

SUPERINDUSTRIA Y COMERCIO

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Dependencia: 2820 DIVISION DE NUEVAS CREACIONES

Señores

DIVISION DE NUEVAS CREACIONES

Superintendencia de Industria y Comercio

Ciudad.-

02-01-09-

Re.: Expediente No. 2000 - 26.001

Solicitud de Patente

Debidamente traducida, adjunto acompaño la copia oficial de la solicitud de patente básica Estadounidense No. 60/128.687, la cual le solicito agregar a este expediente para los efectos legales respectivos.

De Ud. atentamente,

GERMAN CASTILLO GRAU

T.P. 2978

\$3 39

**ESTADOS UNIDOS DE AMÉRICA**

**A TODOS A QUIENES LLEGUE EL PRESENTE:**

**DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS**

Oficina de Marcas y Patentes

20 de marzo de 2000.

LA PRESENTE CERTIFICA QUE LA ADJUNTA ES UNA COPIA TOMADA DE LOS REGISTROS DE LA OFICINA DE MARCAS Y PATENTES DE LOS ESTADOS UNIDOS, DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE ABAJO IDENTIFICADA Y LA CUAL CUMPLE CON LOS REQUISITOS PARA QUE LE SEA CONFERIDA UNA FECHA DE RADICACIÓN SEGÚN 35 USC 111.

**NÚMERO DE SOLICITUD** 60/128,687

**FECHA DE RADICACIÓN:** 09 de abril de 1999

Por Autoridad del

COMISIONADO DE MARCAS Y PATENTES

(FIRMA) T. WALLACE

Oficial de Certificaciones

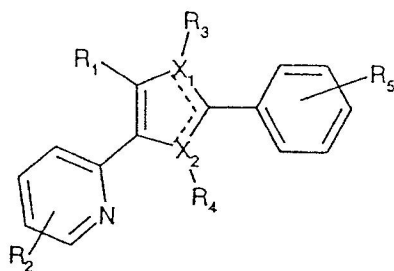
(SELLO DORADO DE OBLEA)

\* \* \* \*

## COMPUESTOS Y MÉTODOS

### REIVINDICACIONES:

1. Un método para inhibir el trayecto de señalización de TGF- $\beta$  en mamíferos que consiste en administrar a un mamífero, una cantidad inhibidora terapéuticamente efectiva de un compuesto de la fórmula (I), o una sal farmacéuticamente aceptable de lo mismo:



(I)

en donde:

$R^1$  es naftil, antracenil o fenil, en donde fenil es sustituido opcionalmente con sustituyentes sin carga formal seleccionados del grupo compuesto por halo, -O-alquil $C_{1-4}$ , -S-alquil $C_{1-4}$ , alquil $C_{1-4}$ , O-(CH $_2$ ) $_n$ -Ph, S-(CH $_2$ ) $_n$ -Ph, ciano, fenil y CO $_2$ R, en donde R es alquil $C_{1-4}$  y n es 0-3 o  $R^1$  es fenil fusionado con un anillo cíclico aromático o no aromático de 5 a 7 miembros en donde dicho anillo cíclico que contiene opcionalmente hasta dos heteroátomos, independientemente seleccionado de N, O y S;

$R_2$  es H, NH(CH $_2$ ) $_n$ -Ph, NH-alquil;

$R_3$  es H, OH, alquil $C_{1-6}$  o cicloalquil $C_{3-7}$ , cuando  $X_1$  es N o C;

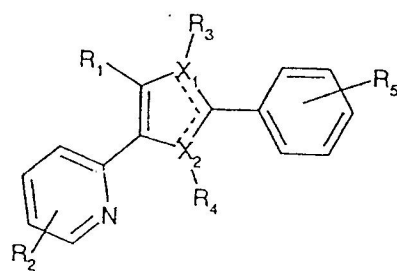
$R_4$  es H, o OH, cuando  $X_2$  es n O c;

$R_5$  es CO $_2$ H, CONH $_2$ , CN, NO $_2$ , S-alquil $C_{1-4}$ , SO-alquil $C_{1-4}$ , O-alquil $C_{1-4}$ , SONH $_2$ , CONHOH, NH $_2$ , CHO, CH $_2$ OH, CH $_2$ OH, CH $_2$ NH $_2$  o CO $_2$ R', en donde R' es alquil $C_{1-6}$ ;

$X_1$  es N o C, o  $X_1$  es S u O, cuando  $X_2$  es N o C; y

$X_2$  es N o C, o  $X_2$  es S u O cuando  $X_1$  es N o C;

2. El método de la reivindicación 1, en donde la inhibición del trayecto de señalización TGF- $\beta$  ocurre mediante la inhibición de la fosforilación de smad2 o smad3.
3. El método de la reivindicación 2, en donde la fosforilación de smad2 o smad3 ocurre mediante el receptor ALK5.
4. Un método para tratar una enfermedad mediada por el receptor ALK5 en mamíferos, que consisten administrar a un mamífero que requiera dicho tratamiento, de una cantidad terapéuticamente efectiva de un compuesto de la fórmula (I) o una sal farmacéuticamente aceptable de lo mismo.



(I)

en donde:

R<sub>1</sub> es naftil, antracenil o fenil, en donde fenil es sustituido opcionalmente sustituido con sustituyentes sin carga formal seleccionados del grupo compuesto por halo, -O-alkilC<sub>1-4</sub>, -S-alkilC<sub>1-4</sub>, alkilC<sub>1-4</sub>, O-(CH<sub>2</sub>)<sub>n</sub>-Ph, S-(CH<sub>2</sub>)<sub>n</sub>-Ph, ciano, fenil y CO<sub>2</sub>R, en donde R es alkilC<sub>1-4</sub> y n es 0-3 o R<sup>1</sup> es fenil fusionado con un anillo cíclico aromático o no aromático de 5 a 7 miembros en donde dicho anillo cíclico que contiene opcionalmente hasta dos heteroátomos, independientemente seleccionado de N, O y S;

R<sub>2</sub> es H, NH(CH<sub>2</sub>)<sub>n</sub>-Ph, NH-alkil;



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$R_3$  es H, OH, alquil $C_{1-6}$  o cicloalquil $C_{3-7}$ , cuando  $X_1$  es N o C;

$R_4$  es H, o OH, cuando  $X_2$  es N o C;

$R_5$  es  $CO_2H$ ,  $CONH_2$ , CN,  $NO_2$ , S-alquil $C_{1-4}$ , SO-alquil $C_{1-4}$ , O-alquil $C_{1-4}$ ,  $SONH_2$ ,  $CONHOH$ ,  $NH_2$ , CHO,  $CH_2OH$ ,  $CH_2NH_2$  o  $CO_2R'$ , en donde  $R'$  es alquil $C_{1-6}$ ;

$X_1$  es N o C, o  $X_1$  es S u O, cuando  $X_2$  es N o C; y

$X_2$  es N o C, o  $X_2$  es S u O cuando  $X_1$  es N o C.

5. El método de la reivindicación 1, en donde el compuesto de la fórmula (I) es un compuesto seleccionado de:

4-[4(3,4-etilendioxfenil)-5-(2-piridil)-1H-imidazol-2-il]benzonitrilo;

4-[4(4-metoxifenil)-5-(2-piridil)-1H-imidazol-2-il]benzonitrilo;

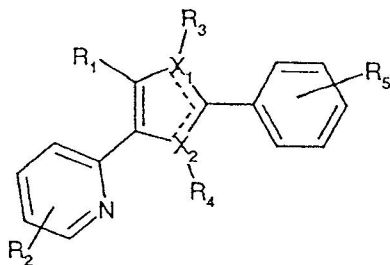
4-[4(4-fluorofenil)-5-(2-piridil)-1H-imidazol-2-il]nitrobenceno; y

4-[4(4-fluorofenil)-5-(2-piridil)-1H-imidazol-2-il]analina.

6. El método de la reivindicación 4, en donde la enfermedad es seleccionada de enfermedad renal crónica, enfermedad renal aguda, curación de heridas, artritis, osteoporosis, enfermedad renal, falla cardíaca congestiva, úlceras, trastornos oculares, heridas de la córnea, nefropatía diabética, mala función neurológica, enfermedad de Alzheimer, condiciones tróficas, aterosclerosis, cualquier enfermedad en donde fibrosis sea un componente mayor, y restenosis.

7. Un compuesto que es 4-[4(4-Fluorofenil)-5-(2-piridil)-1H-imidazol-2-il]ácido benzóico.

8. Un compuesto de la fórmula (I), o una sal farmacéuticamente aceptable del mismo:



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en donde:

$R_1$  es naftil antracenil o fenil, en donde fenil es sustituido opcionalmente con sustituyentes sin carga formal seleccionados del grupo compuesto por halo, -O-alkil $C_{1-4}$ , -S-alkil $C_{1-4}$ , alkil $C_{1-4}$ , O-(CH $_2$ ) $_n$ -Ph, S-(CH $_2$ ) $_n$ -Ph, ciano, fenil y CO $_2$ R, en donde R es alkil $C_{1-4}$  y  $n$  es 0-3 o  $R^1$  es fenil fusionado con un anillo cíclico aromático o no aromático de 5 a 7 miembros en donde dicho anillo cíclico que contiene opcionalmente hasta dos heteroátomos, independientemente seleccionado de N, O y S;

$R_2$  es H, NH(CH $_2$ ) $_n$ -Ph, NH-alkil;

$R_3$  es H, OH, alkil $C_{1-6}$  o cicloalkil $C_{3-7}$ , cuando  $X_1$  es N o C;

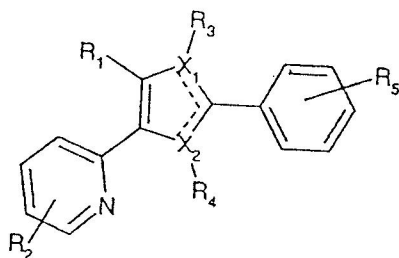
$R_4$  es H, o OH, cuando  $X_2$  es N o C;

$R_5$  es CO $_2$ H, CONH $_2$ , CN, NO $_2$ , S-alkil $C_{1-4}$ , SO-alkil $C_{1-4}$ , O-alkil $C_{1-4}$ , SONH $_2$ , CONHOH, NH $_2$ , CHO, CH $_2$ OH, CH $_2$ OH, CH $_2$ NH $_2$  o CO $_2$ R', en donde R' es alkil $C_{1-6}$ ;

$X_1$  es N o C, o  $X_1$  es S u O, cuando  $X_2$  es N o C; y

$X_2$  es N o C, o  $X_2$  es S u O cuando  $X_1$  es N o C.

9. Un método para el tratamiento de una condición asociada con exceso de smad3 en mamíferos, que consiste en administrar a un mamífero que requiera tal tratamiento, una cantidad terapéuticamente efectiva de un compuesto de la fórmula (I) o una sal farmacéuticamente aceptable de lo mismo:



(I)

en donde:

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$R_1$  es naftil, antracénil o fenil, en donde fenil es sustituido opcionalmente con sustituyentes sin carga formal seleccionados del grupo compuesto por halo, -O-alkil $C_{1-4}$ , -S-alkil $C_{1-4}$ , alkil $C_{1-4}$ , O-(CH $_2$ ) $_n$ -Ph, S-(CH $_2$ ) $_n$ -Ph, ciano, fenil y CO $_2$ R, en donde R es alkil $C_{1-4}$  y n es 0-3 o R $^1$  es fenil fusionado con un anillo cíclico aromático o no aromático de 5 a 7 miembros en donde dicho anillo cíclico que contiene opcionalmente hasta dos heteroátomos, independientemente seleccionado de N, O y S;

$R_2$  es H, NH(CH $_2$ ) $_n$ -Ph, NH-alkil;

$R_3$  es H, OH, alkil $C_{1-6}$  o cicloalkil $C_{3-7}$ , cuando X $_1$  es N o C;

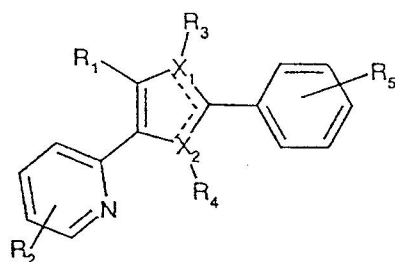
$R_4$  es H, o OH, cuando X $_2$  es n O c;

$R_5$  es CO $_2$ H, CONH $_2$ , CN, NO $_2$ , S-alkil $C_{1-4}$ , SO-alkil $C_{1-4}$ , O-alkil $C_{1-4}$ , SONH $_2$ , CONHOH, NH $_2$ , CHO, CH $_2$ OH, CH $_2$ OH, CH $_2$ NH $_2$  o CO $_2$ R', en donde R' es alkil $C_{1-6}$ ;

X $_1$  es N o C, o X $_1$  es S u O, cuando X $_2$  es N o C; y

X $_2$  es N o C, o X $_2$  es S u O cuando X $_1$  es N o C

10. Un método para el tratamiento de una condición asociada con exceso de smad2 en mamíferos, que consiste en administrar a un mamífero que requiera tal tratamiento una cantidad terapéuticamente efectiva de un compuesto de la fórmula (I) o una sal farmacéuticamente aceptable de lo mismo:



(I)

en donde:

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$R_1$  es naftil, antracenil o fenil, en donde fenil es sustituido opcionalmente con sustituyentes sin carga formal seleccionados del grupo compuesto por halo, -O-alquil $C_{1-4}$ , -S-alquil $C_{1-4}$ , alquil $C_{1-4}$ , O-(CH $_2$ ) $_n$ -Ph, S-(CH $_2$ ) $_n$ -Ph, ciano, fenil y CO $_2$ R, en donde R es alquil $C_{1-4}$  y n es 0-3 o R $^1$  es fenil fusionado con un anillo cíclico aromático o no aromático de 5 a 7 miembros en donde dicho anillo cíclico que contiene opcionalmente hasta dos heteroátomos, independientemente seleccionado de N, O y S;

$R_2$  es H, NH(CH $_2$ ) $_n$ -Ph, NH-alquil;

$R_3$  es H, OH, alquil $C_{1-6}$  o cicloalquil $C_{3-7}$ , cuando X $_1$  es N o C;

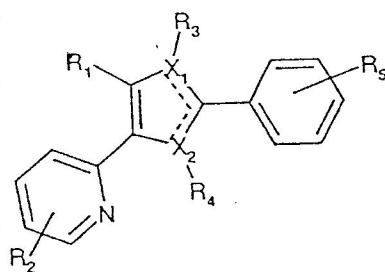
$R_4$  es H, o OH, cuando X $_2$  es n O c;

$R_5$  es CO $_2$ H, CONH $_2$ , CN, NO $_2$ , S-alquil $C_{1-4}$ , SO-alquil $C_{1-4}$ , O-alquil $C_{1-4}$ , SONH $_2$ , CONHOH, NH $_2$ , CHO, CH $_2$ OH, CH $_2$ OH, CH $_2$ NH $_2$  o CO $_2$ R', en donde R' es alquil $C_{1-6}$ ;

X $_1$  es N o C, o X $_1$  es S u O, cuando X $_2$  es N o C; y

X $_2$  es N o C, o X $_2$  es S u O cuando X $_1$  es N o C

11. Un método para inhibir la formación de matriz en mamíferos, que consiste en administrar a un mamífero que requiera tal tratamiento una cantidad terapéuticamente efectiva de un compuesto de la fórmula (I) o una sal farmacéuticamente aceptable de lo mismo:



(I)

en donde:

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$R_1$  es naftil, antracenil o fenil, en donde fenil es sustituido opcionalmente con sustituyentes sin carga formal seleccionados del grupo compuesto por halo, -O-alquil $C_{1-4}$ , -S-alquil $C_{1-4}$ , alquil $C_{1-4}$ , O-(CH $_2$ ) $_n$ -Ph, S-(CH $_2$ ) $_n$ -Ph, ciano, fenil y CO $_2$ R, en donde R es alquil $C_{1-4}$  y n es 0-3 o R $^1$  es fenil fusionado con un anillo cíclico aromático o no aromático de 5 a 7 miembros en donde dicho anillo cíclico que contiene opcionalmente hasta dos heteroátomos, independientemente seleccionado de N, O y S;

$R_2$  es H, NH(CH $_2$ ) $_n$ -Ph, NH-alquil;

$R_3$  es H, OH, alquil $C_{1-6}$  o cicloalquil $C_{3-7}$ , cuando X $_1$  es N o C;

$R_4$  es H, o OH, cuando X $_2$  es n O c;

$R_5$  es CO $_2$ H, CONH $_2$ , CN, NO $_2$ , S-alquil $C_{1-4}$ , SO-alquil $C_{1-4}$ , O-alquil $C_{1-4}$ , SONH $_2$ , CONHOH, NH $_2$ , CHO, CH $_2$ OH, CH $_2$ OH, CH $_2$ NH $_2$  o CO $_2$ R', en donde R' es alquil $C_{1-6}$ ;

X $_1$  es N o C, o X $_1$  es S u O, cuando X $_2$  es N o C; y

X $_2$  es N o C, o X $_2$  es S u O cuando X $_1$  es N o C

PA 221398



# THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

March 20, 2000

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 60/128,687

FILING DATE: April 09, 1999



By Authority of the  
COMMISSIONER OF PATENTS AND TRADEMARKS

*T. Wallace*  
T. WALLACE

Certifying Officer

04/09/99



Jc649 U.S. PTO

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I Hereby Certify That This Paper Or Fee Is Being Deposited With The United States Postal Service "Express Mail Post Office To Addressee" Service Under 37 CFR 1.10 On The Date Indicated Above And Is Addressed To: Assistant Commissioner for Patents, Washington, D.C. 20231.

Name Of Person Mailing Paper Or Fee

(Type Or Print) J. WERNERSignature [Signature]

52 APPROV

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**PROVISIONAL APPLICATION COVER SHEET**

This is a request for filing a PROVISIONAL APPLICATION for PATENT under 37 CFR 1.53(c).

Docket No.

P50909P

**INVENTOR(s) / APPLICANT(s)**

| Last Name | First Name | Middle Initial | Residence (City and Either State or Foreign Country) |
|-----------|------------|----------------|------------------------------------------------------|
| Burgess   | Joelle     | L.             | Phoenixville, PA 19460                               |
| Callahan  | James      | F.             | Philadelphia, PA 19111                               |

**TITLE OF THE INVENTION (280 characters max)**

COMPOUNDS AND METHODS

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Country

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**ENCLOSED APPLICATION PARTS (check all that apply)**

|                                                   |                  |    |                                                     |
|---------------------------------------------------|------------------|----|-----------------------------------------------------|
| <input checked="" type="checkbox"/> Specification | Number of Pages  | 22 | <input type="checkbox"/> Small Entity Statement     |
| <input checked="" type="checkbox"/> Figures       | Number of Sheets | 2  | <input checked="" type="checkbox"/> Other (specify) |
|                                                   |                  |    | 1 page abstract                                     |

**METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT**☒ The Commissioner is hereby authorized to charge filing fees and credit Deposit Account No. 19-2570PROVISIONAL FILING  
FEE AMOUNT (\$)**\$150.00**

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.☐ Yes, the name of the U.S. Government agency and the Government contract number are: \_\_\_\_\_Respectfully submitted,  
Signature:Nora Stein-Fernandez  
Nora Stein-Fernandez

Date:

April 9, 1999

Registration No.:

36,689

O Additional inventors are being named on separately numbered sheets attached hereto.

**PROVISIONAL APPLICATION FILING ONLY**

SEND TO: Assistant Commissioner for Patents, Box Provisional Application, Washington, D.C. 20231.

Document3

Jc541 U.S. PTO

60/128687



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COMPOUNDS AND METHODSFIELD OF THE INVENTION

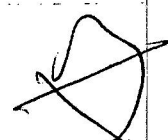
This invention relates to pyridyl substituted triarylimidazoles, and pharmaceutical compositions containing them, which are inhibitors of the transforming growth factor, ("TGF")- $\beta$  signaling pathway, in particular, the phosphorylation of smad2 or smad3 by the type I or activin-like kinase ("ALK")-5 receptor, methods for their preparation and their use in medicine, specifically to the treatment and prevention of a disease state mediated by this pathway.

BACKGROUND OF THE INVENTION

TGF- $\beta$ 1 is the prototypic member of a family of cytokines including the TGF- $\beta$ s, activins, inhibins, bone morphogenetic proteins and Müllerian-inhibiting substance, that signal through a family of single transmembrane serine/threonine kinase receptors. These receptors can be divided in two classes, the type I or activin like kinase (ALK) receptors and type II receptors. The ALK receptors are distinguished from the type II receptors in that the ALK receptors (a) lack the serine/threonine rich intracellular tail, (b) possess serine/threonine kinase domains that are very homologous between type I receptors, and (c) share a common sequence motif called the GS domain, consisting of a region rich in glycine and serine residues. The GS domain is at the amino terminal end of the intracellular kinase domain and is critical for activation by the type II receptor. Several studies have shown that TGF- $\beta$  signaling requires both the ALK and type II receptors. Specifically, the type II receptor phosphorylates the GS domain of the type I receptor for TGF- $\beta$ , ALK5, in the presence of TGF- $\beta$ . The ALK5, in turn, phosphorylates the cytoplasmic proteins smad2 and smad3 at two carboxy terminal serines (see Fig. 1). Generally it is believed that in many species, the type II receptors regulate cell proliferation and the type I receptors regulate matrix production. Therefore, preferred compounds of this invention are selective in that they inhibit the type I receptor and thus matrix production, and not the type II receptor mediated proliferation.

Activation of the TGF- $\beta$ 1 axis and expansion of extracellular matrix are early and persistent contributors to the development and progression of chronic renal disease and vascular disease. Border W.A., Noble N.A., *N Engl J Med*, Nov. 10, 1994; 331(19):1286-92. Further, TGF- $\beta$ 1 plays a role in the formation of fibronectin and plasminogen activator inhibitor-1, components of



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- sclerotic deposits, through the action of smad3 phosphorylation by the TGF- $\beta$ 1 receptor ALK5. Zhang Y., Feng X.H., Derynck R., *Nature*, Aug. 27, 1998; 394(6696):909-13; Usui T., Takase M., Kaji Y., Suzuki K., Ishida K., Tsuru T., Miyata K., Kawabata M., Yamashita H., *Invest Ophthalmol Vis Sci*, Oct. 1998; 39(11):1981-9.

Progressive fibrosis in the kidney and cardiovascular system is a major cause of suffering and death and an important contributor to the cost of health care. Chronic renal disease is characterized by the progressive loss of renal function due to glomerular, tubular or interstitial disease. There are also well over 30 million individuals in the United States at risk for atherosclerosis-related cardiovascular disease. While lipid-lowering therapy is the predominant treatment, only 30% of atherosclerosis patients have elevated lipids. Thus there is a present need for new agents that prevent sclerosis, remodeling of the intima, and restenosis.

- More specifically, the complex architecture and filtration function of the kidney make it particularly vulnerable to the consequences of fibrosis. TGF- $\beta$ 1 has been implicated in many renal fibrotic disorders. Border W.A., Noble N.A., *N Engl J Med*, Nov 10, 1994; 331(19):1286-92. TGF- $\beta$ 1 is elevated in acute and chronic glomerulonephritis Yoshioka K., Takemura T., Murakami K., Okada M., Hino S., Miyamoto H., Maki S., *Lab Invest*, Feb. 1993; 68(2):154-63, diabetic nephropathy Yamamoto, T., Nakamura, T., Noble, N.A., Ruoslahti, E., Border, W.A., (1993) *PNAS* 90:1814-1818., allograft rejection, HIV nephropathy and angiotensin-induced nephropathy Border W.A., Noble N.A., *N Engl J Med*, Nov. 10, 1994; 331(19):1286-92. In these diseases the levels of TGF- $\beta$ 1 expression coincide with the production of extracellular matrix. Three lines of evidence suggest a causal relationship between TGF- $\beta$ 1 and the production of matrix. First, normal glomeruli, mesangial cells and non-renal cells can be induced to produce extracellular-matrix protein and inhibit protease activity by exogenous TGF- $\beta$ 1 in vitro. Second, neutralizing anti-bodies against TGF- $\beta$ 1 can prevent the accumulation of extracellular matrix in nephritic rats. Third, TGF- $\beta$ 1 transgenic mice or in vivo transfection of the TGF- $\beta$ 1 gene into normal rat kidneys resulted in the rapid development of glomerulosclerosis. Kopp J.B., Factor V.M., Mozes M., Nagy P., Sanderson N., Bottinger E.P., Klotman P.E., Thorgeirsson S.S., *Lab Invest*, June 1996; 74(6):991-1003. Thus, inhibition of TGF- $\beta$ 1 activity is indicated as a therapeutic intervention in chronic renal disease.

TGF- $\beta$ 1 and its receptors are increased in injured blood vessels and are indicated in neointima formation following balloon angioplasty Saltis J.,

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- Agrotis A., Bobik A., *Clin Exp Pharmacol Physiol*, Mar. 1996; 23(3):193-200. In addition TGF- $\beta$ 1 is a potent stimulator of smooth muscle cell ("SMC") migration in vitro and migration of SMC in the arterial wall is a contributing factor in the pathogenesis of atherosclerosis and restenosis. Moreover, in
- 5 multivariate analysis of the endothelial cell products against total cholesterol, TGF- $\beta$  receptor ALK5 correlated with total cholesterol ( $P < 0.001$ ) Blann A.D., Wang J.M., Wilson P.B., Kumar S., *Atherosclerosis*, Feb. 1996; 120(1-2):221-6. Furthermore, SMC derived from human atherosclerotic lesions have an increased ALK5/TGF- $\beta$  type II receptor ratio. Because TGF- $\beta$ 1 is over-
- 10 expressed in fibroproliferative vascular lesions, receptor-variant cells would be allowed to grow in a slow, but uncontrolled fashion, while overproducing extracellular matrix components McCaffrey T.A., Consigli S., Du B., Falcone D.J., Sanborn T.A., Spokojny A.M., Bush H.L., Jr., *J Clin Invest*, Dec. 1995; 96(6):2667-75. TGF- $\beta$ 1 was immunolocalized to non-foamy macrophages in
- 15 atherosclerotic lesions where active matrix synthesis occurs, suggesting that non-foamy macrophages may participate in modulating matrix gene expression in atherosclerotic remodeling via a TGF- $\beta$ -dependent mechanism. Therefore, inhibiting the action of TGF- $\beta$ 1 on ALK5 is also indicated in atherosclerosis and restenosis.
- 20 Surprisingly, it has now been discovered that a class of non-peptide compounds, in particular 2-pyridyl substituted triarylimidazoles of formula (I), function as potent and selective non-peptide inhibitors of ALK5 kinase and therefore, have utility in the treatment and prevention of various disease states mediated by ALK5 kinase mechanisms, such as chronic renal disease, acute
- 25 renal disease, wound healing, arthritis, osteoporosis, kidney disease, congestive heart failure, ulcers, ocular disorders, corneal wounds, diabetic nephropathy, impaired neurological function, Alzheimer's disease, trophic conditions, atherosclerosis, any disease wherein fibrosis is a major component, including, but not limited to lung fibrosis and liver fibrosis, and restenosis.

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#### SUMMARY OF THE INVENTION

In one aspect, the present invention is to novel compounds of formula (I) which have been found to be modulators of the TGF- $\beta$ 1 signaling pathway, particularly as inhibitors of the ALK5 receptor.

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In another aspect, the present invention is to pharmaceutical compositions comprising a compound of formula (I), and a pharmaceutically acceptable carrier therefor.

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In still another aspect, the present invention is to a method of inhibiting the TGF- $\beta$  signaling pathway in mammals, for example, inhibiting the phosphorylation of smad2 or smad3 by the type I or activin-like kinase ALK5 receptor.

- 5 In yet another aspect, the present invention is to a method of inhibiting matrix formation in mammals by inhibiting the TGF- $\beta$  signaling pathway, for example, inhibiting the phosphorylation of smad2 or smad3 by the type I or activin-like kinase ALK5 receptor.

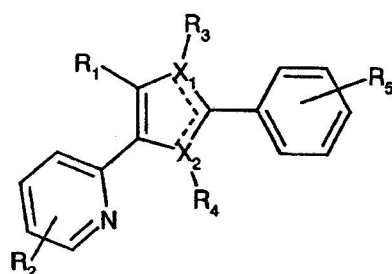
- 10 In still yet another aspect, the present invention is to a method of treating a disease mediated by the ALK5 receptor in mammals, comprising administering to a mammal in need of such treatment, an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof. ALK5-mediated disease states, include, but are not limited to, chronic renal disease, acute renal disease, wound healing, arthritis, osteoporosis, kidney  
15 disease, congestive heart failure, ulcers, ocular disorders, corneal wounds, diabetic nephropathy, impaired neurological function, Alzheimer's disease, trophic conditions, atherosclerosis, any disease wherein fibrosis is a major component, including, but not limited to lung fibrosis and liver fibrosis, and restenosis.

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#### DETAILED DESCRIPTION OF THE INVENTION

- It has now been discovered that novel pyridyl substituted triarylimidazoles of formula (I) are ALK5 receptor modulators. It has also now been discovered that selective inhibition of ALK5 receptor mechanisms  
25 by treatment with the receptor modulators of formula (I), or a pharmaceutically acceptable salt thereof, represents a novel therapeutic and preventative approach to the treatment of a variety of disease states, including, but not limited to, chronic renal disease, acute renal disease, wound healing, arthritis, osteoporosis, kidney disease, congestive heart failure, ulcers, ocular  
30 disorders, corneal wounds, diabetic nephropathy, impaired neurological function, Alzheimer's disease, trophic conditions, atherosclerosis, any disease wherein fibrosis is a major component, including, but not limited to lung fibrosis and liver fibrosis, and restenosis.

- 35 A preferred group of compounds for use herein are those compounds of the formula (I) or a pharmaceutically acceptable salt thereof:



(I)

wherein:

$R_1$  is naphthyl, anthracenyl, or phenyl, wherein phenyl is optionally substituted with substituents without formal charge selected from the group consisting of halo,  $-O-C_{1-4}alkyl$ ,  $-S-C_{1-4}alkyl$ ,  $C_{1-4}alkyl$ ,  $O-(CH_2)_n-Ph$ ,  $S-(CH_2)_n-Ph$ , cyano, phenyl, and  $CO_2R$ , wherein  $R$  is  $C_{1-4}alkyl$  and  $n$  is 0-3 or  $R_1$  is phenyl fused with an aromatic or non-aromatic cyclic ring of 5-7 members wherein said cyclic ring optionally contains up to two heteroatoms, independently selected from N, O, and S;

$R_2$  is H,  $NH(CH_2)_n-Ph$ ,  $NH-alkyl$ ;

$R_3$  is H, OH,  $C_{1-6}alkyl$ , or  $C_{3-7}cycloalkyl$ , when  $X_1$  is N or C;

$R_4$  is H, or OH, when  $X_2$  is N or C;

$R_5$  is  $CO_2H$ ,  $CONH_2$ , CN,  $NO_2$ ,  $S-C_{1-4}alkyl$ ,  $SO-C_{1-4}alkyl$ ,  $O-C_{1-4}alkyl$ ,  $SONH_2$ ,  $CONHOH$ ,  $NH_2$ , CHO,  $CH_2OH$ ,  $CH_2NH_2$ , or  $CO_2R'$ ,

wherein  $R'$  is  $C_{1-6}alkyl$ ;

$X_1$  is N or C, or  $X_1$  is S or O when  $X_2$  is N or C; and

$X_2$  is N or C, or  $X_2$  is S or O when  $X_1$  is N or C.

As used herein, the double bond indicated by the dotted lines of formula (I), represent the possible tautomeric ring forms of the compounds falling within the scope of this invention. It will be understood that when either  $X_1$  or  $X_2$  are carbon, then the double bond could be either to the carbon or the heteroatom. When  $X_1$  and  $X_2$  are both carbon, then the double bond could be to either  $X_1$  or  $X_2$ . When  $X_1$  and  $X_2$  are both heteroatoms, then the double bond is to the unsubstituted heteroatom.

Suitably  $R_1$  is naphthyl, anthracenyl, or phenyl, wherein phenyl is optionally substituted with substituents without formal charge selected from the group consisting of halo,  $-O-C_{1-4}alkyl$ ,  $-S-C_{1-4}alkyl$ ,  $C_{1-4}alkyl$ ,  $O-(CH_2)_n-Ph$ ,  $S-(CH_2)_n-Ph$ , cyano, phenyl, and  $CO_2R$ , wherein  $R$  is  $C_{1-4}alkyl$  and  $n$  is 0-3 or  $R_1$  is phenyl fused with an aromatic or non-aromatic cyclic ring of 5-7 members wherein said cyclic ring optionally contains up to two heteroatoms, independently selected from N, O, and S. Preferably  $R_1$  is phenyl optionally substituted with substituents selected from the group

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consisting of halo, -O-C<sub>1-4</sub>alkyl, -S-C<sub>1-4</sub>alkyl, phenyl, or R<sub>1</sub> is phenyl fused with an aromatic or non-aromatic cyclic ring of 5-7 members wherein said cyclic ring optionally contains up to two heteroatoms, independently selected from N, O, S.

5 Suitably R<sub>2</sub> is H, NH(CH<sub>2</sub>)<sub>n</sub>-Ph, NH-alkyl. Preferably, R<sub>2</sub> is positioned ortho to the nitrogen of the pyridyl ring. More preferably R<sub>2</sub> is H.

Suitably R<sub>3</sub> is H, OH, C<sub>1-6</sub>alkyl, or C<sub>3-7</sub>cycloalkyl, when X<sub>1</sub> is N or C. Depending on the nature of the tautomeric substitution, it will be understood that the atom attached to R<sub>3</sub> may be unsubstituted. Preferably R<sub>3</sub> is H or the atom attached to R<sub>3</sub> is unsubstituted.

Suitably R<sub>4</sub> is H, or OH, when X<sub>2</sub> is N or C. As with R<sub>3</sub>, depending on the nature of the tautomeric substitution, it will be understood that the atom attached to R<sub>4</sub> may be unsubstituted. Preferably R<sub>4</sub> is H or the atom attached to R<sub>4</sub> is unsubstituted.

15 Suitably R<sub>5</sub> is CO<sub>2</sub>H, CONH<sub>2</sub>, CN, NO<sub>2</sub>, S-C<sub>1-4</sub>alkyl, SO-C<sub>1-4</sub>alkyl, O-C<sub>1-4</sub>alkyl, SONH<sub>2</sub>, CONHOH, NH<sub>2</sub>, CHO, CH<sub>2</sub>OH, CH<sub>2</sub>NH<sub>2</sub>, or CO<sub>2</sub>R', wherein R' is C<sub>1-6</sub>alkyl. Preferably R<sub>5</sub> is CO<sub>2</sub>H, CONH<sub>2</sub>, CN, NO<sub>2</sub>, SONH<sub>2</sub>, CONHOH, NH<sub>2</sub>, CHO, CH<sub>2</sub>OH, CH<sub>2</sub>NH<sub>2</sub>.

Suitably X<sub>1</sub> is N or C or X<sub>1</sub> is S or O when X<sub>2</sub> is N or C. Preferably X<sub>1</sub> is N.

Suitably X<sub>2</sub> is N or C or X<sub>2</sub> is S or O when X<sub>1</sub> is N or C. Preferably X<sub>2</sub> is N.

Among the preferred compounds of this invention is a compound of formula (I) selected from the group consisting of:

25 4-[4(4-Fluorophenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzoic acid;  
4-[4(3,4-ethylenedioxyphenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzoic acid;

4-[4(3,4-ethylenedioxyphenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzonitrile;

30 4-[4(4-methoxyphenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzonitrile;  
4-[4(4-fluorophenyl)-5-(2-pyridyl)-1H-imidazol-2yl]nitrobenzene; and  
4-[4(4-fluorophenyl)-5-(2-pyridyl)-1H-imidazol-2yl]aniline.

The term "C<sub>1-4</sub>alkyl" is used herein at all occurrences to mean a straight or branched chain radical of 1 to 4 carbon atoms, unless the chain length is limited thereto, including, but not limited to methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, and the like.

35 The terms "halo" or "halogen" are used interchangeably herein to mean radicals derived from the elements chlorine, fluorine, iodine and bromine.

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The term "aryl" is used herein at all occurrences to mean 5-14-membered substituted or unsubstituted aromatic ring(s) or ring systems which may include bi- or tri-cyclic systems, including, but not limited to phenyl, naphthyl, and the like.

- 5 The terms "-O-C<sub>1-4</sub>alkyl" or "alkoxy" are used herein interchangeably to mean a straight or branched chain radical of 1 to 4 carbon atoms, unless the chain length is limited thereto, bonded to an oxygen atom, including, but not limited to, methoxy, ethoxy, n-propoxy, isopropoxy, and the like.

- 10 The term "ALK5 inhibitor" is used herein at all occurrences to mean a compound, other than inhibitory smads, e.g., smad6 and smad7, which selectively inhibits the ALK5 receptor preferentially over p38 or type II receptors.

- 15 The term "ALK5 mediated disease state" is used herein at all occurrences to mean any disease state which is mediated (or modulated) by ALK5, for example a disease which is modulated by the inhibition of the phosphorylation of smad 2/3 in the TGF- $\beta$  signaling pathway.

The term "ulcers" is used herein at all occurrences to include, but not to be limited to, diabetic ulcers, chronic ulcers, gastric ulcers, and duodenal ulcers.

- 20 Suitably, pharmaceutically acceptable salts of formula (I) include, but are not limited to, salts with inorganic acids such as hydrochloride, sulfate, phosphate, diphosphate, hydrobromide, and nitrate, or salts with an organic acid such as malate, maleate, fumarate, tartrate, succinate, citrate, acetate, lactate, methanesulfonate, p-toluenesulfonate, palmitate, salicylate, and  
25 stearate.

#### **Brief Description of the Figures:**

- Fig. 1 shows the amino acid alignment of the last 20 amino acids at the carboxy terminal end of smad1, 2, 3 and 5. Smad2 is phosphorylated  
30 sequentially first on Ser<sup>467</sup> and second on Ser<sup>465</sup>.

Fig. 2 depicts a model of the TGF- $\beta$  signaling pathway.

#### **Formulation of Pharmaceutical Compositions**

- 35 The pharmaceutically effective compounds of this invention (and the pharmaceutically acceptable salts thereof) are administered in conventional dosage forms prepared by combining a compound of formula (I) ("active ingredient") in an amount sufficient to treat chronic renal disease, acute renal disease, wound healing, arthritis, osteoporosis, kidney disease, congestive

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heart failure, ulcers, ocular disorders, corneal wounds, diabetic nephropathy, impaired neurological function, Alzheimer's disease, trophic conditions, atherosclerosis, any disease wherein fibrosis is a major component, including, but not limited to lung fibrosis and liver fibrosis, and restenosis, ("ALK5-mediated disease states") with standard pharmaceutical carriers or diluents according to conventional procedures well known in the art. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation.

The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl monostearate or glyceryl distearate alone or with a wax.

A wide variety of pharmaceutical forms can be employed. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid carrier will vary widely but preferably will be from about 25 mg to about 1000 mg. When a liquid carrier is used, the preparation will be in the form of a syrup, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampule or nonaqueous liquid suspension.

The active ingredient may also be administered topically to a mammal in need of treatment or prophylaxis of ALK5 mediated disease states. The amount of active ingredient required for therapeutic effect on topical administration will, of course, vary with the compound chosen, the nature and severity of the disease state being treated and the mammal undergoing treatment, and is ultimately at the discretion of the physician. A suitable dose of an active ingredient is 1.5 mg to 500 mg for topical administration, the most preferred dosage being 10 mg to 100 mg, for example 5 to 25 mg administered two or three times daily.

By topical administration is meant non-systemic administration and includes the application of the active ingredient externally to the epidermis, to the buccal cavity and instillation of such a compound into the ear, eye and nose, and where the compound does not significantly enter the blood stream. By systemic administration is meant oral, intravenous, intraperitoneal and intramuscular administration.

While it is possible for an active ingredient to be administered alone as the raw chemical, it is preferable to present it as a pharmaceutical formulation.



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The active ingredient may comprise, for topical administration, from 0.001% to 10% w/w, e.g. from 1% to 2% by weight of the formulation although it may comprise as much as 10% w/w but preferably not in excess of 5% w/w and more preferably from 0.1% to 1% w/w of the formulation.

5        The topical formulations of the present invention, both for veterinary and for human medical use, comprise an active ingredient together with one or more acceptable carrier(s) therefor and optionally any other therapeutic ingredient(s). The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

10       Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of inflammation such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose.

15       Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous or alcoholic solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by  
20       autoclaving or maintaining at 98-100°C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable  
25       solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

      Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods  
30       similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

      Creams, ointments or pastes according to the present invention are semi-  
35       solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy basis. The basis may comprise



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hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol. The formulation may  
5 incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surfactant such as esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as siliceous silicas, and other ingredients such as lanolin, may also be included.

- 10 The active ingredient may also be administered by inhalation. By "inhalation" is meant intranasal and oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques. The daily dosage amount of the active ingredient administered by inhalation is from about  
15 0.1 mg to about 100 mg per day, preferably about 1 mg to about 10 mg per day.

- In one aspect, this invention relates to a method of treating chronic renal disease, acute renal disease, wound healing, arthritis, osteoporosis, kidney disease, congestive heart failure, ulcers, ocular disorders, corneal wounds, diabetic nephropathy, impaired neurological function, Alzheimer's  
20 disease, trophic conditions, atherosclerosis, any disease wherein fibrosis is a major component, including, but not limited to lung fibrosis and liver fibrosis, and restenosis, all in mammals, preferably humans, which comprises administering to such mammal an effective amount of a ALK5 receptor modulator, in particular, a compound as depicted in formula (I).

- 25 By the term "treating" is meant either prophylactic or therapeutic therapy. Such formula (I) compound can be administered to such mammal in a conventional dosage form prepared by combining the formula (I) compound with a conventional pharmaceutically acceptable carrier or diluent according to known techniques. It will be recognized by one of skill in the art that the form  
30 and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The formula (I) compound is administered to a mammal in need of treatment for chronic renal disease, acute renal disease, wound healing, arthritis, osteoporosis, kidney disease,  
35 congestive heart failure, ulcers, ocular disorders, corneal wounds, diabetic nephropathy, impaired neurological function, Alzheimer's disease, trophic conditions, atherosclerosis, any disease wherein fibrosis is a major component, including, but not limited to lung fibrosis and liver fibrosis, and restenosis, in

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an amount sufficient to decrease symptoms associated with these disease states. The route of administration may be oral or parenteral.

The term parenteral as used herein includes intravenous, intramuscular, subcutaneous, intra-rectal, intravaginal or intraperitoneal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred. The daily parenteral dosage regimen will preferably be from about 30 mg to about 300 mg per day of active ingredient. The daily oral dosage regimen will preferably be from about 100 mg to about 2000 mg per day of active ingredient.

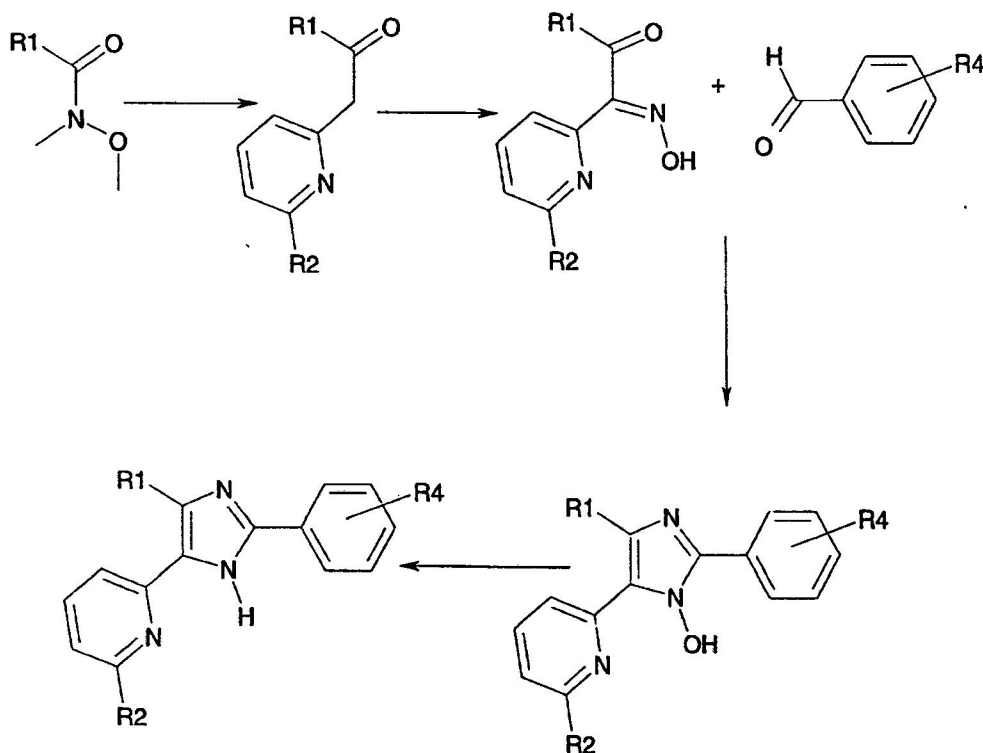
It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a formula (I) compound will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular mammal being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of the formula (I) compound given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

#### Methods of Preparation

The compounds of formula (I) can be prepared by art-recognized procedures from known or commercially available starting materials. If the starting materials are unavailable from a commercial source, their synthesis is described herein, or they can be prepared by procedures known in the art.

Specifically, compounds of formula (I) may be prepared according to Scheme 1. Using the method detailed in U.S. Patent 3,940,486, N-methoxy-N-methylaryl amide was alkylated with the anion generated from a 2(6)-methylpyridine to give a ketone. The ketone was treated with sodium nitrite to form the oxime which is condensed with an aldehyde and  $\text{NH}_4\text{OAc}$  to give a hydroxy imidazole. The hydroxy imidazole was then reduced with triphenylphosphite in a method described in (US Pat. 5,656,644) to give the corresponding imidazole.

#### Scheme 1



The invention will now be described by reference to the following examples which are merely illustrative and are not to be construed as a limitation of the scope of the present invention. In the Examples, mass spectra were performed using an Hitachi Perkin-Elmer RMU-6E with chemical ionization technique (CI) or a Micromass Platform II instrument with electrospray (ES) ionization technique.

## 10 EXAMPLES

### Example 1

#### Preparation of 2-(4-Cyanophenyl)-4-(4-fluorophenyl)-N-1-hydroxy-5-(2-pyridyl)-imidazole

The title compound was prepared using the method U.S. Patent 3,940,486 teaches to prepare 2-(t-butyl)-4-(phenyl)-N-1-hydroxy-5-(4-pyridyl)imidazole except using 2-hydroxyimino-2-(2-pyridyl)-(4-fluoro)-acetophenone and 4-cyanobenzaldehyde. ESMS (M+H)<sup>+</sup>=357.1.

### Example 2

#### Preparation of 4-[4(4-Fluorophenyl)-5-(2-pyridyl)-1H-imidazol-2-yl]benzonitrile

The title compound was prepared using the procedure U.S. Patent 5,656,644 (example 1) teaches to prepare 2-(4-cyanophenyl)-4-(4-

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fluorophenyl)-5-(4-pyridyl)-1H-imidazole except using 2-(4-cyanophenyl)-4-(4-fluorophenyl)-N-1-hydroxy-5-(2-pyridyl)-imidazole. ESMS (M+H)<sup>+</sup>=341.2.

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### Example 3

#### Preparation of 4-[4(4-Fluorophenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzoic acid

The title compound was prepared using the procedure U.S. Patent 5,656,644 (example 9) teaches to prepare 4-[4-(4-fluorophenyl)-5-(4-pyridyl)-1H-imidazol-2yl]benzoic acid except using 4-[4(4-fluorophenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzonitrile. ESMS (M+H)<sup>+</sup>=360.1.

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### Example 4

#### Preparation of Methyl 4-[4(4-Fluorophenyl)-5-(2-pyridyl)-imidazol-2yl]benzoate

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Material from Example 3 was heated at reflux in a solution of methanol/sulfuric acid for 18 h. The solution was concentrated to remove the methanol and the resulting aqueous solution was extracted with chloroform to yield the title compound. ESMS (M+H)<sup>+</sup>=374.2.

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### Example 5

#### Preparation of Ethyl 4-[4(4-Fluorophenyl)-5-(2-pyridyl)-imidazol-2yl]benzoate

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Material from Example 3 was heated at reflux in a solution of ethanol/sulfuric acid for 18 h. The solution was concentrated to removed the ethanol and the resulting aqueous solution was extracted with chloroform to yield the title compound. ESMS (M+H)<sup>+</sup>=388.2.

### Example 6

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#### Preparation of 2-(4-Cyanophenyl)-4-(3,4-methylenedioxyphenyl)-N-1-hydroxy-5-(2-pyridyl)-imidazole

The title compound was prepared using the Example 1 except using 2-hydroxyimino-2-(2-pyridyl)-(2, 3-methylenedioxy)-acetophenone and 4-cyanobenzaldehyde.

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### Example 7

#### Preparation of 4-[4(3,4-methylenedioxyphenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzonitrile

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The title compound was prepared using the procedure of Example 2 except replacing 2-(4-cyanophenyl)-4-(4-fluorophenyl)-N-1-hydroxy-5-(2-pyridyl)-imidazole with 2-(4-cyanophenyl)-4-(3,4-methylenedioxyphenyl)-N-1-hydroxy-5-(2-pyridyl)-imidazole. ESMS (M+H)<sup>+</sup>= 367.1.

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#### Example 8

#### Preparation of 4-[4(4-Fluorophenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzoic acid

The title compound was prepared using the procedure of Example 3 except replacing 4-[4-(4-fluorophenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzonitrile with 4-[4-(3,4-methylenedioxyphenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzonitrile. ESMS (M+H)<sup>+</sup>= 386.1.

#### Biological Data:

#### 15 Method for evaluating ALK5 kinase phosphorylation of smad3

Basic Flash-Plates (NEN Life Sciences) were coated by pipetting 100 micro liter of 0.1 molar sodium bicarbonate (pH 7.6), containing 150 nanograms of the fusion protein glutathion-S-transferase-smad3/100 micro liter of coating buffer. Plates were covered and incubated at room temperature for 10-24 hours. Then the plates were washed 2 times with 200 micro liter of coating buffer (0.1 molar sodium bicarbonate) and allowed to air dry for 2-4 hours.

For the phosphorylation reaction each well received 90 microliter containing 50 millimolar HEPES buffer (pH 7.4); 5 millimolar MgCl<sub>2</sub>; 1 millimolar CaCl<sub>2</sub>; 1 millimolar dithiothreitol; 100 micromolar guanosine triphosphate; 0.5 micro Ci/well gamma<sup>33</sup>P-adenosine triphosphate (NEN Life Sciences) and 400 nanograms of a fusion protein of glutathion-S-transferase at the N-terminal end of the kinase domain of ALK5 (GST-ALK5). Background counts were measured by not adding any GST-ALK5. Inhibitors of ALK5 were evaluated by determining the activity of the enzyme in the presence of various compounds. Plates were incubated for 3 hours at 30°C. After incubation the assay buffer was removed by aspiration and the wells were washed 3 times with 200 microliter cold 10 millimolar sodium pyrophosphate in phosphate buffered saline. The last wash was aspirated and blotted plate dry. Plate was then counted on a Packard TopCount.

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**Inhibition of Matrix Markers: Western Blot Protocol**

Data confirming activity in the enzyme assay was obtained as follows.

Cells were grown to near confluence in flasks, starved overnight and treated with TGF-beta and compounds. Cells were washed at 24 or 48 hours after treatment with ice cold phosphate buffered saline, then 500 microliter of 2X loading buffer was added to plate and cells were scraped and collected in microcentrifuge tube. (2X loading buffer: 100 mM Tris-Cl, pH6.8, 4% sodium dodecyl sulfate, 0.2% bromophenol blue, 20% glycerol, 5% beta-mercapto-ethanol). Cells were lysed in tube and vortexed. Sample was boiled for 10 minutes. 20 microliters of sample was loaded on 7.5% polyacrylamide gel (BioRad) and electrophoresed.

Size fractionated proteins in gel were transferred to nitrocellulose membrane by semidry blotting. Membrane was blocked overnight with 5% powdered milk in phosphate buffer saline (PBS) and 0.05% Tween-20 at 4 degrees C. After 3 washes with PBS/Tween membranes were incubated with primary antibody for 4 hours at room temperature. After three washes with PBS/Tween membrane was incubated with secondary antibody for 1 hour at room temperature. Finally, a signal was visualized with ECL detection kit from Amersham.

The compounds of this invention show ALK5 receptor modulator activity having IC<sub>50</sub> values in the range of 0.0001 to 100 μM. The full structure/activity relationship has not yet been established for the compounds of this invention. However, given the disclosure herein, one of ordinary skill in the art can utilize the present assays in order to determine which compounds of formula (I) are modulators of the ALK5 receptor and which inhibit with an IC<sub>50</sub> value in the range of 0.0001 to 100 μM.

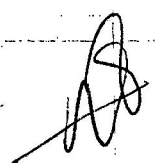
All publications, including, but not limited to, patents and patent applications cited in this specification, are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration it is believed that one skilled in the art can, given the preceding description, utilize the present invention to its fullest extent.

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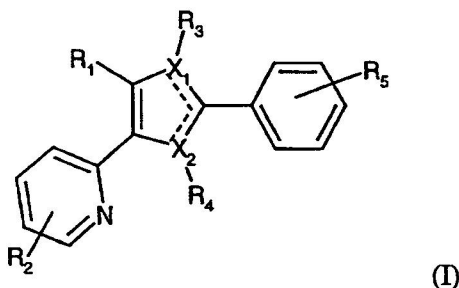
Therefore any examples are to be construed as merely illustrative and not a limitation on the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

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What is claimed is:

1. A method of inhibiting the TGF- $\beta$  signaling pathway in mammals, comprising administering to a mammal, a therapeutically effective inhibiting amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof:



wherein:

R<sub>1</sub> is naphthyl, anthracenyl, or phenyl, wherein phenyl is optionally substituted with substituents without formal charge selected from the group consisting of halo, -O-C<sub>1-4</sub>alkyl, -S-C<sub>1-4</sub>alkyl, C<sub>1-4</sub>alkyl, O-(CH<sub>2</sub>)<sub>n</sub>-Ph, S-(CH<sub>2</sub>)<sub>n</sub>-Ph, cyano, phenyl, and CO<sub>2</sub>R, wherein R is C<sub>1-4</sub>alkyl and n is 0-3 or R<sub>1</sub> is phenyl fused with an aromatic or non-aromatic cyclic ring of 5-7 members wherein said cyclic ring optionally contains up to two heteroatoms, independently selected from N, O, and S;

R<sub>2</sub> is H, NH(CH<sub>2</sub>)<sub>n</sub>-Ph, NH-alkyl;

R<sub>3</sub> is H, OH, C<sub>1-6</sub>alkyl, or C<sub>3-7</sub>cycloalkyl, when X<sub>1</sub> is N or C;

R<sub>4</sub> is H, or OH, when X<sub>2</sub> is N or C;

R<sub>5</sub> is CO<sub>2</sub>H, CONH<sub>2</sub>, CN, NO<sub>2</sub>, S-C<sub>1-4</sub>alkyl, SO-C<sub>1-4</sub>alkyl, O-C<sub>1-4</sub>alkyl, SONH<sub>2</sub>, CONHOH, NH<sub>2</sub>, CHO, CH<sub>2</sub>OH, CH<sub>2</sub>NH<sub>2</sub>, or CO<sub>2</sub>R', wherein R' is C<sub>1-6</sub>alkyl;

X<sub>1</sub> is N or C, or X<sub>1</sub> is S or O when X<sub>2</sub> is N or C; and

X<sub>2</sub> is N or C, or X<sub>2</sub> is S or O when X<sub>1</sub> is N or C.

2. The method of claim 1, wherein inhibition of the TGF- $\beta$  signaling pathway occurs through inhibition of the phosphorylation of smad2 or smad3.

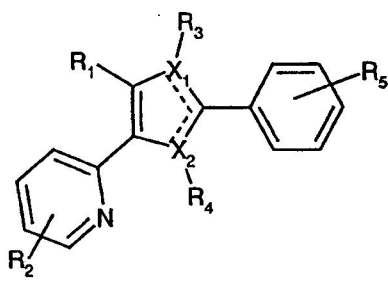
3. The method of claim 2, wherein the phosphorylation of smad2 or smad3 occurs through the ALK5 receptor.

4. A method for treating a disease mediated by the ALK5 receptor in mammals, comprising administering to a mammal in need of such treatment, a



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therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof:



(I)

wherein:

R<sub>1</sub> is naphthyl, anthracenyl, or phenyl, wherein phenyl is optionally substituted with substituents without formal charge selected from the group consisting of halo, -O-C<sub>1-4</sub>alkyl, -S-C<sub>1-4</sub>alkyl, C<sub>1-4</sub>alkyl, O-(CH<sub>2</sub>)<sub>n</sub>-Ph, S-(CH<sub>2</sub>)<sub>n</sub>-Ph, cyano, phenyl, and CO<sub>2</sub>R, wherein R is C<sub>1-4</sub>alkyl and n is 0-3 or R<sub>1</sub> is phenyl fused with an aromatic or non-aromatic cyclic ring of 5-7 members wherein said cyclic ring optionally contains up to two heteroatoms, independently selected from N, O, and S;

R<sub>2</sub> is H, NH(CH<sub>2</sub>)<sub>n</sub>-Ph, NH-alkyl;

R<sub>3</sub> is H, OH, C<sub>1-6</sub>alkyl, or C<sub>3-7</sub>cycloalkyl, when X<sub>1</sub> is N or C;

R<sub>4</sub> is H, or OH, when X<sub>2</sub> is N or C;

R<sub>5</sub> is CO<sub>2</sub>H, CONH<sub>2</sub>, CN, NO<sub>2</sub>, S-C<sub>1-4</sub>alkyl, SO-C<sub>1-4</sub>alkyl, O-C<sub>1-4</sub>alkyl, SONH<sub>2</sub>, CONHOH, NH<sub>2</sub>, CHO, CH<sub>2</sub>OH, CH<sub>2</sub>NH<sub>2</sub>, or CO<sub>2</sub>R', wherein R' is C<sub>1-6</sub>alkyl;

X<sub>1</sub> is N or C, or X<sub>1</sub> is S or O when X<sub>2</sub> is N or C; and

X<sub>2</sub> is N or C, or X<sub>2</sub> is S or O when X<sub>1</sub> is N or C.

5. The method of claim 1 wherein the compound of formula (I) is a compound selected from:

4-[4(4-Fluorophenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzoic acid;

4-[4(3,4-ethylenedioxyphenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzoic acid;

4-[4(3,4-ethylenedioxyphenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzonitrile;

4-[4(4-methoxyphenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzonitrile;

4-[4(4-fluorophenyl)-5-(2-pyridyl)-1H-imidazol-2yl]nitrobenzene; and

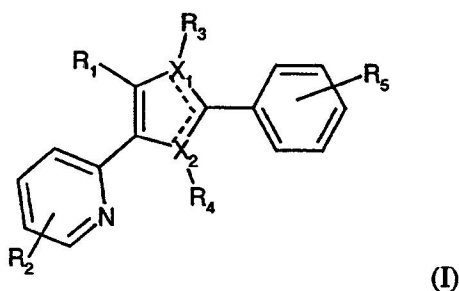
4-[4(4-fluorophenyl)-5-(2-pyridyl)-1H-imidazol-2yl]aniline.

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6. The method of claim 4, wherein the disease is selected from chronic renal disease, acute renal disease, wound healing, arthritis, osteoporosis, kidney disease, congestive heart failure, ulcers, ocular disorders, corneal wounds, diabetic nephropathy, impaired neurological function, Alzheimer's disease, trophic conditions, atherosclerosis, any disease wherein fibrosis is a major component, and restenosis.

7. A compound which is 4-[4(4-Fluorophenyl)-5-(2-pyridyl)-1H-imidazol-2yl]benzoic acid.

8. A compound of formula(I), or a pharmaceutically acceptable salt thereof:



wherein:

R<sub>1</sub> is naphthyl, anthracenyl, or phenyl, wherein phenyl is optionally substituted with substituents without formal charge selected from the group consisting of halo, -O-C<sub>1-4</sub>alkyl, -S-C<sub>1-4</sub>alkyl, C<sub>1-4</sub>alkyl, O-(CH<sub>2</sub>)<sub>n</sub>-Ph, S-(CH<sub>2</sub>)<sub>n</sub>-Ph, cyano, phenyl, and CO<sub>2</sub>R, wherein R is C<sub>1-4</sub>alkyl and n is 0-3 or R<sub>1</sub> is phenyl fused with an aromatic or non-aromatic cyclic ring of 5-7 members wherein said cyclic ring optionally contains up to two heteroatoms, independently selected from N, O, and S;

R<sub>2</sub> is H, NH(CH<sub>2</sub>)<sub>n</sub>-Ph, NH-alkyl;

R<sub>3</sub> is H, OH, C<sub>1-6</sub>alkyl, or C<sub>3-7</sub>cycloalkyl, when X<sub>1</sub> is N or C;

R<sub>4</sub> is H, or OH, when X<sub>2</sub> is N or C;

R<sub>5</sub> is CO<sub>2</sub>H, CONH<sub>2</sub>, CN, NO<sub>2</sub>, S-C<sub>1-4</sub>alkyl, SO-C<sub>1-4</sub>alkyl, O-C<sub>1-4</sub>alkyl, SONH<sub>2</sub>, CONHOH, NH<sub>2</sub>, CHO, CH<sub>2</sub>OH, CH<sub>2</sub>NH<sub>2</sub>, or CO<sub>2</sub>R', wherein R' is C<sub>1-6</sub>alkyl;

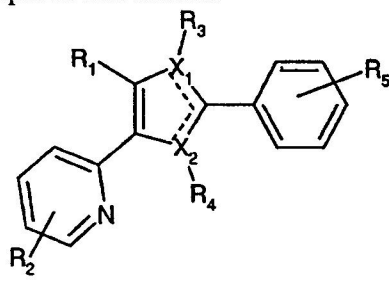
X<sub>1</sub> is N or C, or X<sub>1</sub> is S or O when X<sub>2</sub> is N or C; and

X<sub>2</sub> is N or C, or X<sub>2</sub> is S or O when X<sub>1</sub> is N or C.

9. A method of treating a condition associated with excess smad3 in mammals, comprising administering to a mammal in need of such treatment a

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therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof:



wherein:

R<sub>1</sub> is naphthyl, anthracenyl, or phenyl, wherein phenyl is optionally substituted with substituents without formal charge selected from the group consisting of halo, -O-C<sub>1-4</sub>alkyl, -S-C<sub>1-4</sub>alkyl, C<sub>1-4</sub>alkyl, O-(CH<sub>2</sub>)<sub>n</sub>-Ph, S-(CH<sub>2</sub>)<sub>n</sub>-Ph, cyano, phenyl, and CO<sub>2</sub>R, wherein R is C<sub>1-4</sub>alkyl and n is 0-3 or R<sub>1</sub> is phenyl fused with an aromatic or non-aromatic cyclic ring of 5-7 members wherein said cyclic ring optionally contains up to two heteroatoms, independently selected from N, O, and S;

R<sub>2</sub> is H, NH(CH<sub>2</sub>)<sub>n</sub>-Ph, NH-alkyl;

R<sub>3</sub> is H, OH, C<sub>1-6</sub>alkyl, or C<sub>3-7</sub>cycloalkyl, when X<sub>1</sub> is N or C;

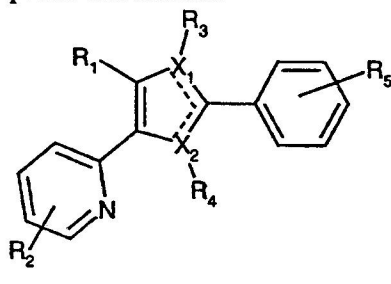
R<sub>4</sub> is H, or OH, when X<sub>2</sub> is N or C;

R<sub>5</sub> is CO<sub>2</sub>H, CONH<sub>2</sub>, CN, NO<sub>2</sub>, S-C<sub>1-4</sub>alkyl, SO-C<sub>1-4</sub>alkyl, O-C<sub>1-4</sub>alkyl, SONH<sub>2</sub>, CONHOH, NH<sub>2</sub>, CHO, CH<sub>2</sub>OH, CH<sub>2</sub>NH<sub>2</sub>, or CO<sub>2</sub>R', wherein R' is C<sub>1-6</sub>alkyl;

X<sub>1</sub> is N or C, or X<sub>1</sub> is S or O when X<sub>2</sub> is N or C; and

X<sub>2</sub> is N or C, or X<sub>2</sub> is S or O when X<sub>1</sub> is N or C.

10. A method of treating a condition associated with excess smad2 in mammals, comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof:



wherein:

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R<sub>5</sub> is CO<sub>2</sub>H, CONH<sub>2</sub>, CN, NO<sub>2</sub>, S-C<sub>1-4</sub>alkyl, SO-C<sub>1-4</sub>alkyl, O-C<sub>1-4</sub>alkyl, SONH<sub>2</sub>, CONHOH, NH<sub>2</sub>, CHO, CH<sub>2</sub>OH, CH<sub>2</sub>NH<sub>2</sub>, or CO<sub>2</sub>R', wherein R' is C<sub>1-6</sub>alkyl;

X<sub>1</sub> is N or C, or X<sub>1</sub> is S or O when X<sub>2</sub> is N or C; and

X<sub>2</sub> is N or C, or X<sub>2</sub> is S or O when X<sub>1</sub> is N or C.

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ABSTRACT OF THE DISCLOSURE

This invention relates to novel 2-pyridyl substituted triarylimidazoles of formula (I), which are inhibitors of the ALK5 receptor, and pharmaceutical compositions containing them. In addition, this invention relates to the treatment and prevention of disease states mediated by ALK5, including, but not limited to, tissue fibrosis, chronic renal disease, acute renal disease, wound healing, arthritis, osteoporosis, kidney disease, congestive heart failure, ulcers, ocular disorders, corneal wounds, diabetic nephropathy, impaired neurological function, Alzheimer's disease, trophic conditions, atherosclerosis, any disease wherein fibrosis is a major component, including, but not limited to lung fibrosis and liver fibrosis, and restenosis.

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Figure 1

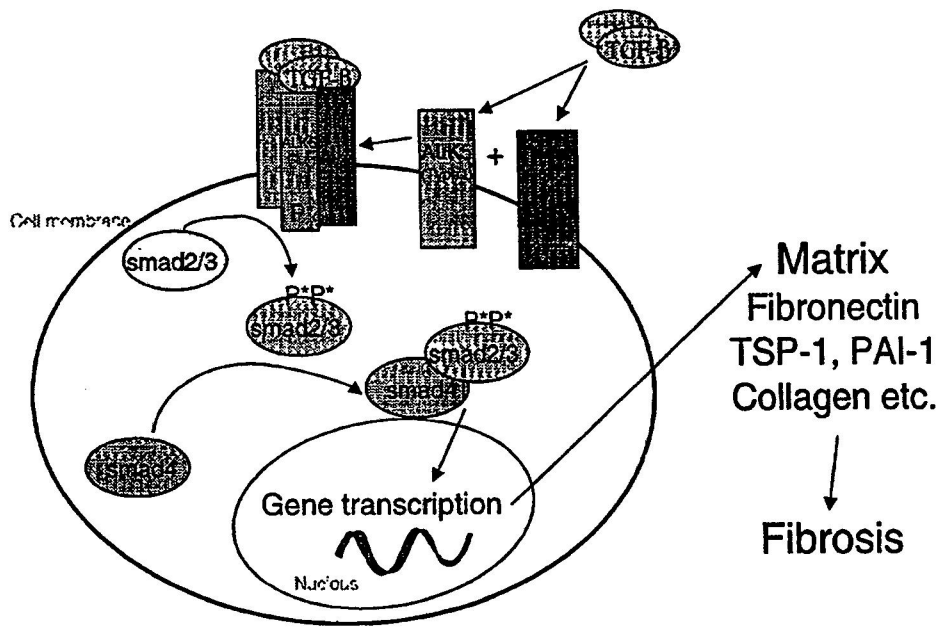
SMAD1 --WLDKV LTQMG SPHNP ISSVS  
SMAD2 --WLDKV LTQMG SPSVR CSSMS  
SMAD3 --WLDKV LTQMG SPSIR CSSVS  
SMAD5 --WLDKV LTQMG SPLNP ISSVS

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Figure 2

Model of the TGF- $\beta$  signaling pathway



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Industria y Comercio

SUPERINTENDENCIA

Expediente 00-26001

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Auto 4022

LA JEFE DE DIVISION DE NUEVAS CREACIONES

TENIENDO EN CUENTA:

Que a la fecha ya obra la copia certificada por la Administración, de la cual se reivindica prioridad en la presente solicitud, con lo cual, la solicitud de privilegio de patente que se tramita bajo el expediente indicado en la referencia, reúne los requisitos de forma establecidos en los artículos 13 y 14 de la Decisión 344 de la Comisión del Acuerdo de Cartagena y los artículos 3 y 4 del Decreto 117 de 1994.

DISPONE:

**ARTICULO PRIMERO:** Reconocer personería al doctor GERMAN CASTILLO GRAU, como apoderado de la sociedad solicitante, en los términos y para los efectos del poder conferido.

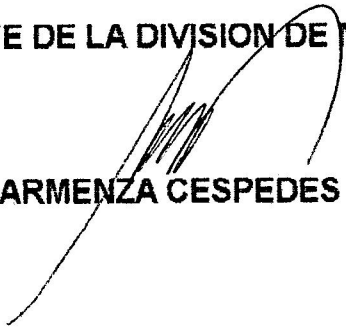
**ARTICULO SEGUNDO:** Enviar el extracto de la solicitud a la Oficina de Comunicaciones para efecto de su publicación en la Gaceta de Propiedad Industrial, conforme lo establece el artículo 23 de la Decisión 344 de la Comisión del Acuerdo de Cartagena.

CUMPLASE

90 MAR 2005

Dado en Bogotá D.C., a los \_\_\_\_\_

LA JEFE DE LA DIVISION DE NUEVAS CREACIONES,

  
ALIX CARMENZA CESPEDES DE VERGEL

Bogotá D.C., 09-03-2005 el extracto de esta solicitud fue publicado en el N° 503 (de fecha 27-04-01) de la Gaceta de la Propiedad Industrial. El término hábil señalado por la ley expiró el 30-07-01 y SI ☐ NO ☒ se presentaron observaciones por parte de terceros.