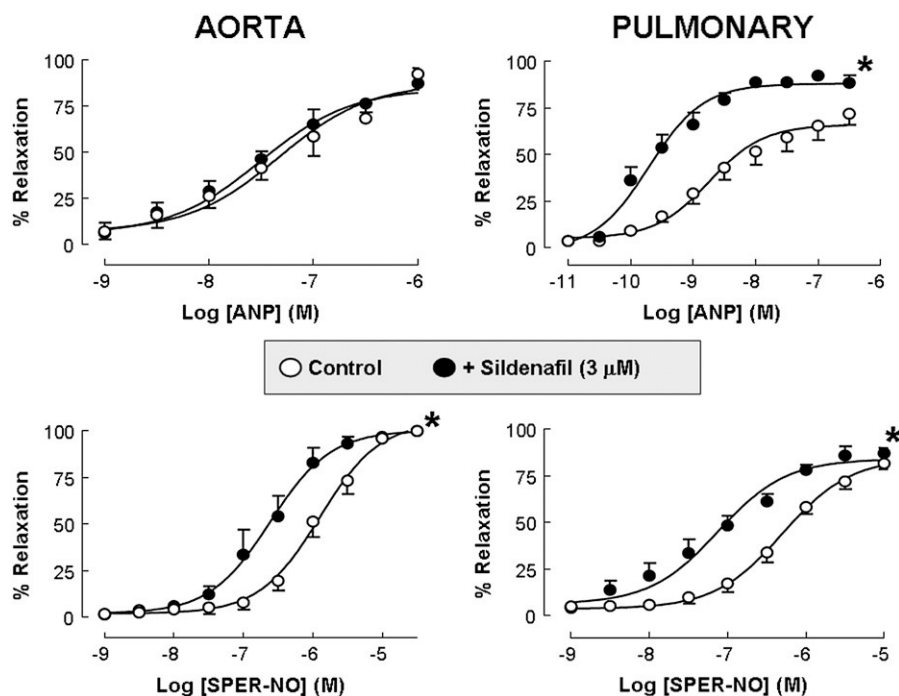


**Figure 1.** Concentration response curves to atrial natriuretic peptide (ANP) and N-[4-[1-(3-aminopropyl)-2-hydroxy-2-nitrosohydrazino]-butyl]-1,3-propanediamine (SPER-NO) in rat aorta (left panels) and pulmonary artery (right panels) in the absence (open circles) and presence of sildenafil (3  $\mu$ M; 30 min incubation; solid circles). Relaxation is expressed as mean  $\pm$  SEM percentage reversal of U46619-induced tone (\* $P < 0.05$  vs. control by two-way ANOVA comparing the entire concentration range; ANP aorta,  $n = 12$ ; ANP pulmonary,  $n = 11$ ; SPER-NO aorta,  $n = 8$ ; SPER-NO pulmonary  $n = 11$ ).

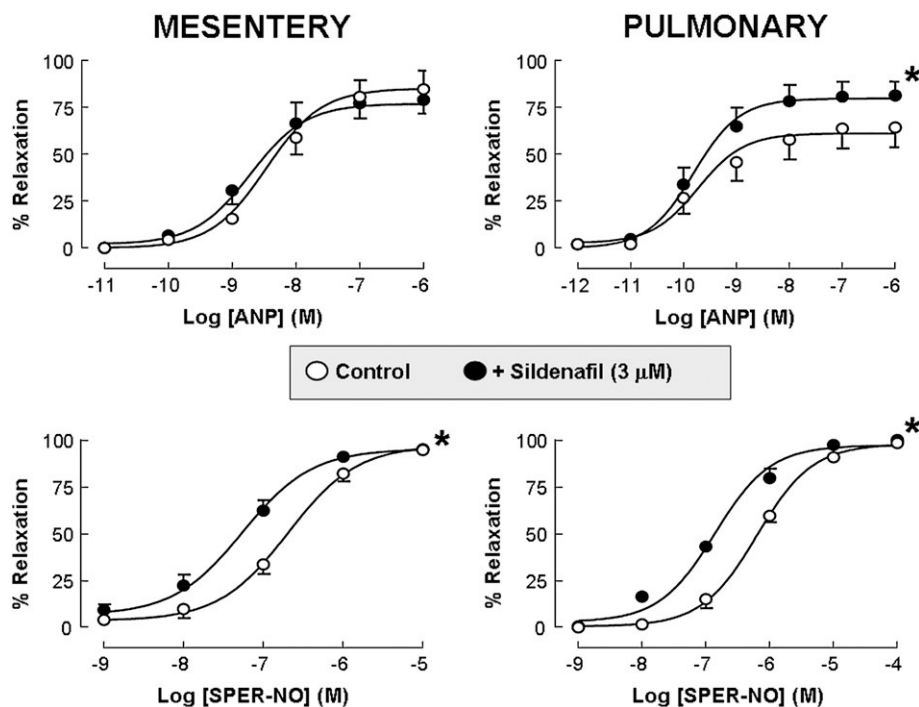


**Figure 2.** Concentration–response curves to ANP and SPER-NO in rat mesenteric (left panels) and pulmonary (right panels) small arteries in the absence (open circles) and presence of sildenafil (3  $\mu$ M; 30 min incubation; solid circles). Relaxation is expressed as mean  $\pm$  SEM percentage reversal of U46619-induced tone (\* $P$  < 0.05 vs. control by two-way ANOVA comparing the entire concentration range; ANP mesenteric,  $n$  = 9, ANP pulmonary,  $n$  = 7, SPER-NO mesenteric,  $n$  = 8, SPER-NO pulmonary,  $n$  = 5).

( $P$  < 0.01; Figure 4). Similarly, 2 weeks of hypoxia produced a doubling of the RVSP in untreated hypoxic rats ( $38.72 \pm 2.0$  mm Hg) compared with normoxia control animals ( $18.8 \pm 2.3$  mm Hg). Single treatment with ecadotril ( $41.3 \pm 1.8$  mm Hg) failed to reduce RVSP, but the sildenafil ( $36.17 \pm 1.8$  mm Hg) and sildenafil + ecadotril group showed reduced RVSP ( $32.5 \pm 1.4$  mm Hg;  $P$  < 0.05 vs. sildenafil and ecadotril alone; Figure 4).

#### Effect of Combination Therapy on Cardiac Hypertrophy

There were no significant changes in total ventricle weight across the groups (Figure 5). Untreated hypoxic animals showed a significant increase in RV/LV+S ratio (normoxia,  $0.30 \pm 0.01$ ; hypoxia,  $0.51 \pm 0.01$ ). Treatment with sildenafil ( $0.39 \pm 0.01$ ) or

ecadotril ( $0.43 \pm 0.01$ ) significantly reduced RV hypertrophy. However, combination treatment of sildenafil plus ecadotril caused an additional reduction in RV hypertrophy ( $0.37 \pm 0.01$ ;  $P$  < 0.05 vs. hypoxia, sildenafil alone and ecadotril alone; Figure 5).

#### Dose–Response Relationship between PDE5 Inhibition and NEP Inhibition in Hypoxia-induced PH

To establish a dose–response relationship for the combination therapy in PH, we conducted additional experimentation to determine the efficacy of lower sildenafil and higher ecadotril doses in alleviating the hemodynamic aberrations and cardiac hypertrophy that underscore the disease. In this case, reducing the sildenafil dose to 10 mg/kg/day produced a smaller effect on

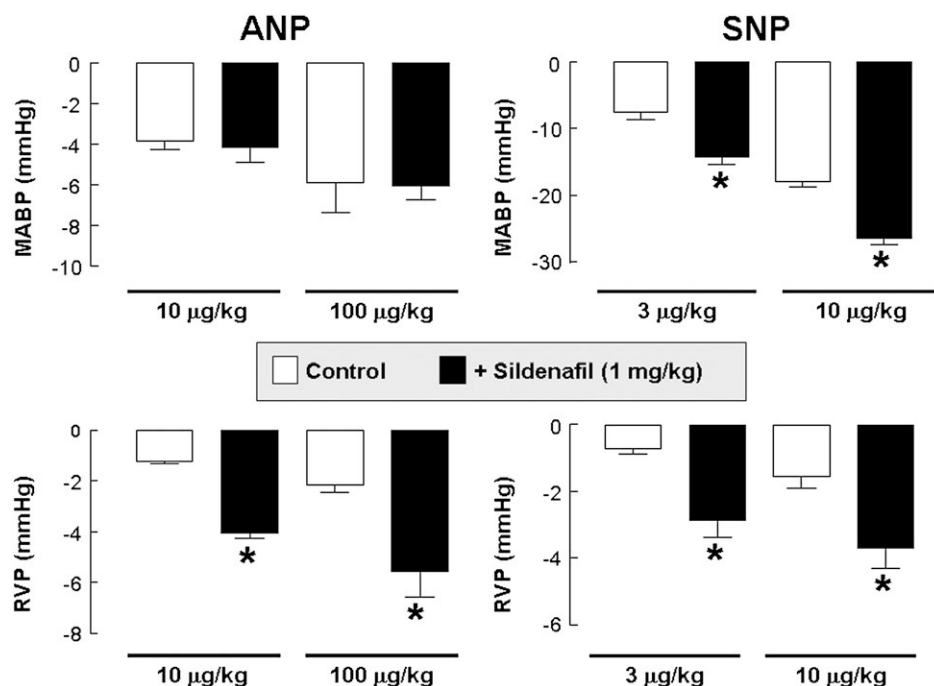


**Figure 3.** Changes in mean arterial blood pressure (MABP) (upper panels) and right ventricular pressure (RVP) (lower panels) in anesthetized mice in response to bolus injections (intravenous) of ANP (10 and 100  $\mu$ g/kg; left panels) and sodium nitroprusside (SNP) (3 and 10  $\mu$ g/kg; right panels) in the absence (open bars) and presence (solid bars) of sildenafil (1 mg/kg, intravenously; \* $P$  < 0.05 vs. corresponding control;  $n$  = 5 for each condition).

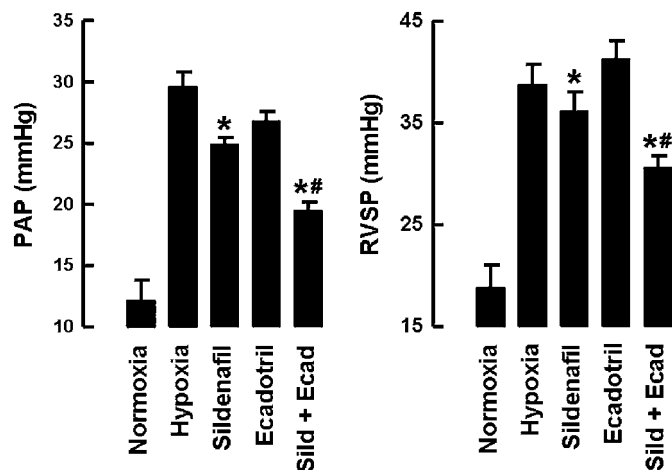


**Figure 4.** Mean pulmonary artery pressure (PAP; left panel) and right ventricular systolic pressure (RVSP; right panel) in control (normoxic) rats ( $n = 6$ ) and animals exposed to 2 weeks of hypoxia (10%  $O_2$ ;  $n = 14$ ) in the absence and presence of sildenafil (30 mg/kg/d;  $n = 14$ ), ecdotril (60 mg/kg/d;  $n = 10$ ), or sildenafil plus ecdotril ( $n = 20$ ). \* $P < 0.05$  versus hypoxic control; # $P < 0.05$  versus sildenafil and ecdotril alone.

Ppa and RV hypertrophy (Figure 6). Likewise, raising the dose of ecdotril to 90 mg/kg/day increased its activity in lowering Ppa (Figure 6). However, at all doses tested, combination therapy was more efficacious than either drug alone in reducing Ppa and, at the higher doses, RV hypertrophy (Figure 6). Importantly, all of the sildenafil and ecdotril combinations did not significantly reduce MABP (Figure 6).

#### Effect of Combination Therapy on Pulmonary Remodeling

Normoxia animals showed only a modest degree of pulmonary muscularization ( $14.09 \pm 1.9\%$ ), which was significantly increased by 2 weeks of hypoxia ( $74.07 \pm 3.1\%$ ). Treatment with sildenafil ( $65.87 \pm 2.7\%$ ) and ecdotril ( $69.83 \pm 1.2\%$ ) did not show significant attenuation of this hypoxia-induced muscularization. However, the combination of sildenafil plus ecdotril produced a significant decrease in the percentage of muscularized arteries ( $64.79 \pm 1.5\%$ ;  $P < 0.05$ ; Figure 7).

#### Effect of Combination Therapy on Plasma and Lung cGMP Concentrations

Sildenafil alone, and the combination of sildenafil plus ecdotril, significantly increased plasma cGMP levels compared with

hypoxic controls (Figure 8), whereas in lung biopsies only the combination treatment caused a significant rise in cGMP concentrations (Figure 8). Moreover, the increase in cGMP content was significantly negatively correlated to Ppa ( $r = -0.39$ ,  $P < 0.0001$ ) only in the combination (sildenafil + ecdotril) group (Figure 8).

#### Effect of Combination Therapy on Plasma ANP Concentrations

Ecdotril alone, and the combination of sildenafil plus ecdotril, significantly increased plasma ANP levels compared with hypoxic controls (Figure 8).

## DISCUSSION

Herein we attempted to unravel the endogenous mechanisms underlying the relative pulmonary selectivity of PDE5 inhibitors in PH and used an animal model of hypoxia-induced PH to determine if this pulmonary-centric profile of activity can be exploited to improve drug treatment of the disease. First, we assessed *in vitro* and *in vivo* the relative responsiveness of isolated aortic and pulmonary conduit and resistance arteries to the vasodilators NO and ANP in the absence and presence of sildenafil. An important and unexpected pattern of reactivity emerged that clearly explains the pulmonary selectivity of PDE5 inhibitors: in the pulmonary conduit and resistance arteries (i.e., pulmonary artery and pulmonary small arteries), the vasodilator activity of ANP and the NO donor SPER-NO was significantly augmented in the presence of sildenafil. This is consistent with a cGMP-dependent mechanism of relaxation invoked by both mediators, which is physiologically curtailed by the activity of PDE5, as has been previously described (24). In contrast, in the systemic conduit and resistance vasculature (i.e., aorta and mesenteric small arteries), whereas relaxant responses to SPER-NO were augmented in the presence of sildenafil, the vasoactive potency of ANP remained unchanged during PDE5 inhibition. To determine whether the studies with isolated arteries were parallel *in vivo*, we assessed the responsiveness of MABP and RVP to soluble guanylate cyclase (sGC) and particulate guanylate cyclase (pGC) activation in anesthetized mice. In this series of experiments, both SNP (NO donor) and ANP caused dose-dependent decreases in MABP and RVP. After sildenafil treatment, RVP reductions in responses to both SNP and ANP were enhanced, mirroring the augmentation observed *in vitro*. In contrast, sildenafil treatment significantly increased the fall in MABP in response

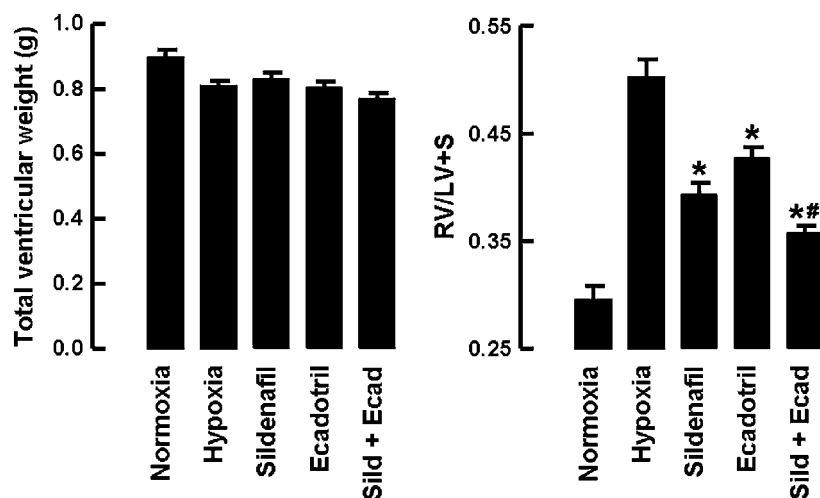


**Figure 5.** Total ventricular weight (left panel) and right ventricle: left ventricle plus septum ratio (RV/LV+S; right panel) in control (normoxic) rats ( $n = 6$ ) and animals exposed to 2 weeks of hypoxia (10%  $O_2$ ;  $n = 14$ ) in the absence and presence of sildenafil (30 mg/kg/d;  $n = 14$ ), ecdotril (60 mg/kg/d;  $n = 10$ ), or sildenafil plus ecdotril ( $n = 20$ ). \* $P < 0.05$  versus hypoxic control; # $P < 0.05$  versus sildenafil and ecdotril alone.

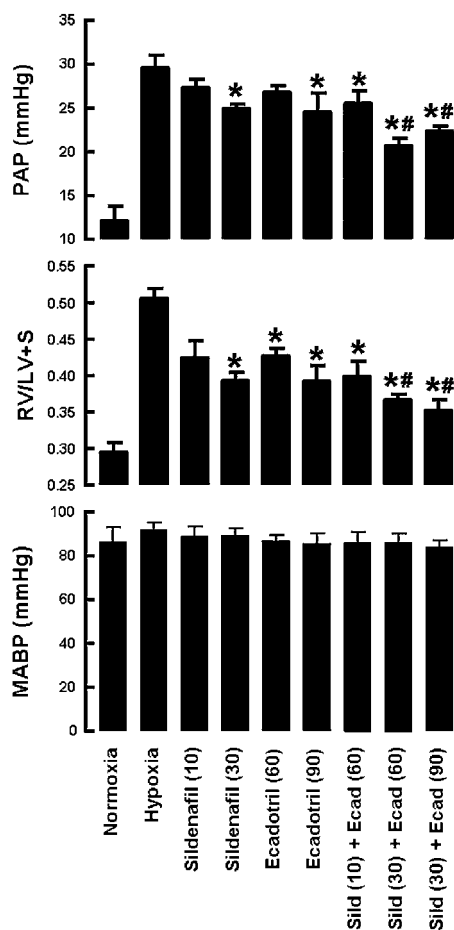


**Figure 6.** Mean pulmonary artery pressure (PAP; upper panel), right ventricle:left ventricle plus septum ratio (RV/LV+S; middle panel), and mean arterial blood pressure (MABP) (lower panel) in control (normoxic) rats ( $n = 6$ ) and animals exposed to 2 weeks of hypoxia (10%  $O_2$ ;  $n = 14$ ) in the absence and presence of sildenafil (10 or 30 mg/kg/d;  $n = 6$  and 14), ecdotril (60 or 90 mg/kg/d;  $n = 10$  and 6), or sildenafil plus ecdotril (10 and 60 mg/kg/d [ $n = 6$ ], 30 and 60 mg/kg/d [ $n = 20$ ], or 30 and 90 mg/kg/d [ $n = 6$ ]). \* $P < 0.05$  versus hypoxic control; # $P < 0.05$  versus sildenafil and ecdotril alone.

to SNP, but did not alter systemic blood pressure responses to ANP. This lack of effect of sildenafil on systemic vasodilator response to ANP (but not SNP) reiterated the observations made *in vitro* and confirmed the pulmonary-specific synergy between natriuretic peptides and PDE5 inhibitors. Indeed, a recent *in vivo* study has reported that, although sildenafil does augment the systemic hypotensive effect of ANP, this effect is mild compared with the concomitant pulmonary dilatation, and therefore severe systemic hypotension should not be a problem with this combination (25).

The above observations suggest that generation of cGMP by sGC and pGC (i.e., in response to NO and natriuretic peptides, respectively), at least in the systemic circulation, can be regulated independently and does not result in changes in a single cellular pool of cGMP. These findings fit well with previous reports revealing differential effects of pGC and sGC activation on functions within the same cells (26, 27). Indeed, recent studies have provided the rationale for such discrepant effects of the two cGMP-generating pathways (28, 29). First, it has been proposed that localization of GC enzymes within specialized structures (e.g., caveoli, vesicles, or endoplasmic

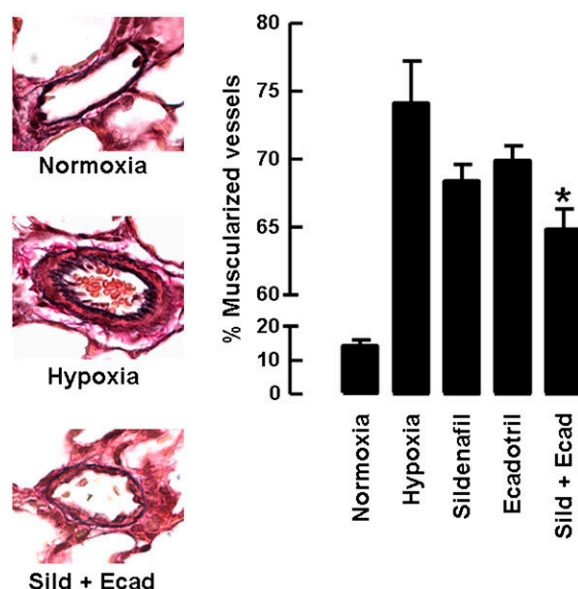


**Figure 7.** Percentage of muscularized vessels (right panel) in control (normoxic) rats ( $n = 6$ ) and animals exposed to 2 weeks of hypoxia (10%  $O_2$ ;  $n = 6$ ) in the absence and presence of sildenafil (30 mg/kg/d;  $n = 6$ ), ecdotril (60 mg/kg/d;  $n = 6$ ), or sildenafil plus ecdotril ( $n = 8$ ). \* $P < 0.05$  versus hypoxic control. The left panel depicts representative light microscopic images ( $\times 80$  original magnification) of pulmonary arteries from normoxic, hypoxic, and combination (sildenafil plus ecdotril)-treated animals; the hypoxic vessels exhibit a marked muscularization that is reduced in the presence of sildenafil plus ecdotril.

reticulum) acts as a barrier to limit the spread of cGMP, similar to mechanisms suggested for cAMP (30). Second, different PDE isoforms might be involved in preventing the diffusion of cGMP. These theses are supported by the observation that, in cardiac cells, the pGC/membrane cGMP pool is controlled exclusively by PDE2, whereas the soluble GC/cytosolic cGMP pool is controlled by PDE5 (28, 29). Our data suggest that not only does intracellular compartmentalization of cGMP occur in vascular smooth muscle but this differs between the systemic and pulmonary vasculature. Moreover, because the  $EC_{50}$  values (i.e., potency), and relative shift in the presence of sildenafil, for SPER-NO-dependent relaxation of both the pulmonary and systemic vessels are equivalent, this suggests that it is not simply greater PDE5 expression/activity in the pulmonary circulation (which has been suggested to occur in PH [31, 32]) that is responsible for the differential effect on ANP-dependent responses.

The mechanism or mechanisms responsible for regulation of ANP-dependent cGMP-mediated responses in the systemic circulation remain unclear and merit further attention. At present, it is reasonable to assume that an alternate PDE isoform fulfills this capacity. Possible candidates include PDE1 (regulates ANP-mediated dilatation in porcine aorta and has been linked to nitrate tolerance [33]), PDE2 (plays a key role in regulating cGMP production in the central nervous system [34], heart [28], and adrenal cortex [35]), and PDE3 (found in the heart, vascular smooth muscle, and platelets, and may link cGMP and cAMP pathways [36]). Whatever the case may be, this differential activity of PDE5 inhibitors on the vasoactive properties of ANP gives rise to the possibility of exerting pulmonary-specific dilation by combining a PDE5 inhibitor and a natriuretic peptide “enhancer” (i.e., exogenous

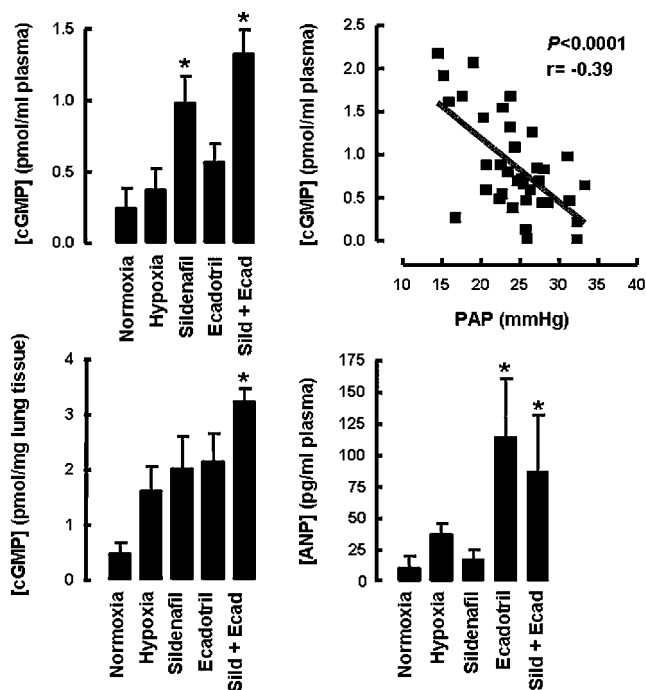


**Figure 8.** Cyclic guanosine-3',5'-monophosphate (cGMP) accumulation in the plasma (upper left panel) and lung (lower left panel), and ANP concentrations in the plasma (lower right panel) in control (normoxic) rats ( $n = 6$ ) and animals exposed to 2 weeks of hypoxia (10%  $O_2$ ;  $n = 14$ ) in the absence and presence of sildenafil (30 mg/kg/d;  $n = 14$ ), ecdotril (60 mg/kg/d;  $n = 10$ ), or sildenafil plus ecdotril ( $n = 20$ ). \* $P < 0.05$  versus hypoxic control. Correlation between cGMP and mean pulmonary artery pressure (PAP) (upper right panel) in rats exposed to 2 weeks of hypoxia (10%  $O_2$ ) in the presence of sildenafil (30 mg/kg/d) plus ecdotril (60 mg/kg/d;  $n = 36$ ).

natriuretic peptide or drug increasing endogenous natriuretic peptide levels) and exploiting the synergistic activity of these two pathways, which is absent in the systemic vasculature.

Having established the pulmonary-specific regulation of ANP-dependent responses by PDE5 inhibitors, we used a well-defined model of hypoxia-induced PH (11) to assess the efficacy of a combination treatment for PH based on a PDE5 inhibitor (sildenafil) plus an ANP enhancer. Endogenous ANP levels increase in hypoxia, possibly as a compensatory measure, although this is insufficient to prevent the development of PH (37). Administration of exogenous ANP has been shown to reduce hypoxia-induced PH (38). However, the short half-life (0.5–5 min) and negligible oral bioavailability make ANP a poor candidate for drug therapy. We therefore chose to increase endogenous ANP levels by inhibiting NEP activity with the selective drug ecdotril (39). NEP is a membrane-bound zinc metalloproteinase that degrades a number of biologically active peptides including ANP and endothelin (ET)-1 (40). Metabolic breakdown by NEP is one of two primary routes of degradation of natriuretic peptides (the second is via binding to NPR-C, internalization, and lysosomal breakdown [41]). Ecdotril is an orally active prodrug of thiorphan that has been shown to potentiate the antihypertensive, diuretic, and natriuretic effects of ANP (39).

Our results demonstrate a clear synergistic activity of sildenafil and ecdotril in alleviating many indices of disease severity in an animal model of (hypoxia-induced) PH. Using a dose of sildenafil that is similar to that used clinically, we demonstrate that monotherapy with a PDE5 inhibitor or an

NEP inhibitor partially reduces the hemodynamic and structural changes associated with the disease (11, 18); the magnitude of the protective effect of both drugs was akin to previous studies using a rat model of hypoxia-induced PH (18, 42, 43). However, in accord with our hypothesis based on pharmacologic observations made *in vitro* and *in vivo*, a combination treatment regime of sildenafil plus ecdotril has synergistic activity in PH over a range of doses (albeit at submaximally effective doses of the individual agents). This is true not only for a reduction in  $\overline{Ppa}$  (and right ventricular systolic pressure) but for RV hypertrophy and pulmonary vascular remodeling as well. The added value of this dual therapy was substantiated by the observation that both plasma and lung cGMP concentrations were significantly elevated in the combination group only, and that efficacy (i.e., reduction in  $\overline{Ppa}$ ) correlated with cGMP levels, suggesting that the combination treatment was targeting the cGMP production where it was required, the pulmonary vasculature; accordingly, the combination therapy had no significant effect on MABP. Our data therefore give credence to the idea that a combined PDE5 inhibitor plus NEP inhibitor regime may offer therapeutic benefit in the treatment of PH above and beyond sildenafil alone.

These findings are consistent with, and advance, previous studies demonstrating that ANP synergistically potentiates pulmonary vasodilation in sildenafil-treated rats exposed to acute hypoxia (44) and that the pulmonary selectivity of sildenafil is lost in NPR-A knockout animals (23). Interestingly, in the present study, plasma ANP levels in the presence of sildenafil alone (in contrast to both ecdotril and the combination treatment) were not significantly increased in response to hypoxia; as such, the beneficial effects of sildenafil in PH may not fully exploit the synergy between PDE5 inhibition and the natriuretic peptide system that we have identified (hence the added benefit of the NEP inhibitor). Undoubtedly, NEP inhibition will augment the biological activity of a number of vasoactive peptides, principally ET-1 and bradykinin. However, because ET-1 is a potent vasoconstrictor, and bradykinin produces vasodilation predominantly via activation of NO-dependent pathways, it is likely that the primary beneficial effect of NEP inhibition in PH is augmentation of endogenous natriuretic peptide levels.

In conclusion, these data suggest that the pulmonary selectivity of PDE5 inhibitors in PH is a result of the differential ability of PDE5 to regulate ANP-mediated dilation in the pulmonary, but not systemic, vasculature. This gives rise to the possibility of effecting organ- or vessel-specific alterations in the vasodilator activity of ANP, which may not only represent an important physiologic regulatory mechanism but also be of therapeutic benefit in PH. In accord, we demonstrate that the combination of natriuretic peptide augmentation (i.e., ecdotril) and PDE5 inhibition (i.e., sildenafil) selectively produces pulmonary vasodilation in the absence of systemic hypotension and this strategy is synergistically effective in improving several indices of disease severity in an animal model of PH. Indeed, a recent study has revealed synergistic activity between sildenafil and BNP in patients with PH (45), suggesting this phenomenon is likely to occur in the human vasculature. Thus, this drug combination could be a novel therapeutic avenue for PH, and given the availability of both agents as licensed drugs, there are realistic translational opportunities.

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