

CASTELLANOS & Co.  
ABOGADOS  
Calle 94 A No. 7 A - 09  
Bogotá, D.C., COLOMBIA

286  
~~286~~  
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 04-120497- -00010-0000

Fecha: 2008-07-15 14:49:09 Dep. 2020 NUECREACIONE  
Tra. 11 PATENTEYII Eve: 379 FASENACIONALI  
Act. 444 RESPUESTAREQUE Folios: 12

Señora

Jefe de la División de Nuevas Creaciones

Superintendencia de Industria y Comercio

Ministerio de Comercio, Desarrollo Industria y Turismo

E.

S.

D.

Ref.: Expediente No. 04.120.497

Solicitud de Patente PCT entrada fase nacional para: **DERIVADOS DE N-((3-OXO-2,3-DIHIDRO-1H-ISOINDOL-1-IL)ACETIL)GUANIDINA EN CALIDAD DE INHIBIDORES DE NHE-1 PARA EL TRATAMIENTO DE INFARTO Y LA ANGINA DE PECHO**

Solicitante: **SANOFI-AVENTIS DEUTSCHLAND GMBH**

### RESPUESTA A EXAMEN DE FONDO

**LUIS PATIÑO LEIVA**, mayor de edad, identificado y domiciliado como aparece al pie de mi firma, en atención a su oficio No. 3172 notificado el 29 de febrero de 2008, para el cual solicite una prórroga el día 28 de mayo de 2008, atentamente me permito dar repuesta al mismo de la siguiente manera:

#### **Claridad de las reivindicaciones y unidad de invención**

*Ese despacho considera que el capítulo reivindicatorio se presenta de forma muy amplia puesto que se hace referencia a términos de categorías funcionales y químicas que no permiten determinar con precisión el contenido y alcance de la invención. Además, concluye que la invención no tiene unidad de invención.*

Con el objeto de superar las objeciones por claridad, presento un nuevo pliego reivindicatorio en el cual el compuesto de la reivindicación 1 se ha limitado a los sustituyentes definidos en la anterior reivindicación 3. Adicionalmente, para R1 o R2 se incluye el sustituyente SO<sub>2</sub>R7 en donde R7 es alquilo de 1, 2, 3 o 4 átomos de carbono.

287  
~~287~~

**CASTELLANOS & Co.**  
ABOGADOS  
Calle 94 A No. 7 A - 09  
Bogotá, D.C., COLOMBIA

Los compuestos de fórmula I así definidos, están enteramente sustentados por los ejemplos 1 a 56b de la solicitud.

Por otro lado, se han eliminado algunos compuestos de las anteriores reivindicaciones 4 y 5, los cuales no están cubiertos por la nueva reivindicación 1.

En conclusión, el nuevo pliego reivindicatorio tal y como se modificó sí cumple con el requisito de claridad y concisión estipulado por el artículo 30 de la Decisión 486 y con el requisito de unidad de invención del artículo 25 de dicha Decisión, puesto que para el compuesto reivindicado se define una lista limitada de posibles sustituyentes.

#### **Reivindicaciones relativas a un uso**

Las objeciones sobre reivindicaciones de uso se deben tener por superadas toda vez que las reivindicaciones 7 y 10 a 18 se han eliminado del pliego reivindicatorio.

#### **Reivindicaciones de proceso**

En atención al requerimiento de ese Despacho, en las nuevas reivindicaciones 7 a 10 se han incluido las condiciones de proceso necesarias para llevar a cabo las reacciones de cada etapa, las cuales están debidamente soportadas por la descripción.

Cumplido así con lo dispuesto, solicito que se realice el examen de patentabilidad a la presente invención toda vez que se han superado las objeciones hechas a la claridad de la solicitud, de tal manera que es posible compararla y diferenciarla del estado de la técnica para verificar los requisitos de patentabilidad y, a su vez, es posible determinar sin ambigüedad el alcance de la invención.

#### **Anexos**

Nuevo capítulo reivindicatorio  
Formulario de modificaciones  
Recibo correspondiente

2

De la Señora Jefe

*Luis Pantoja Leyva*

Luis Pantoja Leyva

C.C. No. 2.935.816 de Bogotá

T.P.A. No. 2598 del C. Sup. de la Judicatura

Calle 94ª # 7ª-09

Teléfono: 8187420

15 JUL. 2008

FORMULARIO ÚNICO DE CORRECCIONES Y MODIFICACIONES

1

SOLICITUD DE

☐

Corrección de Error Material

☒

Modificación a una solicitud

2

Nombre: SANOFI-AVENTIS DEUTSCHLAND GMBH

IDENTIFICACIÓN

Dirección: Brüningstrasse 50  
Frankfurt am main  
Alemania

C.C.

☐

NIT

☐

Domicilio: Frankfurt am main  
Alemania

C.E.

☐

Otro

☐

Lugar de Constitución: Alemania

Cual

Teléfono:

Fax:

Número

E-mail:

3

REPRESENTANTE  
O APODERADO

Nombre: LUIS PATIÑO LEYVA

IDENTIFICACIÓN

Dirección: Calle 94A # 7A-09

C.C.

☒

NIT

☐

Teléfono: 610 74 20 Fax: 610 74 80

C.E.

☐

Otro

☐

E-mail: [info@castellanosyco.com](mailto:info@castellanosyco.com)

Cual

Número

2.930.616

TPA

2698

4

Número de Expediente:

04.120.497

5

Descripción de la corrección o modificación solicitada:

Presento nuevo capítulo reivindicatorio sobre el cual solicito se realice el examen de patentabilidad

6

Comprobante de pago No.:

03.52.138

Fecha:

01/07/2008

7

Anexos:

☒

Comprobante de pago de la tasa.

☐

Poderes si fuere el caso, junto con la sustitución de los mismos a mi favor.

☐

Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas

☒

Nuevo capítulo reivindicatorio

8

NOMBRE:

LUIS PATIÑO LEYVA

FIRMA:

CC 2.930.616 de Bogotá - TPA 2698-06J

20160 200  
290

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO**

NET : 000174089-2

RECIBO OFICIAL DE CAJA : 05 - 52,130  
FECHA : JULIO 1 DE 2009

**1000 CONVENCIÓN 1000**

| DEPOSITANTE        | TIPO PAGO  | BANCO           | CUENTA   | No. PAGU | FECHA PAG  | VR. PAGU     |
|--------------------|------------|-----------------|----------|----------|------------|--------------|
| OLVARO CASTELLANOS | CONVENCIÓN | BANCO DE BOGOTÁ | 01274487 | 07475    | 07/07/2009 | 4,471,000.00 |

**1000 CONCEPTO 1000**

| CANT. | RENTISTICO  | CONCEPTO                        | TOTAL CONCEPTO |
|-------|-------------|---------------------------------|----------------|
| 1     | 00005-01-05 | CREDITO DE MODALIDAD Y MODIFICA | 103,000.00     |
|       |             | 12 MARCAS Y LINEAS COMERCIALES  | 103,000.00     |
|       |             | TOTAL                           | 103,000.00     |

BON: CIENTO TRES MIL PESOS

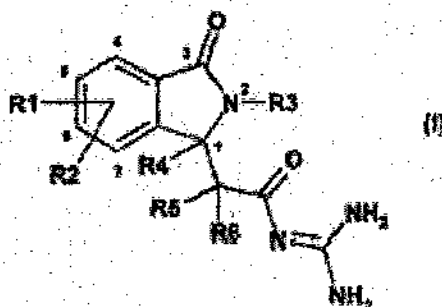
RESPONSABLE :

RECIBO DE CAJA APLICADO AL EFICIENTE No.

291  
291

# REIVINDICACIONES MODIFICADAS

1. Compuesto de la fórmula I



En el cual

R1 y R2 son, independientemente uno de otro, hidrógeno, alquilo que tiene 1, 2, 3 ó 4 átomos de carbono, F, Cl, Br, I, OH, alcoxi que tiene 1, 2, 3 o 4 átomos de carbono, polifluoroalquilo que tiene 1, 2, 3 o 4 átomos de carbono o polifluoroalcoxi que tiene 1, 2 o 3 átomos de carbono, o SO<sub>2</sub>R7 en donde R7 es alquilo que tiene 1, 2, 3 ó 4 átomos de carbono,

Estando R1 y R2 opcionalmente sustituidos por un alquilo lineal o ramificado que tiene 1, 2, 3 ó 4 átomos de carbono;

R3 es un grupo del tipo Alk-R8 o cicloalquilo que tiene 3, 4, 5, 6, 7 ó 8 átomos de carbono, en el cual cicloalquilo está sustituido opcionalmente por uno o más sustituyentes seleccionados del grupo F o Cl,

Alk es un alquilo que tiene 1, 2, 3, 4 o 5 átomos de carbono en una cadena lineal o ramificada,

R8 es hidrógeno, fenil, cicloalquilo que tiene 3, 4, 5, 6, 7 o 8 átomos de carbono o polifluoroalquilo que tiene 1, 2, 3 ó 4 átomos de carbono;

R4, R5 y R6 son, independientemente uno del otro, hidrógeno o un alquilo lineal o ramificado que tiene 1, 2, 3 o 4 átomos de carbono;

y mezclas racémicas, enantiómeros y diastereoisómeros de los mismos y sus mezclas, sus tautómeros y sus sales farmacéuticamente aceptables.

2. Un compuesto de acuerdo con la reivindicación 1, caracterizado porque se selecciona de:

- N-[2-(2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il)-2-metilpropionil] guanidina,
- N-[2-(2-ciclopropilmetil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il)-2-metil- propionil] guanidina,
- N- [ (3-oxo-2- (3, 3, 3-trifluoropropil)-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,
- N- [ (3-oxo-2- (4, 4, 4-trifluorobutil)-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,
- N- [ (3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,
- N-[(2-isobutil-7-metil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,

N-[(4-hidroxi-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(5-hidroxi-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(6-hidroxi-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(7-hidroxi-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(4, 7-dicloro-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(4-fluoro-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(5-fluoro-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(6-fluoro-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(4, 5-difluoro-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(6,7-difluoro-2-isobutil-3-oxo-2,3-dihidro-1H-isoindol-1-il)acetil] guanidina,  
 N-[(2-isobutil-1-metil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina  
 y mezclas racémicas, enantiómeros y diastereoisómeros de los mismos y sus mezclas,  
 sus tautómeros y sus sales farmacéuticamente aceptables.

3. Un compuesto de acuerdo con la reivindicación 1, caracterizado porque se selecciona de:

N-[(2-metil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(2-etil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(3-oxo-2-propil-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(2-(3-oxo-2-propil-2,3-dihidro-1H-isoindol-1-il)propionil] guanidina,  
 N-[(2-isopropil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(2-(2-butil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) propionil] guanidina,  
 N-[(2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(2-(2-isobutil-3-oxo-2,3-dihidro-1H-isoindol-1-il)propionil] guanidina,  
 N-[(2-ciclopropilemtil-3-oxo-2,3-dihidro-1H-isoindol-1-il)acetil] guanidina,  
 N-[(2-bencil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(3-oxo-2-(2, 2, 2-trifluoroetil)-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(2-isobutil-4-metil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(2-isobutil-5-metil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(2-isobutil-6-metil-3-oxo-2,3-dihidro-1H-isoindol-1-il)acetil] guanidina,  
 N-[(5-terc-butil-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(6-terc-butil-2-isobutil-3-oxo-2,3-dihidro-1H-isoindol-1-il)acetil] guanidina,  
 N-[(2-isobutil-5-isopropoxi-3-oxo-2,3-dihidro-1H-isoindol-1-il)acetil] guanidina,  
 N-[(5-cloro-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(6-cloro-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(5-cloro-2-ciclopropilmetil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(6-cloro-2-ciclopropilmetil-3-oxo-2,3-dihidro-1H-isoindol-1-il)acetil] guanidina,  
 N-[(5-cloro-3-oxo-2-(2, 2, 2-trifluoroetil)-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(6-cloro-3-oxo-2-(2, 2, 2-trifluoroetil)-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(5-cloro-3-oxo-2-(3, 3, 3-trifluoropropil)-2, 3-dihidro-1 H-isoindol-1-il) acetil]-  
 guanidina,

- N-[(6-cloro-3-oxo-2-(3, 3, 3-trifluoropropil)-2, 3-dihidro-1 H-isoindol-1-il) acetil]- guanidina,  
 N- [ (5-cloro-3-oxo-2- (4, 4, 4-trifluorobutil)-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(6-cloro-3-oxo-2-(4, 4, 4-trifluorobutil)-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N- [ (5, 6-dicloro-2-isobutil-3-oxo-2, 3-dihidro- 1 H-isoindol-1-il) acetil] guanidina,  
 N- [ (7-fluoro-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N- [ (4, 7-difluoro-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N- [ (5-bromo-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(6-bromo-2-isobutil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(2-isobutil-3-oxo-5-trifluorometil-2, 3-dihidro-1 H-isoindol-1-il) acetil] guanidina,  
 N-[(2-isobutil-3-oxo-6-trifluorometil-2,3-dihidro-1H-isoindol-1-il)acetil] guanidina,  
 N-[(2-ciclopropilmetil-3-oxo-5-trifluorometil-2, 3-dihidro-1 H-isoindol-1-il) acetil]- guanidina,  
 N-[(2-ciclopropilmetil-3-oxo-6-trifluorometil-2,3-dihidro-1H-isoindol-1-il)acetil]- guanidina,  
 N- [ (3-oxo-2- (2, 2, 2-trifluoroetil)-6-trifluorometil-2, 3-dihidro-1 H-isoindol-1-il) acetil]-  
 guanidina,  
 N- [ (3-oxo-2- (2, 2, 2-trifluoro-etil)-5-trifluorometil-2, 3-dihidro-1 H-isoindol-1-il)- acetil]-  
 guanidina,  
 N- [ (3-oxo-5-trifluorometil-2- (3, 3, 3-trifluoropropil)-2, 3-dihidro-1 H-isoindol-1-il) acetil]-  
 guanidina,  
 N- [ (3-oxo-6-trifluorometil-2- (3, 3, 3-trifluoropropil)-2, 3-dihidro-1 H-isoindol-1-il) acetil]-  
 guanidina,  
 N- [ (3-oxo-2- (4, 4, 4-trifluorobutil)-5-trifluorometil-2, 3-dihidro-1 H-isoindol-1-il) acetil]-  
 guanidina,  
 N- [ (3-oxo-2- (4, 4, 4-trifluorobutil)-6-trifluorometil-2, 3-dihidro-1 H-isoindol-1-il) acetil]-  
 guanidina,  
 N- {2- [6-Metanosulfonil-3-oxo-2- (2, 2, 2-trifluoro-etil)-2, 3-dihidro-1 H-isoindol-1-il]- acetil]-  
 guanidina,  
 N- [2- (2-ciclopropilmetil-6-metanesulfonil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il)- acetil]-  
 guanidinium,  
 N- {2- [5, 6-difluoro-3-oxo-2- (3, 3, 3-trifluoro-propil)-2, 3-dihidro-1 H-isoindol-1-il]-acetil]-  
 guanidina,  
 N- {2- [5, 6-dicloro-3-oxo-2- (3, 3, 3-trifluoro-propil)-2, 3-dihidro-1 H-isoindol-1-il]-acetil]-  
 guanidina,  
 N- [2- (5, 6-dicloro-2-ciclopropilmetil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il)-acetil]- guanidina,  
 N- [2- (5, 6-dicloro-2-ciclopropil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il)-acetil]-guanidina,  
 N- {2- [5, 6-dicloro-3-oxo-2- (2, 2, 2-trifluoro-etil)-2,3-dihidro-1H-isoindol-1-il]- acetil]-  
 guanidina,  
 N- {2- [5, 6-difluoro-3-oxo-2- (2, 2, 2-trifluoro-etil)-2, 3-dihidro-1 H-isoindol-1-il]-acetil]-  
 guanidina

y mezclas racémicas, enantiómeros y diastereoisómeros de los mismos y sus mezclas,  
 sus tautómeros y sus sales farmacéuticamente aceptables.

4. Un compuesto de acuerdo con la reivindicación 1, caracterizado porque se selecciona de:

(R)- N-{2-[6-Metanosulfonil-3-oxo-2-(2, 2, 2-trifluoro-etil)-2,3-dihidro-1H-isoindol-1-il]-acetil}-guanidina,

(S)- N-{2-[6-Metanosulfonil-3-oxo-2-(2, 2, 2-trifluoro-etil)-2, 3-dihidro-1 H-isoindol-1-il]-acetil}-guanidina,

(R)- N-[2-(2-ciclopropilemtil-3-oxo-6-trifluorometil-2,3-dihidro-1H-isoindol-1-il)- acetil]-guanidina,

(S)- N-[2-(2-ciclopropilemtil-3-oxo-6-trifluorometil-2,3-dihidro-1H-isoindol-1-il)- acetil]-guanidina,

(R)-N- {2- [5, 6-difluoro-3-oxo-2- (3, 3, 3-trifluoro-propil)-2, 3-dihidro-1 H-isoindol-1-il]-acetil}-guanidina,

(S)-N- {2- [5, 6-difluoro-3-oxo-2- (3, 3, 3-trifluoro-propil)-2, 3-dihidro-1 H-isoindol-1-il]-acetil}-guanidina,

(R)-N- {2- [5, 6-dicloro-3-oxo-2- (3, 3, 3-trifluoro-propil)-2, 3-dihidro-1 H-isoindol-1-il]-acetil}-guanidina,

(S)-N- {2- [5, 6-dicloro-3-oxo-2- (3, 3, 3-trifluoro-propil)-2, 3-dihidro-1 H-isoindol-1-il]-acetil}-guanidina,

(R)-N- [2- (5, 6-dicloro-2-ciclopropilmetil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il)-acetil]-guanidina,

(S)-N- [2- (5, 6-dicloro-2-ciclopropilmetil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il)-acetil]-guanidina,

(R)-N- [2- (5, 6-dicloro-2-ciclopropil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il)-acetil]- guanidina,

(S)-N- [2- (5, 6-dicloro-2-ciclopropil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il)-acetil]- guanidina,

(R)-N- {2- [5, 6-dicloro-3-oxo-2- (2, 2, 2-trifluoro-etil)-2, 3-dihidro-1H-isoindol-1-il]- acetil}-guanidina,

(S)-N- {2- [5, 6-dicloro-3-oxo-2- (2, 2, 2-trifluoro-etil)-2,3-dihidro-1H-isoindol-1-il]- acetil}-guanidina,

(R)-N- {2- [3-oxo-2- (2, 2, 2-trifluoro-etil)-6-trifluorometil-2, 3-dihidro-1H-isoindol-1-il]-acetil}-guanidina,

(S)-N- {2- [3-oxo-2- (2, 2, 2-trifluoro-etil)-6-trifluorometil-2,3-dihidro-1H-isoindol-1-il]- acetil}-guanidina,

(R)-N- {2- [3-oxo-2- (2, 2, 2-trifluoro-etil)-5-trifluorometil-2,3-dihidro-1H-isoindol-1-il]- acetil}-guanidina,

(S)-N- {2- [3-oxo-2- (2, 2, 2-trifluoro-etil)-5-trifluorometil-2, 3-dihidro-1 H-isoindol-1-il]-acetil}-guanidina,

(R)-N- [2- (6-cloro-3-oxo-2- (3, 3, 3-trifluoropropil)-2, 3-dihidro-1 H-isoindol-1-il) acetil]-guanidina,

(S)-N- [2- (6-cloro-3-oxo-2- (3, 3, 3-trifluoropropil)-2,3-dihidro-1H-isoindol-1-il)acetil]-guanidina,

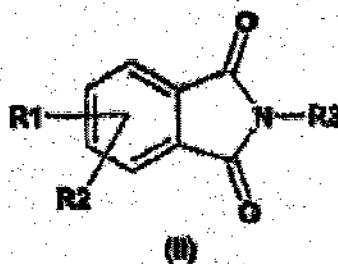
- (R)-N- [2- (5-cloro-3-oxo-2- (3, 3, 3-trifluoropropil)-2, 3-dihidro-1 H-isoindol-1-il) acetil]-guanidina,
- (S)-N- [2- (5-cloro-3-oxo-2- (3, 3, 3-trifluoropropil)-2, 3-dihidro-1 H-isoindol-1-il) acetil]-guanidina,
- (R)- N-{2-[3-oxo-6-trifluorometil-2-(3, 3, 3-trifluoro-propil)-2, 3-dihidro-1 H-isoindol-1- il]-acetil}-guanidina,
- (S)-N- {2- [3-oxo-6-trifluorometil-2- (3, 3, 3-trifluoro-propil)-2, 3-dihidro-1 H-isoindol-1- il]-acetil}-guanidina,
- (R)-N- {2- [3-oxo-5-trifluorometil-2- (3, 3, 3-trifluoro-propil)-2, 3-dihidro-1 H-isoindol-1- il]-acetil}-guanidina,
- (S)-N- {2- [3-oxo-5-trifluorometil-2- (3, 3, 3-trifluoro-propil)-2, 3-dihidro-1 H-isoindol-1- il]-acetil}-guanidina,
- (R)-N- {2- [6-cloro-3-oxo-2- (4, 4, 4-trifluoro-butil)-2, 3-dihidro-1 H-isoindol-1-il]-acetil}-guanidina,
- (S)-N- {2- [6-cloro-3-oxo-2- (4, 4, 4-trifluoro-butil)-2, 3-dihidro-1 H-isoindol-1-il]-acetil}-guanidina,
- (R)-N- {2- [5-cloro-3-oxo-2- (4, 4, 4-trifluoro-butil)-2, 3-dihidro-1 H-isoindol-1-il]-acetil}-guanidina,
- (S)-N- {2- [5-cloro-3-oxo-2- (4, 4, 4-trifluoro-butil)-2, 3-dihidro-1 H-isoindol-1-il]-acetil}-guanidina,
- (R)-N- [2- (6-cloro-2-ciclopropilmetil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il)-acetil]- guanidina,
- (S)-N- [2- (6-cloro-2-ciclopropilmetil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il)-acetil]- guanidina,
- (R)-N- [2- (5-cloro-2-ciclopropilmetil-3-oxo-2, 3-dihidro-1H-isoindol-1-il)-acetil]- guanidina,
- (S)-N- [2- (5-cloro-2-ciclopropilmetil-3-oxo-2, 3-dihidro-1 H-isoindol-1-il)-acetil]- guanidina

5. Una composición farmacéutica para uso humano, veterinario y/o fitoprotector que comprende una cantidad eficaz de un compuesto de la fórmula I y/o sus sales farmacéuticamente aceptables de acuerdo con una o más de las reivindicaciones 1 a 4, junto con un medio farmacéuticamente aceptable.

6. Una composición farmacéutica para uso humano, veterinario y/o fitoprotector que comprende una cantidad eficaz de un compuesto de la fórmula I y/o sus sales farmacéuticamente aceptables de acuerdo con una o más de las reivindicaciones 1 a 4, junto con un medio farmacéuticamente aceptable en combinación con otros ingredientes farmacológicamente activos o medicamentos.

7. Un proceso para la preparación de los compuestos de la formula I como se define en las reivindicaciones 1 a 4, en donde R4 y R6 son hidrógeno, caracterizado porque:

a) un hidruro complejo se hace reaccionar con una ftalamida (de la fórmula II, en la que R1, R2 y R3 tienen la misma definición que en la reivindicaciones 1, a una temperatura entre 0° y la temperatura de ebullición de la mezcla de reacción,

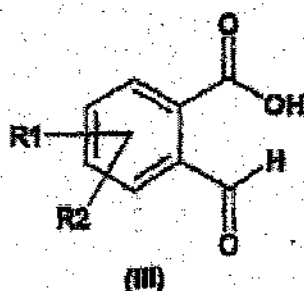


b) el producto obtenido se hace reaccionar luego con una alcóxicarbonilmetileno-trifenil-fosforano en tolueno a una temperatura entre 20° y la temperatura de ebullición de la mezcla de reacción, o con un fosonoacetato de trialquilo y una base, a una temperatura entre 0° y la temperatura de ebullición de la mezcla de reacción,

c) el producto obtenido se hace reaccionar con cloruro de guanidinio y una base a una temperatura entre 20° y la temperatura de ebullición de la mezcla de reacción o con guanidina v.g., en un alcohol que tiene 1, 2, 3 o 4 átomos de carbono, a una temperatura entre 20° y la temperatura de ebullición de la mezcla de reacción.

8. Un proceso para la preparación de los compuestos de la fórmula I como se define en las reivindicaciones 1 a 4, en donde R4 y R6 son hidrógeno, caracterizado porque:

a) un compuesto de fórmula III (R1 y R2 tienen la misma definición de las reivindicaciones 1 a 4) se hace reaccionar con alcóxicarbonilmetileno-trifenil-fosforano en tolueno a una temperatura entre 20° y la temperatura de ebullición de la mezcla de reacción, o con un fosonoacetato de trialquilo y una base a una temperatura entre 0° y la temperatura de ebullición de la mezcla de reacción,



b) el producto obtenido se hace reaccionar con una amina de fórmula R3NH<sub>2</sub> (R3 la misma definición de las reivindicaciones 1 a 4) y una carbodiimida a una temperatura entre 0° y la temperatura de ebullición de la mezcla de reacción,

c) el producto obtenido se hace reaccionar con cloruro de guanidinio y una base a una temperatura entre 20° y la temperatura de ebullición de la mezcla de reacción o, con

guanidina e.g., en un alcohol que tiene 1, 2, 3 o 4 átomos de carbono a una temperatura entre 20° y la temperatura de ebullición de la mezcla de reacción.

9. Un proceso para la preparación de los compuestos de la fórmula I como se define en las reivindicaciones 1 a 4, en donde R<sub>4</sub> es alquilo y R<sub>6</sub> es hidrógeno, caracterizado porque:

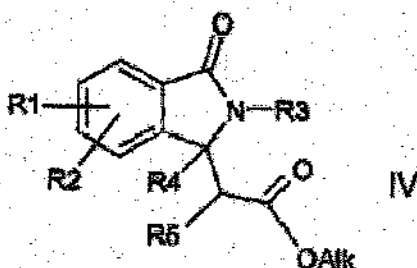
a) una ftalamida (de la fórmula II como se define en la reivindicación 7) se hace reaccionar con un haluro de alquilmagnesio o con un reactivo de alquil-litio en un éter a una temperatura entre 0° y la temperatura de ebullición de la mezcla de reacción

b) el producto obtenido se hace reaccionar luego con un alcóxicarbonilmetileno-trifenilfosforano en tolueno a una temperatura entre 20° y la temperatura de ebullición de la mezcla de reacción, o con 1-etoxi-1-trimetilsiloxietileno y un ácido de Lewis a una temperatura entre -78°C y 20°C.

c) el producto obtenido se hace reaccionar con cloruro de guanidinio y una base a una temperatura entre 20° y la temperatura de ebullición de la mezcla de reacción o, con guanidina e.g., en un alcohol que tiene 1, 2, 3 o 4 átomos de carbono a una temperatura entre 20° y la temperatura de ebullición de la mezcla de reacción.

10. Un proceso para la preparación de los compuestos de la formula I como se define en las reivindicaciones 1 a 4, en donde R6 es un grupo alquilo, caracterizado porque:

a) Un compuesto de la formula IV (R1 a R5 tienen las mismas definiciones de la reivindicación 1), se hace reaccionar en la presencia de diisopropilamido de litio, con un R6-Hal, donde Hal es F, Cl, Br o I, a una temperatura entre -78°C y 0°C.



b) el producto obtenido se hace reaccionar con cloruro de guanidinio y una base a una temperatura entre 20° y la temperatura de ebullición de la mezcla de reacción o, con guanidina e.g., en un alcohol que tiene 1, 2, 3 o 4 átomos de carbono a una temperatura entre 20° y la temperatura de ebullición de la mezcla de reacción.

298  
298  
D<sub>2</sub>  
D<sub>1</sub>.

## Discovery of Zoniporide: A Potent and Selective Sodium–Hydrogen Exchanger Type 1 (NHE-1) Inhibitor with High Aqueous Solubility

Angel Guzman-Perez,\* Ronald T. Wester, Mary C. Allen, Janice A. Brown, Allan R. Buchholz, Ewell R. Cook, Wesley W. Day, Ernest S. Hamanaka, Scott P. Kennedy, Delvin R. Knight, Paul J. Kowalczyk, Ravi B. Marala, Christian J. Mularski, William A. Novomisle, Roger B. Ruggeri, W. Ross Tracey and Roger J. Hill

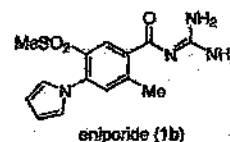
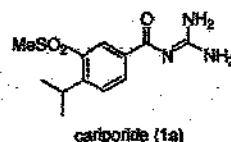
Pfizer Global Research and Development, Pfizer Inc., Eastern Point Road, Groton CT 06340, USA

Received 20 October 2000; accepted 24 January 2001

**Abstract**—Zoniporide (CP-597,396) is a potent and selective inhibitor of NHE-1, which exhibits high aqueous solubility and acceptable pharmacokinetics for intravenous administration. The discovery, synthesis, activities, and rat and dog pharmacokinetics of this compound are presented. The potency and selectivity of zoniporide may be due to the conformation that the molecule adopts due to the presence of a cyclopropyl and a 5-quinolinyl substituent on the central pyrazole ring of the molecule. © 2001 Elsevier Science Ltd. All rights reserved.

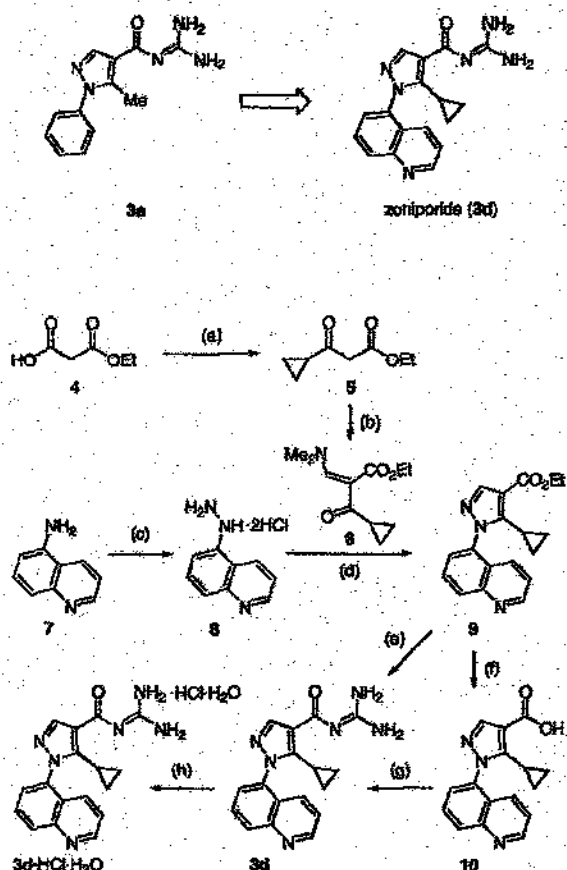
The sodium–hydrogen exchangers (NHEs) are cell membrane transport proteins which exchange extracellular  $\text{Na}^+$  for intracellular  $\text{H}^+$  driven by concentration gradients. NHEs play a key role in intracellular pH regulation, ion-transport and control of cell volume. There are at least six known isoforms that are designated NHE-1 through NHE-6.<sup>1</sup> NHE-1 appears to play a key role in mediating myocardial injury during periods of ischemia and reperfusion. Although there is some debate about the degree of NHE-1 activity during myocardial ischemia, the activation of this exchanger during reperfusion is clearly detrimental to the heart, due to a cascade of increased intracellular  $\text{Na}^+$ , activation of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger, and intracellular  $\text{Ca}^{2+}$  overload. Consequently, inhibition of NHE-1, the sole isoform present in the heart, represents an attractive approach for reducing myocardial injury during ischemia/reperfusion.<sup>2,3</sup> A selective NHE-1 inhibitor is desirable due to the important physiological functions played by the additional NHE isoforms in other tissues. In particular, NHE-2 and NHE-3 appear to have significant roles in the gastrointestinal and renal systems.<sup>1</sup>

The cardioprotective ability of a selective NHE-1 inhibitor could be extremely beneficial in hospital settings; for example, in patients that are at risk for myocardial ischemic injury during cardiac surgery.<sup>2</sup> For surgical settings, an agent with high aqueous solubility that would allow for intravenous administration would be preferred.



Our efforts directed towards the discovery of a selective NHE-1 inhibitor began with the recognition that cariporide (1a)<sup>4</sup> and eniporide (1b),<sup>5</sup> two NHE inhibitors in clinical development, both contain an acylguanidine moiety. The synthesis and screening of a variety of heteroaryl acylguanidines led to the identification of pyrazole 3a as a novel and attractive lead. Herein, we report the modifications in the structure of 3a that led to the discovery of zoniporide (CP-597,396) (3d), a potent and selective NHE-1 inhibitor with the required aqueous solubility and pharmacokinetics for intravenous administration.

\*Corresponding author. Tel.: +1-860-715-0313; fax: +1-860-686-0001; e-mail: angel.guzman-perez@groton.pfizer.com



Scheme 1. Synthesis of zoniporide (3d) (a) (i) TMSCl, pyridine, ether, 23°C, 4 h; (ii) *n*-BuLi (2.5 M in hexanes), -78°C, 75 min; (iii) cyclopropanecarbonyl chloride, DME, -78–23°C, 18 h, 93%; (b) *N,N*-dimethylformamide dimethyl acetal, reflux, 1 h, 91%; (c) (i) NaNO<sub>2</sub>, H<sub>2</sub>O, HCl (aq), 0°C, 1 h; (ii) SnCl<sub>2</sub>·2H<sub>2</sub>O, HCl (aq), 0–23°C, 3 h; (iii) HCl (g), EtOAc, 0°C, 15 min, 97%; (d) Et<sub>3</sub>N, EtOH, reflux, 1 h, 88%; (e) guanidine, EtOH, 80°C, in vacuo, 45 min, 76%; (f) NaOH (2 M aq), MeOH, reflux, 1 h, 56%; (g) (i) SOCl<sub>2</sub>, reflux, 1 h; (ii) guanidine hydrochloride, NaOH (2 M aq), THF, reflux, 1 h, 67%; (h) HCl (conc), THF, 1 h, 82%.

The synthesis of 3d is representative of that used to prepare similar analogues (Scheme 1). Monoethyl malonic ester (4) was converted to β-ketoester 5 by formation of the corresponding trimethylsilyl ester, followed by deprotonation with *n*-butyllithium and acylation with cyclopropanecarbonyl chloride.<sup>6</sup> Treatment of 5 with *N,N*-dimethylformamide dimethyl acetal provided enamine 6 as a single geometric isomer.<sup>7</sup> Hydrazine 8 was prepared by diazotization of amine 7 followed by reduction of the diazonium salt with stannous chloride and was isolated as the dihydrochloride salt.<sup>8</sup> This salt was considerably more stable to storage than the corresponding free base. The condensation of enamine 6 with hydrazine 8 in the presence of triethylamine afforded pyrazole ester 9 in good yield as the only detectable regioisomer.<sup>7</sup> This ester was converted to the acylguanidine by two different methods. In one method, a mixture of the ester and guanidine was heated first in ethanol, and then neat and in vacuo to provide 3d as the free base. Alternatively, the ester was hydrolyzed to the corresponding carboxylic acid 10, which was transformed to the acid chloride using thionyl chloride. The acid chloride was reacted with guanidine hydrochloride under Schotten–Baumann conditions to provide 3d as the free base. In both cases, the free base 3d was converted to the monohydrochloride salt which crystallized as the monohydrate 3d·HCl·H<sub>2</sub>O.<sup>9</sup>

A fluorescence-imaging plate reader (FLIPR) assay was used as our primary screen for NHE-1 inhibitory activity. In this assay, fibroblasts expressing functional human NHE-1 were exposed to an intracellular acidic challenge in the presence of varying concentrations or absence of compound. The rate of NHE-1 mediated recovery of intracellular pH was determined in a 96-well format using the FLIPR and a pH sensitive fluorescent dye. NHE-1 inhibitors reduce the rate of pH recovery. The IC<sub>50</sub> value is the concentration required to inhibit maximal pH recovery by 50%.<sup>10</sup> A similar assay with fibroblasts expressing human NHE-2 and rat NHE-3 was used to determine the inhibition of these isoforms.<sup>10</sup>

Table 1. NHE-1 and NHE-2 inhibitory activity and selectivity of zoniporide and analogues in vitro using a FLIPR based assay

| Compound <sup>a</sup> | R           | Ar          | NHE-1<br>IC <sub>50</sub> (nM) <sup>b</sup> | NHE-2<br>IC <sub>50</sub> (nM) <sup>c</sup> | NHE-1<br>versus NHE-2 <sup>d</sup> |
|-----------------------|-------------|-------------|---------------------------------------------|---------------------------------------------|------------------------------------|
| 3a                    | Me          | Ph          | 830                                         | 3900                                        | 5                                  |
| 3b                    | Cyclopropyl | Ph          | 350                                         | 12,800                                      | 37                                 |
| 3c                    | Cyclopropyl | 1-Naphthyl  | 160                                         | 16,300                                      | 102                                |
| 3d (Zoniporide)       | Cyclopropyl | 5-Quinoliny | 59                                          | 12,000                                      | 203                                |
| 3e                    | Me          | 5-Quinoliny | 860                                         | 41,600                                      | 48                                 |
| 1a (Cariporide)       |             |             | 560                                         | 28,700                                      | 51                                 |
| 1b (Etiporide)        |             |             | 140                                         | 9200                                        | 66                                 |

<sup>a</sup>Experiments carried out using the hydrochloride or mesylate salt forms.

<sup>b</sup>Mean of 9–32 experiments.

<sup>c</sup>Mean of 6–13 experiments.

<sup>d</sup>NHE-1 versus NHE-2 selectivity (NHE-2 IC<sub>50</sub>/NHE-1 IC<sub>50</sub>).

300  
300

As indicated in Table 1, the NHE-1  $IC_{50}$  of lead compound **3a** is somewhat higher than that exhibited by reference compounds cariporide (**1a**) and eniporide (**1b**) in the FLIPR assay, and its NHE-1 versus NHE-2 selectivity is significantly lower. An investigation of the SAR around the methyl group of **3a** led to the identification of compound **3b** bearing a cyclopropyl group in its place. Compound **3b** has improved potency and considerably higher selectivity with respect to **3a**, and is comparable to cariporide and eniporide. Replacement of the phenyl ring with a 1-naphthyl ring provided compound **3c** with even greater potency and selectivity. Unfortunately, the moderate aqueous solubility of compound **3c** was not optimal for developing an intravenous formulation. To overcome this problem, the 1-naphthyl group in **3c** was replaced with hetero-

bicyclic rings. These efforts provided zoniporide (**3d**), which contains a 5-quinoliny ring in place of the 1-naphthyl group. Zoniporide has excellent NHE-1 potency, which is 2- to 9-fold better than cariporide or eniporide, and an NHE-1 versus NHE-2 selectivity that is at least 3-fold higher. Furthermore, it has a rat NHE-3  $IC_{50} > 500,000$  nM, which represents a >8000-fold selectivity against this isoform (Fig. 1). Interestingly, replacement of the cyclopropyl group of zoniporide with the methyl group present in the original lead structure results in a dramatic drop in NHE-1 potency and selectivity (compound **3e**). In fact, the combined effect of incorporating the cyclopropyl and 5-quinoliny groups into this series appears to be synergistic. It is possible that steric interactions between the 5-quinoliny ring and the cyclopropyl ring in zoniporide places both groups in an optimum conformation for both potency and selectivity.

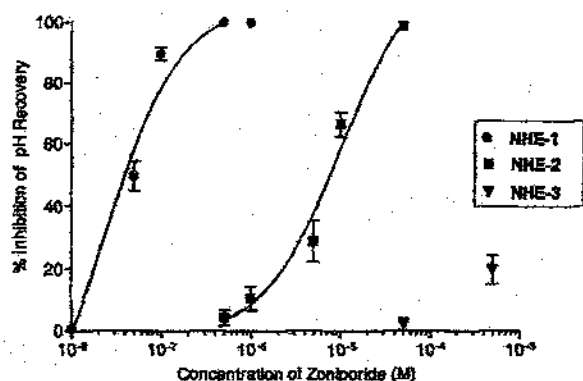


Figure 1. Zoniporide inhibition of human NHE-1, NHE-2, and rat NHE-3-mediated pH recovery in vitro using a FLIPR based assay. Values are means  $\pm$  SE of representative data.

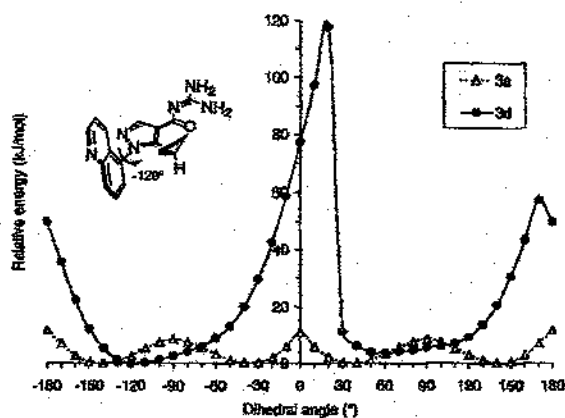


Figure 2. Rotational profile of **3a** about the phenyl-pyrazole bond, and of zoniporide (**3d**) about the quinoline-pyrazole bond. The structure shown represents the lowest energy conformer obtained for **3d**.

Computational molecular modeling<sup>11</sup> supports this hypothesis. Modeling of **3a** shows that the bond connecting the phenyl and pyrazole rings in this compound is conformationally flexible. As shown in Figure 2, even the highest energy rotamers around this bond are readily accessible (8–10 kJ/mol) and no particular conformation is favored (conformational dilution). Modeling of zoniporide (**3d**) provides very different results for the rotamers about the bond joining the quinoline and pyrazole rings (Fig. 2). An energy minimum is obtained at about  $-120^\circ$ , and a valley with relative energy of 4 kJ/mol is observed at approximately  $60^\circ$ . The barriers to rotation of this bond are quite high: approximately 120 and 60 kJ/mol (at dihedral angles of about  $20^\circ$  and  $170^\circ$ , respectively). Thus, zoniporide appears to be considerably more conformationally rigid than **3a**.

The good aqueous solubility of zoniporide makes it well suited for a clinical target of parenteral administration. Crystalline zoniporide-HCl-H<sub>2</sub>O displays high solubility in several vehicles. For example, it has a solubility at room temperature of 5.6 and 8.4 mg of active/mL in sterile water for injection and 5% dextrose for injection, respectively. This is at least partially due to the presence of two distinct ionizable sites in the molecule. The  $pK_a$  of the acylguanidine and of the quinoliny moieties are 7.2 and 3.4, respectively.

The pharmacology of zoniporide is very promising. A  $^{22}Na$  uptake assay confirmed the high in vitro potency ( $IC_{50} = 14$  nM) and selectivity (157-fold and 15,700-fold vs human NHE-2 and rat NHE-3, respectively) of zoniporide.<sup>12</sup> Its high selectivity for NHE-1 versus NHE-2 and NHE-3 diminishes the likelihood of side

Table 2. Pharmacokinetic parameters of zoniporide after intravenous administration in rat and dog<sup>a</sup>

| Species   | $C_0^b$ ( $\mu$ g/mL) | $AUC_{0-\infty}$ ( $\mu$ g-h/mL) | Clp (mL/min/kg) | Vdss (L/kg) | $t_{1/2}$ (h) | Plasma protein binding (%) |
|-----------|-----------------------|----------------------------------|-----------------|-------------|---------------|----------------------------|
| Rat (n=4) | 0.28 (0.08)           | 0.07 (0.01)                      | 237 (28)        | 7.2 (2.2)   | 0.5 (0.1)     | 54                         |
| Dog (n=4) | 0.58 (0.08)           | 0.45 (0.15)                      | 40 (13)         | 2.3 (0.2)   | 0.9 (0.3)     | 55                         |

<sup>a</sup>Values are expressed as mean (standard deviation). Dose 1 mg/kg. All studies used zoniporide-HCl-H<sub>2</sub>O administered as a solution in normal saline.

<sup>b</sup>Represents the estimated plasma concentration at time zero calculated using log-linear back-extrapolation of the first two measured plasma concentrations.

301  
201

effects due to interactions with the latter isoforms. Additionally, zoniporide displays excellent potency in an ex vivo inhibition of human platelet swelling assay ( $IC_{50}=54\text{ nM}$ ), which confirms its activity against endogenous human NHE-1.<sup>12</sup> Furthermore, zoniporide shows robust cardioprotection in the rabbit heart subjected to 30 min of regional ischemia and 2 h of reperfusion, both in vitro and in vivo ( $EC_{50}=0.25$  and  $349\text{ nM}$ , respectively) with a maximum reduction of infarct size of 83 and 75%, respectively, in these models.<sup>13</sup>

Zoniporide displays the pharmacokinetics in rat and dog shown in Table 2, which should allow for intravenous administration. It is noteworthy that the plasma protein binding of zoniporide in these species is moderate. Renal clearance of unchanged drug is low in dogs (13%).

In conclusion, zoniporide is a potent and selective inhibitor of NHE-1, which exhibits high aqueous solubility and acceptable pharmacokinetics for intravenous administration. The potency and selectivity of zoniporide may be due to the conformation that the molecule adopts because of the presence of a cyclopropyl and a 5-quinolinyl substituent on the central pyrazole ring of the molecule. This compound may be useful in protecting the myocardium and other tissues from ischemia/reperfusion injury, and is currently the subject of further investigation.

#### Acknowledgements

The authors are grateful to Leanne M. Buzon, George C. Chang, Shane A. Eisenbeis, Melissa L. Gillaspay, Lisa M. Kath, Jaquelyn L. Klug, George Magnus-Aryitey and Michael R. Makowski for their synthetic contributions to the NHE-1 program. We thank Mark G. Biron, Eugene F. Fiese and Joseph E. Mertz for their pharmaceutical sciences input. The biology advice of Michael L. Mangiapane is gratefully acknowledged.

#### References and notes

- (a) For recent reviews on the structure, function, regulation, isoforms and distribution of NHE, see: Counillon, L.; Pouyssegur, J. *J. Biol. Chem.* **2000**, *275*, 1. (b) Fliegel, L. *J. Thromb. Thrombolysis* **1999**, *8*, 9. (c) Avkiran, M.; Snabaitis, A. K. *J. Thromb. Thrombolysis* **1999**, *8*, 25. (d) Aharonovitz, O.; Grinstein, S. *Drug News Perspect.* **1999**, *12*, 105. (e) Counillon, L.; Touret, N.; Godart, H.; Pouyssegur, J. *Eur. Heart J. Suppl.* **1999**, *1*, K2. (f) Harris, C.; Fliegel, L. *Int. J. Mol. Med.* **1999**, *3*, 315. (g) Diprov, P.; Fliegel, L. *FEBS Lett.* **1998**, *424*, 1. (h) Fliegel, L.; Murtazina, R.; Dibrov, P.; Harris, C.; Moor, A.; Fernandez-Rachubinski, F. A. *Biochem. Cell Biol.* **1998**, *76*, 735. (i) Kinsella, J. L.; Heller, P.; Froehlich, J. P. *Biochem. Cell Biol.* **1998**, *76*, 743. (j) Wakabayashi, S.; Shigekawa, M.; Pouyssegur, J. *Physiol. Rev.* **1997**, *77*, 51. (k) Orlowski, J.; Grinstein, S. *J. Biol. Chem.* **1997**, *272*, 22373.
- For recent reviews on the role of NHE-1 in ischemia/reperfusion injury, see: (a) Karmazyn, M.; Gan, X. T.; Humphreys, R. A.; Yoshida, H.; Kusumoto, K. *Circ. Res.* **1999**, *85*,

- (b) Orlowski, J. *Ann. N.Y. Acad. Sci.* **1999**, *874*, 346. (c) Karmazyn, M. *Ann. N.Y. Acad. Sci.* **1999**, *874*, 326. (d) Gumina, R. J.; Gross, G. J. *J. Thromb. Thrombolysis* **1999**, *8*, 39. (e) Karmazyn, M. *J. Thromb. Thrombolysis* **1999**, *8*, 33. (f) Myers, M. L. *J. Thromb. Thrombolysis* **1999**, *8*, 53. (g) Erhardt, L. R. W. *Am. J. Cardiol.* **1999**, *83*, 23G. (h) Buerke, M.; Rupprecht, H.-J.; von Dahl, J.; Terres, W.; Seyfarth, M.; Schultzeiss, H.-P.; Richardt, G.; Sheehan, F. H.; Drexler, H. *Am. J. Cardiol.* **1999**, *83*, 19G. (i) Avkiran, M. *Am. J. Cardiol.* **1999**, *83*, 10G. (j) Avkiran, M. *Eur. Heart J. Suppl.* **1999**, *1*, K11. (k) Murphy, E.; Cross, H. R.; Steenbergen, C. *Eur. Heart J. Suppl.* **1999**, *1*, K18. (l) Hashimoto, K.; Sugiyama, A.; Xue, Y. X.; Aye, N. N.; Yamada, C.; Chino, D. *Eur. Heart J. Suppl.* **1999**, *1*, K31. (m) Karmazyn, M. *Eur. Heart J. Suppl.* **1999**, *1*, K38. (n) Levitsky, J.; Gurell, D.; Frishman, W. H. *J. Clin. Pharmacol.* **1998**, *38*, 887. (o) Karmazyn, M. *Keio J. Med.* **1998**, *47*, 65. (p) Karmazyn, M. *Cur. Res. Ion Channel Modulat.* **1997**, *3*, 3. (q) Froehlich, O.; Karmazyn, M. *Cardiovasc. Res.* **1997**, *36*, 138.
- For a recent review on NHE inhibitors, see: Kleemann, H. W.; Weichert, A. G. *Drugs* **1999**, *2*, 1009.
- (a) Weichert, A.; Faber, S.; Jansen, H. W.; Scholz, W.; Lang, H. *J. Arzneim.-Forsch.* **1997**, *47*, 1204. (b) Graul, A.; Prous, J.; Castañer, J. *Drugs Future* **1997**, *22*, 1197. (c) Graul, A.; Prous, J.; Castañer, J. *Drugs Future* **1998**, *23*, 1235. (d) Dhein, S.; Salameh, A. *Cardiovasc. Drug Rev.* **1999**, *17*, 134. (e) Scholz, W. *Eur. Heart J. Suppl.* **1999**, *1*, K45. (f) Karmazyn, M. *Expert Opin. Invest. Drugs* **2000**, *9*, 1099.
- Baumgarth, M.; Beier, N.; Gericke, R. *J. Med. Chem.* **1997**, *40*, 2017.
- Taylor, B. C.; Turchi, I. J. *Org. Prep. Proced. Int.* **1978**, *10*, 221.
- Meñozzi, G.; Mosti, L.; Schenone, P. *J. Heterocycl. Chem.* **1987**, *24*, 1669.
- Ferlin, M. G.; Chiarello, G.; Basadonna, O.; Gia, O.; Mobolio, S.; Baccichetti, F.; Carlissare, F. *Farmaco* **1989**, *44*, 1141.
- [1-(Quinolin-5-yl)-5-cyclopropyl-1H-pyrazole-4-carbonyl]-guanidine hydrochloride monohydrate ( $34\text{ HCl}\cdot\text{H}_2\text{O}$ ): mp  $154\text{--}156^\circ\text{C}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  0.42 (m, 2H), 0.59–0.61 (m, 2H), 1.88–1.95 (m, 1H), 7.57 (dd,  $J=9$ , 4 Hz, 1H), 7.67 (d,  $J=4$  Hz, 1H), 7.82 (d,  $J=7$  Hz, 1H), 7.90 (t,  $J=8$  Hz, 1H), 8.22 (d,  $J=8$  Hz, 1H), 8.38 (bs, 2H), 8.69 (bs, 2H), 8.72 (s, 1H), 8.98 (dd,  $J=4$ , 1.4 Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  7.8, 8.0, 114.8, 123.7, 125.3, 126.8, 129.6, 131.7, 131.8, 135.7, 141.6, 148.4, 151.2, 152.2, 156.3, 163.2; APCIMS 321  $[\text{M}+\text{H}]^+$ . Anal. calcd for  $\text{C}_{17}\text{H}_{13}\text{N}_6\text{O}\cdot\text{HCl}\cdot\text{H}_2\text{O}$ : C, 54.47; H, 5.11; N, 22.42; Cl, 9.46. Found: C, 54.36; H, 5.16; N, 22.38; Cl, 9.13.
- Materials: 2,7-Biscarboxyethyl-5,6-carboxyfluorescein acetoxymethyl (BCECF-AM) was purchased from Molecular Probes (Eugene, OR). All other reagents were of analytical grade and obtained commercially. Methods: PS120 fibroblast cells expressing human NHE-1 or rat NHE-3 isoforms were obtained from Professor J. Pouyssegur (Nice, France). Human NHE-2 expressing cells were generated at Pfizer Global Research and Development (Groton, CT). PS120 fibroblasts expressing indicated NHE isoform were grown to confluency in 96-well collagen coated plates. Measurement of intracellular pH (pH<sub>i</sub>) was based on the methodology of: Russ, U.; Balser, C.; Scholz, W.; Albus, U.; Lang, H. J.; Weichert, A.; Scholken, B. A.; Gogelein, H. *Pflugers Arch.* **1996**, *433*, 26. Cells were washed three times with assay buffer (10 mM HEPES, 136 mM NaCl, 5.4 mM KCl, 0.44 mM  $\text{KH}_2\text{PO}_4$ , 0.34 mM  $\text{Na}_2\text{HPO}_4$ , 1 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$  and 5.6 mM glucose, pH 7.4) followed by incubation with 5  $\mu\text{M}$  BCECF-AM in the same buffer for 30 min at  $37^\circ\text{C}$ . Cells were then washed seven times with acid loading buffer (30 mM  $\text{NH}_4\text{Cl}$ , 90 mM choline chloride, 5 mM KCl, 1.8 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$ , 5 mM

glucose, and 15 mM Hepes, pH 7.5). Cells were initially incubated for 1 h with acid loading buffer followed by an additional 5 min incubation in the presence or absence of test compounds. Initial baseline fluorescence was recorded for each well simultaneously using a fluorescence-imaging plate reader (FLIPR). Intracellular acidification was induced by the addition of assay buffer with or without test compound. Intracellular acidification was observed as a marked reduction of BCECF fluorescence. NHE inhibitors prevent recovery from the acidification (measured as fluorescent units). Data analysis: The potency of NHE inhibitors was calculated as a function of its ability to prevent recovery from acidification compared with control. The  $IC_{50}$  value for NHE inhibitors (concentration required to inhibit  $pH_i$  recovery by 50%) was calculated by fitting the data via nonlinear least squares regression analysis to the equation:  $\% \text{ Inhibition} = 100 / [1 + (10^C / 10^X)^D]$ , where  $X = \log [\text{drug concentration}]$ ,  $C$  ( $IC_{50}$ ) =  $\log [\text{drug concentration at 50\% inhibition}]$ , and  $D$  = the Hill coefficient.

302  
302

11. Conformational analyses were performed using the OPLS. A force field with the GB3A solvent approximation as implemented in MacroModel v.7. Low energy conformers were identified via a Monte Carlo search, followed by energy minimization. Rotations were allowed for all single, acyclic and non-terminal bonds. All of the low energy conformers place the cyclopropyl group in the conformation with respect to the pyrazole ring shown in Figure 2. The rotational profiles in Figure 2 were generated using the low energy conformers identified in the Monte Carlo search as the initial structures. The bond of interest was held fixed at  $10^\circ$  increments and a constrained energy minimization was performed on each of these rotamers.

12. Marala, R. B.; Brown, J. A.; Kong, J. X.; Tracey, W. R.; Knight, D. R.; Wester, R. T.; Sun, D.; Kennedy, S. P.; Hamanaka, B. S.; Ruggeri R. B.; Hill, R. J., submitted for publication.

13. Knight, D. R.; Smith, A. H.; Flynn, D. M.; MacAndrew, J. T.; Ellery, S. S.; Kong, J. X.; Marala, R. B.; Wester, R. T.; Guzman-Perez, A.; Hill, R. J.; Magge, W. P.; Tracey, W. R. *J. Pharmacol. Exp. Ther.* 2001, 297, in press.

# Synthesis and Biological Evaluation of Aroylguanidines Related to Amiloride as Inhibitors of the Human Platelet $\text{Na}^+/\text{H}^+$ Exchanger

Didier Laeckmann,<sup>a,\*</sup> Françoise Rogister,<sup>b</sup> Jean-Victor Dejardin,<sup>a</sup>  
Christelle Prosperi-Meys,<sup>c</sup> Joseph Géczy,<sup>b</sup> Jacques Delarge<sup>a</sup> and Bernard Masereel<sup>d</sup>

<sup>a</sup>Department of Medicinal Chemistry, Natural and Synthetic Drugs Research Center, Université de Liège, CHU,  
1 avenue de l'Hôpital, B 36, tour 4, B-4000 Liège, Belgium

<sup>b</sup>Therabel Research S.A., 108 rue Egide Van Ophem, B-1180 Bruxelles, Belgium

<sup>c</sup>Service de Physique Expérimentale, Institut de Physique, B 5, Université de Liège, B-4000 Liège, Belgium

<sup>d</sup>Department of Pharmacy, University of Namur, FUNDP, 61 rue de Bruxelles, B-5000 Namur, Belgium

Received 16 October 2001; accepted 9 January 2002

**Abstract**—Pyridine and benzene bioisosteres of amiloride were synthesized and evaluated for their inhibitory potency against the sodium–hydrogen exchanger (NHE) involved in intracellular pH regulation. The inhibition of NHE was determined by using the platelet swelling assay (PSA) in which the swelling of human platelets was induced by their incubation in an acid buffer (pH 6.7). Additionally, the inhibitory potency of the most active compounds was assessed by measuring the inhibition of the EIPA-sensitive  $^{22}\text{Na}^+$  uptake (UIA) by human platelets after intracellular acidosis. The results indicated that several benzene derivatives and compounds bearing an carbonylguanidine moiety in the *meta* position of the pyridine nitrogen were much more potent than amiloride (PSA:  $\text{IC}_{50}$  = 43.5  $\mu\text{M}$ ; UIA:  $\text{IC}_{50}$  = 100.1  $\mu\text{M}$ ), but less than EIPA, a pyrazine NHE inhibitor (PSA:  $\text{IC}_{50}$  = 0.08  $\mu\text{M}$ ; UIA:  $\text{IC}_{50}$  = 0.5  $\mu\text{M}$ ). In both biological assays (2-amino-5-bromo-pyridine-3-carbonyl)guanidine (32) was the most active molecule (PSA:  $\text{IC}_{50}$  = 0.8  $\mu\text{M}$ ; UIA:  $\text{IC}_{50}$  = 0.8  $\mu\text{M}$ ). Our investigations demonstrated that the replacement of the pyrazine ring of amiloride by a pyridine or a phenyl ring improved the NHE inhibitory potency (phenyl > pyridine > pyrazine). © 2002 Elsevier Science Ltd. All rights reserved.

## Introduction

In many cell types, including platelets, several mechanisms take part in the maintenance of intracellular acid–base homeostasis.<sup>1,2</sup> One of the predominant mechanism by which cells adapt to intracellular acidosis is through the  $\text{Na}^+/\text{H}^+$  antiporter system (NHE). To date, six mammalian isoforms of the  $\text{Na}^+/\text{H}^+$  exchanger have been identified and characterized (NHE-1–NHE-6).<sup>3–6</sup> The most widely distributed subtype is the amiloride-sensitive  $\text{Na}^+/\text{H}^+$  antiporter (NHE-1) which is involved in intracellular pH ( $\text{pH}_i$ ) homeostasis, cell volume control, and in cell signaling.<sup>7</sup> Isoform NHE-1, the main  $\text{Na}^+/\text{H}^+$  exchanger in platelets, mediates the electroneutral exchange of intracellular  $\text{H}^+$  for extracellular  $\text{Na}^+$  with a 1:1 stoichiometric relationship

using the transmembrane  $\text{Na}^+$  gradient established by  $\text{Na}^+/\text{K}^+$  ATPase.<sup>8</sup> The structure and the functional implications of NHE-1 and other isoforms have been recently reviewed.<sup>9,10</sup> The activity of the  $\text{Na}^+/\text{H}^+$  antiporter, especially NHE-1, can be modulated by a number of stimuli such as growth factors, hormones and neurotransmitters, as well as by changes in cell volume and mechanical stimuli.<sup>11</sup> Although the activation of NHE is essential for the restoration of physiological pH, an excessive stimulation of NHE results in an increase of intracellular  $\text{Na}^+$  concentration and a subsequent activation of  $\text{Na}^+/\text{K}^+$  ATPase, with a consecutive increase of energy consumption. Since cellular  $\text{Na}^+$  and  $\text{Ca}^{2+}$  transport is coupled via the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger, which depends on the  $\text{Na}^+$  gradient, the high intracellular  $\text{Na}^+$  levels lead to raised intracellular  $\text{Ca}^{2+}$ .<sup>8,12,13</sup> This cellular  $\text{Ca}^{2+}$  overload is assumed to be involved in ischemic and reperfusion injuries, like arrhythmias, myocardial contracture, stunning and tissue necrosis.<sup>14,15</sup> In addition, the abnormalities in the

\*Corresponding author. Tel.: +32-4-3664381; fax: +32-4-3664362;  
e-mail: d.laeckmann@ulg.ac.be

304  
304

activity of the NHE are implicated in the pathogenesis and/or pathophysiological processes such as essential hypertension,<sup>16–18</sup> diabetes mellitus,<sup>19,20</sup> postischemic dysfunction, and cellular death. Moreover, raised NHE activity in kidney and vascular smooth muscle could play a critical role in the etiology of hypertension,<sup>20–22</sup> vascular smooth muscle cell proliferation<sup>23</sup> and renal disorders.<sup>24</sup> Recently, the inhibition of the NHE has been considered as an useful approach to limit  $\text{Ca}^{2+}$  influx and avoid its deleterious consequences during cardiac ischemia and reperfusion.<sup>25</sup>

Consequently, to attenuate the harmful consequences of an excessive NHE activation, several inhibitors were developed (Fig. 1). Beside its inhibitory potency against the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger and  $\text{Na}^+$  channels, amiloride is a NHE inhibitor. Pharmacomodulation of amiloride generated more potent and more specific NHE inhibitors: the 5-*N*-substituted pyrazine derivatives closely related to the structure of amiloride (Fig. 1). Later, benzoylguanidines were developed (e.g., cariporide, HOE 694, EMD-96785 and FR-183998). These drugs are highly potent and markedly selective inhibitors of NHE-1 without acting on other ion carrier or pH regulatory systems.<sup>26,27</sup>

Pharmacological development of NHE inhibitors for cardiac therapy focused on selective NHE-1 inhibitors. These agents, as well the earlier nonspecific amiloride derivatives, have shown excellent cardioprotective properties. Thus, the NHE inhibitors were expected to be efficient tools for preventing high intracellular  $\text{Na}^+$  levels and concomitant  $\text{Ca}^{2+}$  overload.<sup>28–30</sup>

The success of NHE inhibitors in experimental studies has led to clinical trials in high risk patients with coronary artery disease as well in patients with acute myocardial infarction. These NHE inhibitors attenuated both acute and chronic responses to myocardial post-infarction resulting in the evolution of heart failure.<sup>31–34</sup> These data suggest a potentially effective new therapeutic perspective for the treatment of heart failure with NHE-1 inhibitors.

Since the nature of groups R and R' (Fig. 1) has been extensively studied in the amiloride family, we have examined the influence of bioisosteric replacement of the pyrazine ring by a pyridine or a phenyl, and the effect of other substitutions on the aromatic ring (Fig. 2).

### Chemistry

In a previous work,<sup>35</sup> we reported the preparation of derivatives of amiloride where their pyrazine ring was substituted by a phenyl. Here, pyridine compounds (12, 14, 28, 30–33) structurally related to amiloride were prepared (Table 1). The strictly pyridine bioisosteres of amiloride (12, 28) differ by the position of the pyridinic nitrogen. The preparation of 12 started from 2-amino-6-methylpyridine (Scheme 1). The nitration of this product led to 6-methyl-2-nitraminopyridine 1.<sup>36,37</sup> The one-pot nitration of 1 with an excess of nitric acid (3 equivalents) gave 2-amino-6-methyl-3,5-dinitropyridine 2.<sup>38</sup> The synthesis of 2-hydroxy-6-methyl-3,5-dinitropyridine 3 by diazotization of 2 was carried out under the conditions used by Berrie et al.<sup>39</sup> This step was required by the presence of a high chloride concentration which could not directly generate 2-chloro-6-methyl-3,5-dinitropyridine 4.<sup>40</sup> This latter was obtained by chlorination of 3 with a mixture of  $\text{PCl}_5/\text{OPCl}_3$  according to Czuba method.<sup>41</sup> After reduction of both nitro groups,<sup>42</sup> the 3,5-diamino-2-chloro-6-methylpyridine 5 was acetylated (6), and then oxidized by  $\text{KMnO}_4$  to 3,5-diacetylamino-6-chloro-pyridine-2-carboxylic acid 7.

In a first procedure, this intermediate gave the methyl ester 8 or 9, which could not lead to the desired compound 12. In a second approach, 7 was hydrolyzed in alkaline conditions, providing the 3,5-diamino-6-chloro-pyridine-2-carboxylic acid 10 with high yields (70–75%). The carboxylic derivative 10 was then esterified by means of *N*-*tert*-butyl-5-methylisoxazolium perchlorate (NBI)<sup>43</sup> to give the crotonamide intermediate 11 which finally reacted with guanidine to give 12. A third

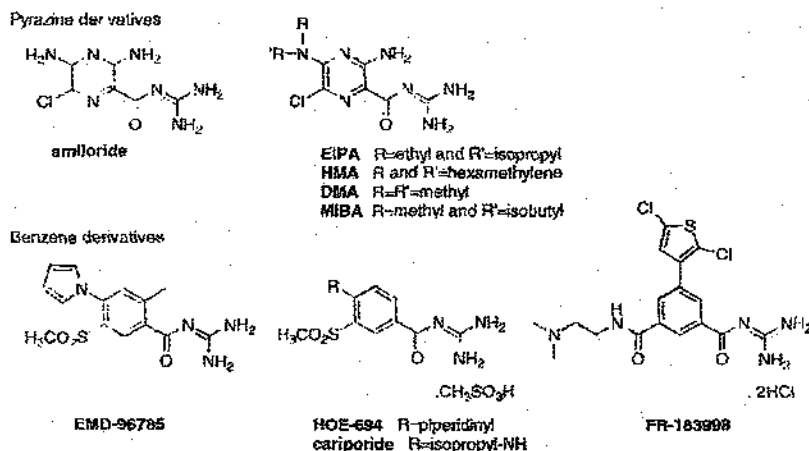


Figure 1. Inhibitors of the  $\text{Na}^+/\text{H}^+$  exchanger.

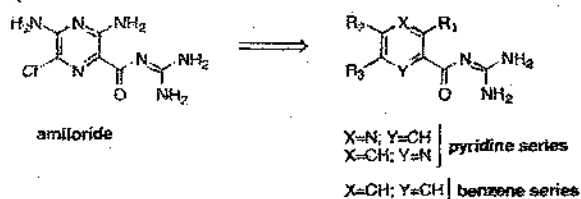


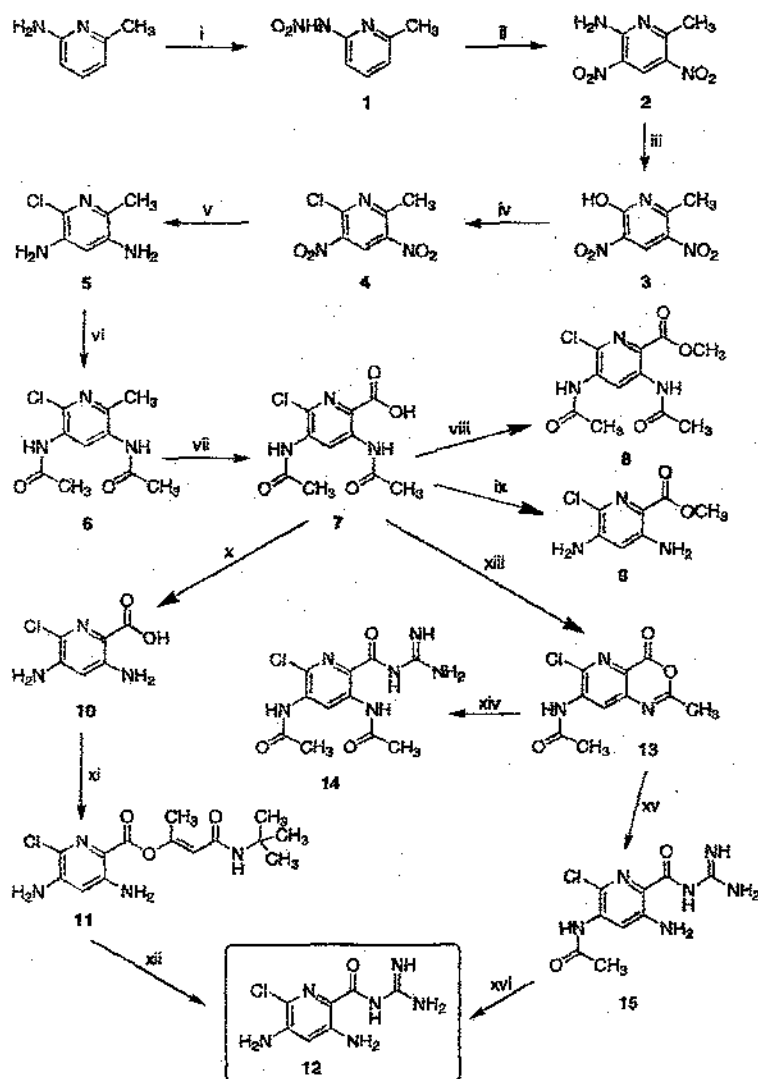
Figure 2. Pyridine and benzene derivatives of amiloride.

pathway involved the cyclization of **7** into *N*-(6-chloro-2-methyl-4-oxo-4H-pyrido[3,2-d]-1,3-oxazin-7-yl)-acetamide **13**, which reacted with guanidine to generate the diacetylated and acetylated carbonylguanidine compounds **14** and **15**, respectively. This latter underwent acidic hydrolysis to give the pyridine bioisostere **12**. However, this hydrolysis occurred with poor yields (25–30%). Alternative pathways<sup>44</sup> such as the synthesis of *N*-hydroxysuccinimide esters or trifluoroacetic protec-

tion of amino groups have also been investigated but did not succeed to reach final compound **12**.

The synthesis of the other pyridine bioisostere **28** started from **16** obtained from 2-methylglutaronitrile following Fritz procedure (Scheme 2).<sup>45</sup>

Although, the preparation of intermediates **17** and **18** was previously described,<sup>46</sup> a mixture of both molecules was always generated, and required fractional crystallization. The 2,5,6-trichloropyridine-3-carboxylic acid **21** was produced by three different ways: the oxidation of **17** by  $\text{KMnO}_4$ , the catalytic oxidation of a mixture of bromide derivatives (**19** and **20**), and the oxydative process of **23** which was obtained from 2-methyleneglutaronitrile according to Mutterer procedure.<sup>47</sup> In the last two cases, the yields were of 50 and 80%, respectively. Indeed, the conversion of a polyhalogenated pyridine to its carboxylic acid derivative by  $\text{KMnO}_4$  is



Scheme 1. Reagents: (i)  $\text{H}_2\text{SO}_4/\text{HNO}_3$ ,  $-20^\circ\text{C}$ ; (ii)  $\text{H}_2\text{SO}_4$ ; (iii)  $\text{NaNO}_2/\text{H}_2\text{SO}_4$ ; (iv)  $\text{PCl}_5/\text{OPCl}_3$ ; (v)  $\text{SnCl}_2/\text{HCl}$ ; (vi)  $\text{Ac}_2\text{O}$ ; (vii)  $\text{KMnO}_4$ ; (viii)  $\text{CH}_2\text{N}_2$ ; (ix)  $\text{CH}_3\text{OH}/\text{H}_2\text{SO}_4$ ; (x)  $\text{NaOH}$ ; (xi)  $\text{Et}_3\text{N}/\text{DMF}/\text{NBI}$ ; (xii) guanidine.HCl/*tert*-BuOK; (xiii)  $\text{Ac}_2\text{O}/\text{EtOAc}$ ; (xiv) guanidine.HCl/Na; (xv) guanidine.HCl/Na/HCl; (xvi)  $\text{HCl}/\text{H}_2\text{O}/\text{EtOH}$ .

known to give low yields.<sup>48,49</sup> As observed,<sup>47</sup> an attempt to prepare the acid **27** by reaction of **21** with  $\text{NH}_4\text{OH}$  led to decarboxylation (**22**). The carboxamide **25** was generated from the acyl chloride **24**, and the substitution of both chlorine atoms gave the 2,6-diamino-5-chloro-pyridine-3-carboxamide **26** with satisfactory yields (65%). Alkaline hydrolysis led to the key intermediate **27**. This latter was then converted by reaction with *N,N'*-carbonyldiimidazole (CDI) into its corresponding ester, which reacted with guanidine to provide the pyridine bioisostere **28**.

Some compounds, related to **28**, where the amino groups are substituted by chlorine were synthesized (Scheme 3).

Hence, the 2,6-dichloropyridine-3-carboxylic acid **29** was prepared by *ortho*-lithiation according to the procedure reported by Radinov.<sup>50</sup> The carboxylic acids **21** and **29** were transformed by condensation with CDI into their corresponding esters, which then reacted with guanidine to provide the desired molecules **31** and **30**, respectively. An attempt to obtain **31** via the methyl ester was unsuccessful. Compound **32** was obtained by bromination of (2-amino-pyridine-3-carbonyl)guanidine as previously described by Cragoe.<sup>51</sup>

Finally, some 'simplified' pyridine (**33**) and benzene (**40**, **42**) derivatives were prepared according to known procedures.<sup>52,53</sup>

## Results and Discussion

To measure the effect of the synthesized compounds on the NHE activity, the human platelet swelling assay (PSA) was used.<sup>54,55</sup> Addition of platelet-rich plasma (PRP) to an acid propionate buffer led to the activation of the NHE which uptakes extracellular  $\text{Na}^+$  and extrudes cytoplasmic  $\text{H}^+$ . To restore iso-osmotic conditions, the cytosolic accumulation of  $\text{Na}^+$  is accompanied by a cell swelling due to the influx of water. This platelet swelling induced a decrease of the optical density (OD) of the platelet suspension.<sup>16,18</sup> This OD decrease is related to the value of extracellular pH. Indeed, at pH 6.7, EIPA (10  $\mu\text{M}$ ), a specific NHE inhibitor, completely abolished the decrease of OD and prevented the platelet swelling (Fig. 3). Other molecules acting on  $\text{Na}^+$  carriers such as ouabain (inhibitor of  $\text{Na}^+/\text{K}^+$  ATPase pump) or furosemide (inhibitor of the symport  $\text{Na}^+ \text{K}^+ 2\text{Cl}^-$ ) were investigated, and failed to modify the OD decrease, and thus the platelet swelling.

Table 1. Chemical structures and inhibitory potency of the platelet  $\text{Na}^+/\text{H}^+$  exchanger

| Compound               | X  | Y  | R <sub>1</sub>      | R <sub>2</sub>                                                                   | R <sub>3</sub>                  | R                             | IC <sub>50</sub> ( $\mu\text{M}$ ) $\pm$ SEM |                                                   |
|------------------------|----|----|---------------------|----------------------------------------------------------------------------------|---------------------------------|-------------------------------|----------------------------------------------|---------------------------------------------------|
|                        |    |    |                     |                                                                                  |                                 |                               | PSA <sup>a</sup>                             | <sup>22</sup> Na <sup>+</sup> uptake <sup>b</sup> |
| <b>12</b>              | CH | N  | NH <sub>2</sub>     | NH <sub>2</sub>                                                                  | Cl                              | H                             | 24.0 $\pm$ 2.0                               |                                                   |
| <b>14</b>              | CH | N  | NHCOCH <sub>3</sub> | NHCOCH <sub>3</sub>                                                              | Cl                              | H                             | 9.9 $\pm$ 0.9                                |                                                   |
| <b>28</b>              | N  | CH | NH <sub>2</sub>     | NH <sub>2</sub>                                                                  | Cl                              | H                             | 1.2 $\pm$ 0.5                                | 1.3 $\pm$ 0.4                                     |
| <b>32</b>              | N  | CH | NH <sub>2</sub>     | H                                                                                | Br                              | H                             | 0.85 $\pm$ 0.05                              | 0.8 $\pm$ 0.1                                     |
| <b>31</b>              | N  | CH | Cl                  | Cl                                                                               | Cl                              | H                             | 2.8 $\pm$ 0.4                                | 5.3 $\pm$ 0.6                                     |
| <b>30</b>              | N  | CH | Cl                  | Cl                                                                               | H                               | H                             | NA                                           |                                                   |
| <b>33</b>              | N  | CH | H                   | H                                                                                | H                               | H                             | NA                                           |                                                   |
| <b>34<sup>d</sup></b>  | CH | CH | NH <sub>2</sub>     | NH <sub>2</sub>                                                                  | Cl                              | H                             | 0.8 $\pm$ 0.08                               | 16.0 $\pm$ 3.8                                    |
| <b>35<sup>d</sup></b>  | CH | CH | NH <sub>2</sub>     | NH <sub>2</sub>                                                                  | Cl                              | C <sub>2</sub> H <sub>5</sub> | NA                                           |                                                   |
| <b>36<sup>d</sup></b>  | CH | CH | NH <sub>2</sub>     | H                                                                                | Cl                              | H                             | 0.5 $\pm$ 0.04                               | 1.8 $\pm$ 0.4                                     |
| <b>37<sup>d</sup></b>  | CH | CH | NH <sub>2</sub>     | H                                                                                | H                               | H                             | NA                                           |                                                   |
| <b>38<sup>d</sup></b>  | CH | CH | NO <sub>2</sub>     | NO <sub>2</sub>                                                                  | Cl                              | H                             | 1.1 $\pm$ 0.3                                | 10.6 $\pm$ 0.1                                    |
| <b>39<sup>d</sup></b>  | CH | CH | Cl                  | H                                                                                | NO <sub>2</sub>                 | H                             | 0.7 $\pm$ 0.4                                | 5.6 $\pm$ 2.1                                     |
| <b>40</b>              | CH | CH | H                   | Cl                                                                               | Cl                              | H                             | 1.3 $\pm$ 0.5                                | 2.7 $\pm$ 0.5                                     |
| <b>41</b>              | CH | CH | Cl                  | H                                                                                | NH <sub>2</sub>                 | H                             | NA                                           |                                                   |
| <b>42</b>              | CH | CH | H                   | H                                                                                | H                               | H                             | NA                                           |                                                   |
| <b>43</b>              | CH | CH | NHCOCH <sub>3</sub> | NO <sub>2</sub>                                                                  | Cl                              | H                             | 0.11 $\pm$ 0.1                               | 3.6 $\pm$ 2.2                                     |
| <b>44</b>              | CH | CH | NHCOCH <sub>3</sub> | NO <sub>2</sub>                                                                  | Cl                              | C <sub>2</sub> H <sub>5</sub> | 24.4 $\pm$ 0.2                               |                                                   |
| <b>45</b>              | CH | CH | NHCOCH <sub>3</sub> | H                                                                                | H                               | H                             | 66.1 $\pm$ 4.0                               |                                                   |
| Amiloride ( <b>1</b> ) | N  | N  | NH <sub>2</sub>     | NH <sub>2</sub>                                                                  | Cl                              | H                             | 43.5 $\pm$ 4.5                               | 100.1 $\pm$ 32                                    |
| EIPA                   | N  | N  | NH <sub>2</sub>     | (C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> NCH(CH <sub>3</sub> ) <sub>2</sub> | Cl                              | H                             | 0.08 $\pm$ 0.03                              | 0.5 $\pm$ 0.1                                     |
| DMA                    | N  | N  | NH <sub>2</sub>     | N(CH <sub>3</sub> ) <sub>2</sub>                                                 | Cl                              | H                             | 0.8 $\pm$ 0.04                               |                                                   |
| HMA                    | N  | N  | NH <sub>2</sub>     | N(CH <sub>3</sub> ) <sub>6</sub>                                                 | Cl                              | H                             | 0.2 $\pm$ 0.01                               | 3.3 $\pm$ 0.4                                     |
| Cariporide             | CH | CH | H                   | (CH <sub>3</sub> ) <sub>2</sub> CH                                               | SO <sub>2</sub> CH <sub>3</sub> | H                             | 0.12 <sup>c</sup>                            |                                                   |
| EMD-96785              | CH | CH | CH <sub>3</sub>     | pyrrole                                                                          | SO <sub>2</sub> CH <sub>3</sub> | H                             | 0.03 <sup>c</sup>                            |                                                   |

<sup>a</sup>Drug concentration to achieve half-maximal inhibition of acid-induced swelling of human platelets.

<sup>b</sup>Drug concentration to achieve half-maximal inhibition of the EIPA-sensitive <sup>22</sup>Na<sup>+</sup> uptake by human platelets. NA = no activity at 10  $\mu\text{M}$ .

<sup>c</sup>Data from ref. 56.

<sup>d</sup>Synthesis of compounds **34–39**, **41**, **43–45** previously described in ref 35.

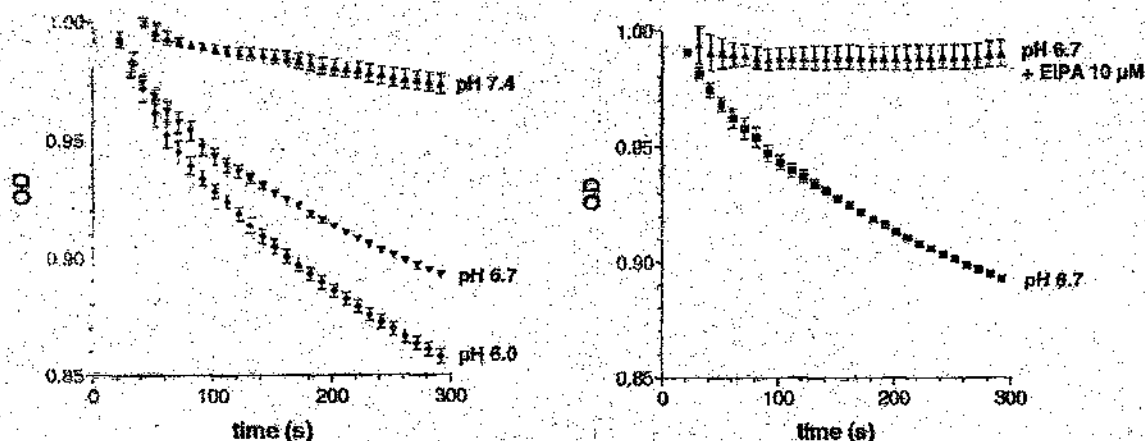


Figure 3. Effect of extracellular pH (left panel) and EIPA (right panel) on the platelet swelling expressed as the decrease of the relative optical density (OD at 680 nm) of a platelet suspension.

μM). Once more, the replacement of the nitro group (39) or of the chlorine (40) in R3 by an amino (41) led to a marked loss of activity. The unsubstituted benzoylguanidine (42) was inactive.

With respect to these considerations, and as observed for the pyridine derivatives, a chloro or a nitro group was required in R3 to inhibit the NHE (c.f., 36, 37 and 41). As shown in the pyridine series, an acetamino in R1 (43–45) enhanced the potency (c.f., 14 and 43). The substitution on the distal nitrogen of the guanidine with an ethyl strongly reduced the biological response (c.f., 43 and 44). These results indicated that the nature of the aromatic ring and the position of the heterocyclic nitrogen strongly influenced the NHE inhibitory potency following the sequence phenyl (34) > pyridine (28 > 12) > pyrazine (amiloride).

For the reference drugs, and for the most active compounds ( $IC_{50} < 3 \mu M$ ), the inhibitory potency of NHE inhibitors was also evaluated by their ability to reduce the EIPA-sensitive  $^{22}Na^+$  uptake by human platelets suspended at pH 6.7. After 5 and 10 min of incubation with EIPA (10 μM), the platelet  $^{22}Na^+$  uptake was reduced by 31 and 33%, respectively. The EIPA concentration-response curve was drawn and led to calcu-

late the concentration required to reduce of 50% ( $IC_{50}$ ) the so-called EIPA-sensitive  $^{22}Na^+$  uptake (Fig. 5).

The *meta*-carbonylguanidine pyridine derivatives (28, 31, 32) were much more potent ( $0.8 < IC_{50} < 5.3 \mu M$ ) than amiloride ( $IC_{50} = 100.1 \mu M$ ). As observed in the platelet swelling assay, 32 remained the most active molecule in this series. The benzene derivatives (34, 36, 38, 39, 40, 43), active in the swelling assay, reduced dose-dependently the EIPA-sensitive  $^{22}Na^+$  uptake. As observed for EIPA and HMA, their  $IC_{50}$  value was 2–30 times higher than in the PSA. In contrast to the results obtained in the PSA, the benzene bioisostere of amiloride (34) was less active than the pyridine bioisostere 28.

### Conclusion

In conclusion, this study demonstrated that the replacement of the pyrazine ring of amiloride by a pyridine or a phenyl ring improved the NHE inhibitory potency.

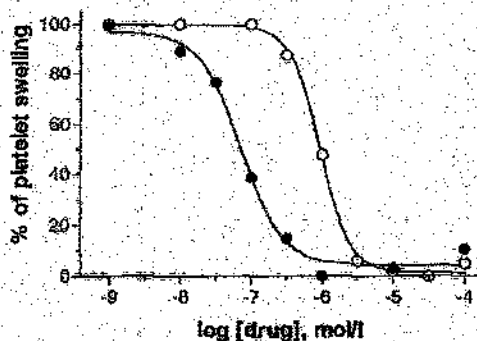


Figure 4. EIPA (●) or 34 (○) reduced the NHE activity expressed as the human platelet swelling induced by an acid buffer (pH 6.7).

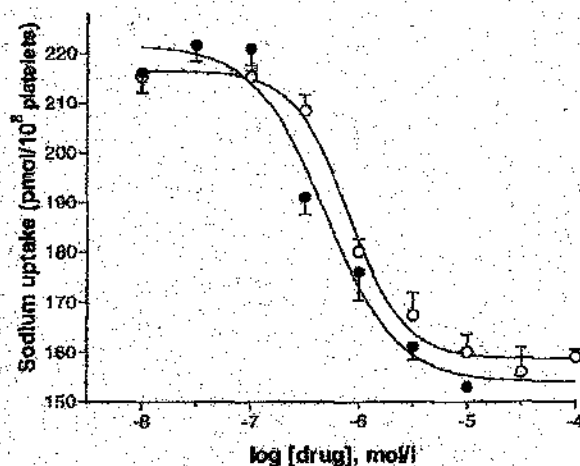


Figure 5. Human platelet sodium uptake inhibited by EIPA (●) or 32 (○). Each point is the mean and SEM of the response in at least three different experiments.

To preserve the biological activity, a nitro or a halide group was required in the R3 position. For the pyridine derivatives, the nitrogen of the heterocycle should be in the *meta* position of the carbonylguanidine side-chain. Taking into account these results, and as compared to the structure of EIPA and other 5-*N*-substituted amiloride derivatives, the synthesis of pyrid-3-yl carbonylguanidine compounds bearing a *N*-substituted nitrogen in R2 should improve the inhibitory potency of NHE. Finally, further functional experiments are warranted to confirm their therapeutic interest.

## Experimental

### Drugs and reagents

Stock solutions (0.5 mM) of DMA, EIPA, HMA (all from Sigma, Belgium) or amiloride derivatives were prepared in DMSO. The final concentration of DMSO was minimized (maximum: 2%) so that it did not affect the measurements.  $^{22}\text{NaCl}$  (37 Mbq) was purchased from NEN (Brussels, Belgium) and ouabain from Sigma (Brussels, Belgium). Furosemide was a generous gift from Pharmachim (Jemeppe, Belgium).

### Chemistry

Melting points were determined on a Büchi-Tottoli apparatus in open capillary tubes and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1750 FT spectrophotometer. The  $^1\text{H}$  NMR spectra were taken on either a Bruker AW-80 (80 MHz) in  $\text{CDCl}_3$  or in  $\text{DMSO}-d_6$  with HMDS as the internal standard and a Bruker AW-400 (400.13 MHz) instrument in  $\text{DMSO}-d_6$  with TMS as the internal standard, at 25 °C; chemical shifts are reported in  $\delta$  values (ppm) relative to internal HMDS or TMS. The abbreviation s=singlet, d=doublet, t=triplet, q=quadruplet, m=multiplet, and br. s=broad singlet are used throughout. For most amiloride analogues, the high number of protons bound to nitrogen atoms increases the occurrence of exchange and greatly influence the shift values. The off-decoupling  $^{13}\text{C}$  NMR spectra were measured with a Bruker DRX 400 Avance spectrometer (100.62 MHz) in  $\text{DMSO}-d_6$  at 25 °C. Chemical shifts are reported in  $\delta$  values (ppm) with TMS as the internal standard. Elemental analyses (C, H, N, S in %) were performed with a Carlo-Erba EA 1108-elemental analyser and were within  $\pm 0.4\%$  of the theoretical values. All reactions were routinely checked by TLC on silica gel Merck 60F 254 plates.

**3,5-Diamino-2-chloro-6-methylpyridine (5).** Portionwise 2-chloro-6-methyl-3,5-dinitropyridine 4 (7.5 g, 35 mmol) was added to a suspension of tin chloride (42 g, 186 mmol) in HCl 12N (110 mL). After stirring for 90 min at room temperature, the mixture was heated at 100 °C for 45 min. After cooling, it was concentrated under reduced pressure, the residue brought to pH 12 with NaOH 5N, and continuously extracted with  $\text{CHCl}_3$  overnight. The organic layer was washed with water, dried and evaporated under reduced pressure to afford a

solid brown residue. The crude product 5 was crystallized from toluene. The precipitate was filtered, washed with petroleum ether 40–60 °C, and dried (3.3 g, 60%); mp 170–172 °C; IR 3460, 3375, 3301, 1618, 1477, 1449, 1427, 1245, 1212, 871, 741  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (80 MHz,  $\text{CDCl}_3 + \text{DMSO}-d_6$ , HMDS),  $\delta$  2.26 (s, 3H,  $\text{CH}_3$ ), 3.49 (s, 2H, 3- $\text{NH}_2$ ), 3.74 (s, 2H, 5- $\text{NH}_2$ ), 6.36 (s, 1H, 4H-pyridine). Anal. calcd for  $\text{C}_6\text{H}_8\text{N}_4\text{Cl}$ : C 45.73, H 5.12, N 26.66; found: C 46.05, H 5.23, N 26.57.

**3,5-Diacetylamino-2-chloro-6-methylpyridine (6).** A solution of 3,5-diamino-2-chloro-6-methylpyridine 5 (7.5 g, 48 mmol) in acetic anhydride (100 mL, 1.06 mol) was stirred for 30 min at room temperature until formation of a white precipitate. The medium was then poured on ice (100 g) and stirred for 30 min. Afterwards, it was neutralized with 30% NaOH to pH 5, and then to pH 7 with saturated  $\text{NaHCO}_3$ . After cooling overnight, the crude compound 6 was filtered, washed with water, and dried. Crystallization from toluene gave the title compound (10.3 g, 90%); mp 238–240 °C; IR 3306, 3270, 3016, 1667, 1536, 1499, 1432, 1373, 1241, 681  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (80 MHz,  $\text{CDCl}_3 + \text{DMSO}-d_6$ , HMDS),  $\delta$  2.07 (s, 6H,  $\text{NH}-\text{CO}-\text{CH}_3$ ), 2.33 (s, 3H,  $\text{CH}_3$ ), 8.26 (s, 1H, 4H-pyridine), 9.48 (s, 2H,  $\text{NH}-\text{CO}-\text{CH}_3$ ). Anal. calcd for  $\text{C}_{10}\text{H}_{12}\text{N}_4\text{O}_2\text{Cl}$ : C 49.70, H 5.00, N 17.39; found: C 49.45, H 5.26, N 17.66.

**3,5-Diacetylamino-6-chloro-pyridine-2-carboxylic acid (7).** A mixture of 3,5-diacetylamino-2-chloro-6-methylpyridine 6 (10 g, 41 mmol) and  $\text{MgSO}_4$  (3 g, 25 mmol) in water (200 mL) was stirred and heated at 80 °C.  $\text{KMnO}_4$  (16 g, 101 mmol) was added portionwise, and the resulting mixture was stirred for 1 h. Afterwards, the excess of  $\text{KMnO}_4$  was reduced by  $\text{NaHSO}_3$ , the manganese dioxide ( $\text{MnO}_2$ ) filtered, and washed with hot water (2  $\times$  50 mL). The combined filtrates were adjusted to pH 1 with 20%  $\text{H}_2\text{SO}_4$ . Left overnight at 4 °C, the formed precipitate was collected, washed with water, and dried. Crystallization from ethanol gave the title compound 7 (6.75 g, 60%); mp 212–215 °C; IR 3533, 3346, 3245, 1712, 1690, 1584, 1537, 1475, 1371, 1247, 1228, 1194, 723, 667  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (80 MHz,  $\text{CDCl}_3 + \text{DMSO}-d_6$ , HMDS),  $\delta$  2.19 (s, 3H, 5- $\text{NH}-\text{CO}-\text{CH}_3$ ), 2.33 (s, 3H, 3- $\text{NH}-\text{CO}-\text{CH}_3$ ), 5.36 (s,  $\text{COOH} + \text{H}_2\text{O}$ ), 9.35 (s, 1H, 5- $\text{NH}-\text{CO}-\text{CH}_3$ ), 9.68 (s, 1H, 4H-pyridine), 11.07 (s, 1H, 3- $\text{NH}-\text{CO}-\text{CH}_3$ ). Anal. calcd for  $\text{C}_{10}\text{H}_{10}\text{N}_4\text{O}_4\text{Cl}$ : C 44.21, H 3.71, N 15.47; found: C 43.96, H 3.98, N 15.76.

**Methyl 3,5-diacetylamino-6-chloro-pyridine-2-carboxylic ester (8).** The decomposition of methylnitrosourea (4.1 g, 40 mmol) into NaOH 5N gave diazomethane, which was extracted with diethyl ether (10 mL). The diazomethane solution was then added dropwise to a solution of 3,5-diacetylamino-6-chloro-pyridine-2-carboxylic acid 7 (2.7 g, 9.9 mmol) in a mixture ethanol–dimethylformamide (1:1, 100 mL), and the temperature was kept under 10 °C. After warming to room temperature, the medium was concentrated under reduced pressure, and diluted with water (100 mL). The pure compound 8 was filtered, washed with water, and dried (1.85 g, 65%); mp 197–199 °C; IR 3321, 3277, 1710, 1688, 1586, 1523,

310  
310

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

2020

Bogotá, D.C., 24/08/2009

Abogado

**Luis Patiño Leyva**

(Apoderado)

**ASUNTO**

Radicación **04 120497**

Trámite 002

Evento 001

Actuación 430

Oficio 9902

Folios 008

Patente de Invención

☒

Patente de Modelo de Utilidad

☐

Vía Nacional

☐

Vía PCT (fase nacional)

☒

Cap. I

☐

Cap. II

☒

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

**1. Documentos estudiados**

1.1. Descripción: Folios: 85 a 198

1.2. Reivindicaciones: Folios: 199 a 212  
Folios: 291 a 297 Memorial de 15/07/2008

1.3. Datos PCT:

|                             |                |
|-----------------------------|----------------|
| Solicitud Internacional:    | PCT/EP03/05279 |
| Capítulo II                 |                |
| Fecha Sol. Internacional:   | 20/05/2003     |
| Publicación. Internacional: | WO03/101450    |
| Fecha Pub. Internacional:   | 11/12/2003     |
| Fecha Inicio Fase Nacional: | 03/01/2005     |
| Solicitud Nacional:         | CO 04 120497   |
| Fecha Sol. Nacional:        | 30/11/2004     |

1.4. Prioridad: FR0206783 de 03/06/2002

1.5. Respuesta del solicitante: Folios 286 a 297 Memorial de 15/07/2008

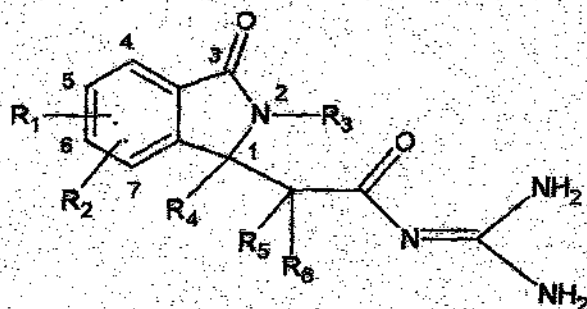
**2. Objeto de la invención**

Título de la solicitud

**Derivados de N-((3-oxo-2,3-dihidro-1H-isoindol-1-il)acetil)guanidina en calidad de inhibidores de NHE-1 para el tratamiento del infarto y la angina de pecho**

Reivindicaciones

**Reivindicaciones 1 a 4:** Se reivindican los compuestos de fórmula (I):



Especialmente, se reclama protección para las especies químicas:

N-[2-(2-isobutil-3-oxo-2,3-dihidro-1H-isoindol-1-il)-2-metilpropionil]guanidina  
 N-[(2-isobutil-7-metil-3-oxo-2,3-dihidro-1H-isoindol-1-il)acetil]guanidina  
 N-[5-cloro-3-oxo-2-(3,3,3-trifluoropropil)-2,3-dihidro-1H-isoindol-1-il)acetil]-guanidina

Entre otros.

**Reivindicaciones 5 y 6:** Se reivindican las composiciones farmacéuticas que contienen compuestos de fórmula (I).

**Reivindicación 7:** Se reivindica el proceso para la obtención de los compuestos de fórmula (I) caracterizado porque comprende las siguientes etapas:

- Un hidruro complejo se hace reaccionar con una ftalimida de fórmula (II).
- El producto obtenido se hace reaccionar luego con un alcóxicarbonilmetileno-trifenilfosforano en tolueno, o con un fosfonoacetato de trialquilo y una base,
- El producto obtenido se hace reaccionar con cloruro de guanidino y una base o con guanidina.

**Reivindicación 8:** Se reivindica el proceso para la obtención de los compuestos de fórmula (I) caracterizado porque comprende las siguientes etapas:

- Un compuesto de fórmula (III) se hace reaccionar con un alcóxicarbonilmetileno-trifenil-fosforano en tolueno o con un fosfonoacetato de trialquilo y una base.
- El producto obtenido se hace reaccionar con una amina de la fórmula  $R_3NH_2$  y una carbodiimida
- El producto obtenido se hace reaccionar con cloruro de guanidino y una base o con guanidina.

**Reivindicación 9:** Se reivindica el proceso para la obtención de los compuestos de fórmula (I) caracterizado porque comprende las siguientes etapas:

- Una ftalimida se hace reaccionar con haluro de alquil magnesio con un reactivo de alquil-litio en un éter
- El producto obtenido se hace reaccionar con un alcóxycarbonilmetileno-trifenilfosforano en tolueno o con 1-etoxi-1-trimetilsiloxietileno y un ácido de Lewis
- El producto obtenido se hace reaccionar con cloruro de guanidinio y una base o con guanidina.

**Reivindicación 10:** Se reivindica el proceso para la obtención de los compuestos de fórmula (I) caracterizado porque comprende las siguientes etapas:

- El compuesto de fórmula (IV) se hace reaccionar en presencia de diisopropilamido de litio
- El producto obtenido se hace reaccionar con cloruro de guanidinio y una base o con guanidina en un alcohol.

#### Sustento Descriptivo

La invención se refiere a nuevos compuestos de isoindolinona de la fórmula (I) que son adecuados como medicamentos anti-arritmicos con un componente cardioprotector para la profilaxis del infarto y el tratamiento del infarto y la angina de pecho. Los compuestos también inhiben de una manera preventiva los procesos patofisiológicos asociados al daño inducido por isquemia, en particular el desencadenamiento de arritmias cardíacas inducidas por isquemia y de la insuficiencia cardíaca.

Los inhibidores del NHE1 de la fórmula (I) tienen un importante efecto antiinflamatorio y pueden utilizarse en la inhibición de los mediadores de la inflamación, además exhiben un efecto reductor de las lipoproteínas del suero factor de riesgo esencial para el desarrollo de lesiones vasculares arterioescleróticas.

- Una vez catalogada la invención ésta se clasificó en la sección: **A 61 K<sup>31</sup>/4035** de la **Clasificación Internacional de Patentes**.

### **3. Determinación del Estado de la Técnica**

Considerando que la fecha de presentación de la primera solicitud de patente de la cual se reivindica prioridad es: 03/06/2002, se procedió a efectuar la búsqueda de documentos relacionados en el estado de la técnica con fecha de publicación anterior a ésta.

| Nº | Documento   | Título                                                                                                          | Fecha de Publicación |
|----|-------------|-----------------------------------------------------------------------------------------------------------------|----------------------|
| D1 | XP002238283 | "Potent and selective inhibition of the human Na <sup>+</sup> /K <sup>+</sup> exchanger isoform NHE1 by a novel | 11/04/2001           |

|    |            |                                                                                                                                                             |               |
|----|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
|    |            | aminoguanidine derivative T-162559"                                                                                                                         |               |
| D2 | Artículo 1 | "Discovery of Zoniporide: A potent and selective sodium-hydrogen exchanger type 1 (NHE-1) inhibitor with high aqueous solubility"                           | Enero de 2001 |
| D3 | Artículo 2 | "Synthesis and Biological evaluation of aroylguanidines related to amiloride as inhibitors of the human platelet Na <sup>+</sup> /H <sup>+</sup> exchanger" | Enero de 2002 |

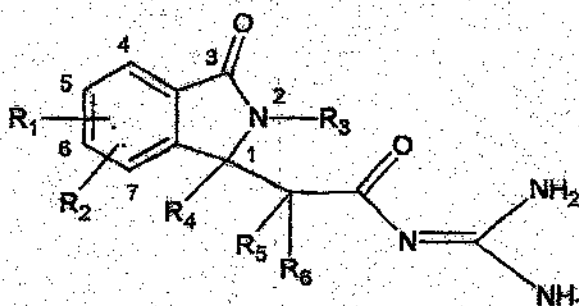
Anterioridad D1 en los Folios 276 a 283, D2 y D3 en los folios 298 a 309.

#### 4. Análisis de patentabilidad (artículo 14 D. 486)

##### 4.1 Nivel inventivo (artículo 18 D. 486)

El nivel inventivo del objeto reclamado se evaluó por comparación entre las características esenciales de los compuestos definidos en el pliego reivindicatorio y los elementos estructurales anticipados por el arte previo.

- Se reivindican compuestos derivados del núcleo de la fórmula (I):



Especialmente, se reclama protección para las especies químicas:

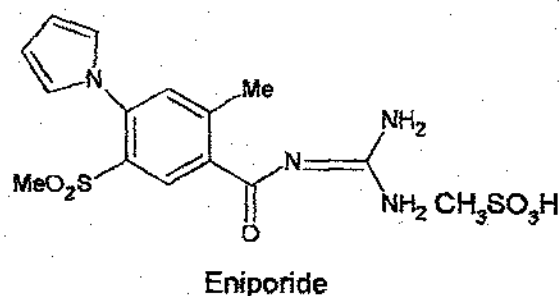
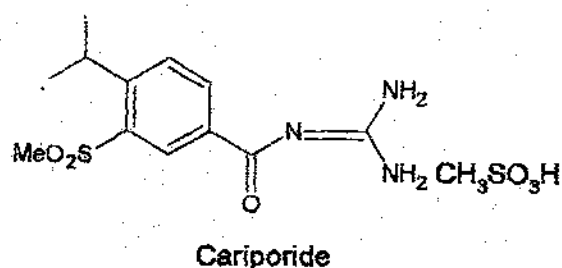
N-[2-(2-isobutil-3-oxo-2,3-dihidro-1H-isoindol-1-il)-2-metilpropionil]guanidina  
 N-[(2-isobutil-7-metil-3-oxo-2,3-dihidro-1H-isoindol-1il)acetil]guanidina  
 N-[5-cloro-3-oxo-2-(3,3,3-trifluoropropil)-2,3-dihidro-1H-isoindol-1-il)acetil]-  
 guanidina

Entre otros.

- El estado del arte previo a la invención presenta las siguientes estructuras:

##### Anterioridad D1:

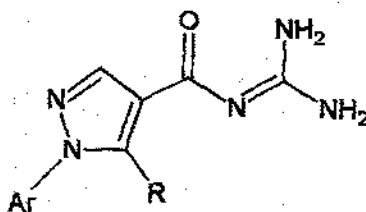
La publicación D1 revela derivados de aminoguanidina que presentan actividad como inhibidores selectivos de la isoforma NHE-1 intercambiador Na<sup>+</sup>/H<sup>+</sup>. Dentro de los compuestos que se encuentran revelados en el folio 277 se encuentran las especies químicas Cariporide y Eniporide:



### Anterioridad D2:

La publicación **D2** hace referencia a la actividad selectiva de Zoniporide como inhibidor de la actividad del intercambiador  $\text{Na}^+/\text{H}^+$  NHE en especial, de los subtipos NHE-1 y NHE-2.

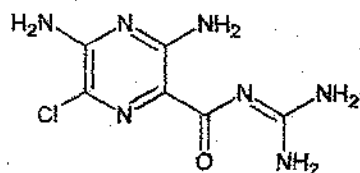
El compuesto zoniporide y sus análogos estructurales se derivan de la estructura de fórmula 3:



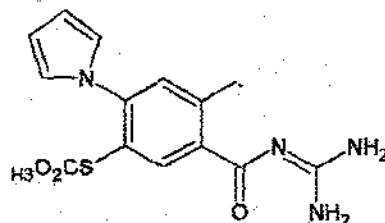
| Compuesto       | R           | Ar           | NHE-1<br>IC <sub>50</sub> (nM) <sup>a</sup> | NH-2<br>IC <sub>50</sub> (nM) <sup>c</sup> | NH-1 vs NHE-2 <sup>d</sup> |
|-----------------|-------------|--------------|---------------------------------------------|--------------------------------------------|----------------------------|
| 3a              | Me          | Fenil        | 830                                         | 3900                                       | 5                          |
| 3b              | Ciclopropil | Fenil        | 350                                         | 12,800                                     | 37                         |
| 3c              | Ciclopropil | 1-nfatil     | 160                                         | 16,300                                     | 102                        |
| 3d (Zoniporide) | Ciclopropil | 5-quinolinil | 59                                          | 12,000                                     | 203                        |
| 3e              | Me          | 5-quinolinil | 860                                         | 41,600                                     | 48                         |
| 1a (Cariporide) |             |              | 560                                         | 28,700                                     | 51                         |
| 1b (Eniporide)  |             |              | 140                                         | 9200                                       | 66                         |

### Anterioridad D3:

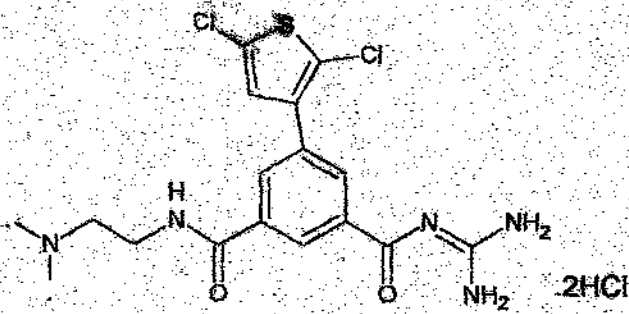
La publicación **D3** hace referencia a compuestos con estructura análoga a la reivindicada, denominados derivados de aroilguanidinas con actividad inhibidora del intercambiador  $\text{Na}^+/\text{H}^+$ . En particular, se presenta el compuesto amiloride y otras estructuras similares que a continuación se relacionan:



AMILORIDE (Derivado de pirazina)



EMD-96785 (Derivado de benceno)



FR-183988

En el folio 308 de la anterioridad D3 se establece que de acuerdo con el estudio efectuado sobre la intercambiabilidad de grupos sobre la estructura del compuesto amiloride se consiguen iguales resultados cuando el anillo es de piridina o cuando el anillo es de benceno, sin que esta situación modifique la potencia inhibitoria del intercambiador  $\text{Na}^+/\text{H}^+$  NHE.

**Diferencias Técnicas:**

Al evaluar los elementos característicos de los compuestos reivindicados con respecto a las enseñanzas del estado de la técnica reveladas en los antecedentes D1 o D2 o D3 es posible concluir lo siguiente:

Con respecto al documento D1 la diferencia estriba en que los derivados de guanidina Cariporide y Eniporide no presentan núcleo de isoindolinona sino de benceno.

Con respecto al documento D2 que presenta derivados de guanidina la diferencia estriba en que el núcleo es un anillo de cinco miembros de pirazol, mientras que en el caso de los compuestos reivindicados el anillo central es de isoindolinona.

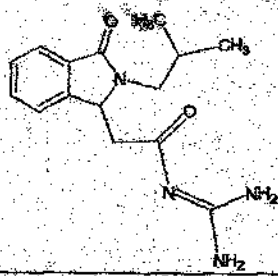
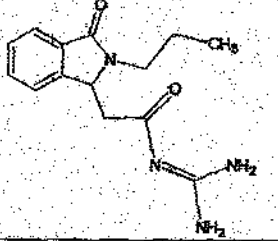
Con relación a las enseñanzas reveladas por el documento D3 la diferencia estriba en que estos compuestos presentan como anillo central estructuras de benceno y pirazina, sin embargo no presentan núcleos fusionados de isoindolinona.

**Del efecto técnico y el problema objetivo:**

De acuerdo con el sustento descriptivo de la solicitud los compuestos que presentan núcleo de isoindol-acetil-guanidina son agentes antiarrítmicos porque inhiben la actividad del intercambiador  $\text{Na}^+/\text{H}^+$  NHE-1. Con el nuevo diseño estructural reivindicado en los folios 291 a 297 se pretende generar una alternativa terapéutica para el manejo de la arritmia cardíaca.

Para demostrar las ventajas que evidencian los compuestos el solicitante adjunta una tabla con las respuestas de actividad IC50 para los ejemplos 1 a 56b, dentro de los que se encuentran estructuras como:

| Compuestos              | IC50      |
|-------------------------|-----------|
| <p><b>Ejemplo 1</b></p> | <p>74</p> |

|                                                                                                           |           |
|-----------------------------------------------------------------------------------------------------------|-----------|
| <p><b>Ejemplo 2</b></p>  | <p>58</p> |
| <p><b>Ejemplo 5</b></p>  | <p>92</p> |

Al respecto es preciso señalar que la diferencia en cuanto a la estructura anular de isoindolinona y su intercambio por anillos de benceno y pirazina no demuestran una ventaja técnica comparativa que evidencie una ventaja inesperada en términos del efecto técnico. Por el contrario, los valores de  $IC_{50}$  de algunos de los ejemplos que acompañan el capítulo descriptivo, como es el caso de las estructuras 1, 2 y 5 demuestran que se requiere la misma concentración para inhibir la actividad del intercambiador NHE-1 que el compuesto Zoniporide, por lo que se concluye que la materia reivindicada carece del requisito de altura inventiva.

Además, los compuestos objeto de reivindicación constituyen una serie bioisómera de la primer serie de guanidinas con actividad sobre el intercambiador  $Na^+/H^+$  NHE y en razón a la comparación efectuada y a la similitud estructural se concluye que los compuestos no cumplen con las disposiciones normativas del artículo 18 de la Decisión 486.

### 5. Conclusión:

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

| N° | Documento N° | Reivindicaciones Afectadas | Requisito que Afecta |
|----|--------------|----------------------------|----------------------|
| D1 | XP002238283  | 1 a 10                     | Nivel Inventivo      |
| D2 | Artículo 1   | 1 a 10                     | Nivel Inventivo      |
| D3 | Artículo 2   | 1 a 10                     | Nivel Inventivo      |

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

317  
317  
Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.

**ALIX CARMENZA CÉSPEDES DE VERGEL**  
Jefe de División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista, de fecha

28 AGO. 2000

**CONCEPTO TÉCNICO No. 2833**

**EXAMINADOR TÉCNICO**

**Código No. 202044**