

Señora

Alix Carmenza Céspedes de Vergel

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

E. _____ S. _____ D. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 03-088515- -00006-0000

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Referencia: Expediente No. 03.088.515

Solicitud de Patente de Invención titulada: "Suspensión de partículas
micronizadas de un compuesto antifugal"

Solicitante: Schering Corporation

RESPUESTA AL AUTO NOTIFICADO POR FIJACIÓN EN LISTA NO. 174,**DEL 25 DE MAYO DE 2007, OFICIO NO. 6402 (EXAMEN DE FONDO)**

ÁLVARO CORREA ORDÓÑEZ, mayor de edad y vecino de Bogotá D.C., identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad en referencia, doy respuesta al auto emanado por ese Despacho dentro del término de prórroga solicitado junto con mi memorial 03088515-00005-0000 de fecha 24 de agosto de 2007:

1. CLARIDAD DE LA SOLICITUD DE PATENTE

El Examinador establece que la solicitud no es clara de acuerdo con lo establecido en el artículo 30 de la Decisión 486, y presenta las siguientes objeciones a las reivindicaciones:

- 1.1. El término “portador líquido farmacéuticamente aceptable” de las reivindicaciones 1, 7, 16 y 18 es muy amplio y puede incluir portadores que no se encuentran revelados en la descripción.
- 1.2. El término “aproximadamente” es muy vago y no delimita de manera correcta la invención.
- 1.3. El término “cantidad antifungal eficaz” es relativa y depende de la condición particular del paciente, por lo tanto es inexacta y no delimita la materia que se pretende proteger.
- 1.4. El término “cantidad eficaz” no delimita de manera correcta la cantidad de espesante, buffer o surfactante que se encuentran en la descripción.
- 1.5. La dependencia entre las reivindicaciones 9 y 7 no es posible porque el ester de sorbitán no se encuentra en el texto de la reivindicación 7.
- 1.6. Finalmente, el Examinador sugiere que se revise la fórmula del compuesto posaconazol.

1.2 Respuesta a las observaciones dirigidas a la solicitud de patente

Para atender a las observaciones del Examinador dirigidas a la solicitud, presento un nuevo capítulo reivindicatorio en donde se realizaron los siguientes cambios:

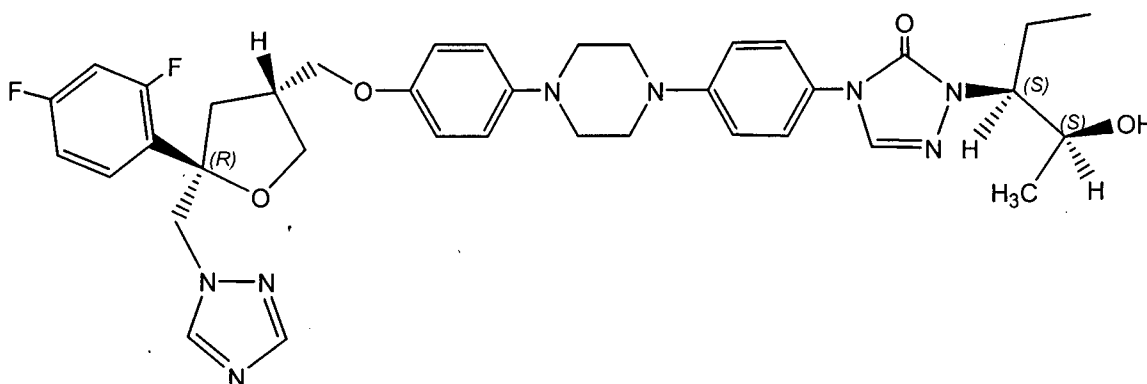
- 1.2.1. Se adicionaron nuevas reivindicaciones dependientes a las reivindicaciones 1 y 16 correspondientes a los portadores: agua purificada, glucosa y glicerina. Ver nuevas reivindicaciones 25 y 26.
- 1.2.2. En cuanto a la reivindicación 18, el solicitante decidió eliminar esta reivindicación.
- 1.2.3. En cuanto a la objeción de la reivindicación 7, no se hicieron modificaciones por cuanto la reivindicación dependiente 14 limita dichos portadores de la reivindicación 7.
- 1.2.4. Se eliminaron los términos “aproximadamente” y “al menos uno” de las reivindicaciones.

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1.2.5. Se añadieron reivindicaciones dependientes a las reivindicaciones independientes para limitar el rango de cantidad antifungal eficaz a 10 a 100 mg/ml de compuesto activo descrito en la especificación. Ver nuevas reivindicaciones 27, 28 y 29.

1.2.6. Se modificó la reivindicación 9 para que sea correcta la dependencia de la reivindicación 8.

1.2.7. En respuesta a la observación sobre la fórmula de posaconazol, el solicitante informa que el compuesto de formula I debe tener un centro asimétrico soportando los grupos hidroxilo y metilo, los cuales son R en lugar de S como se muestra en la estructura. De la misma forma, la unión del grupo 2,4-difluorofenil al furanilo también debe ser dibujada en forma inversa. Por esta razón, se modificó la descripción y las reivindicaciones para corregir estos errores obvios, reemplazando la estructura como actual con la estructura correcta del compuesto de formula I (posaconazol) como se describe abajo:



Soporte para estas correcciones se encuentra en la descripción, por ejemplo, en la página 6, líneas 7-10, la cual muestra que el compuesto de formula I, posaconazole, de la presente suspensión fue preparado como se revela en el Ejemplo 24 de la patente US 5,661,151. Por lo tanto no se añade ningún material nuevo a la solicitud. Adjunto una copia de la US 5,661,151 para su inmediata referencia.

Adicionalmente, el solicitante desea incorporar las siguientes correcciones a las reivindicaciones:

1.2.8. Las reivindicaciones independientes han sido corregidas para incluir el rango de tamaños de las partículas micronizadas basados en lo revelado en página 6, líneas 11-18.

1.2.9. La reivindicación 16 fue modificada para incluir un rango de micronización más amplio y concordante con la especificación y otras reivindicaciones independientes, y se añadió una nueva reivindicación 17 para incluir los rangos preferidos. La antigua reivindicación 17 fue eliminada.

1.2.10. Las reivindicaciones 20 a 24 se han adicionado para reclamar algunas características de la invención que se presentan en la descripción, pero que no habían sido incluidas antes en el capítulo reivindicatorio.

Para que las anteriores modificaciones surtan efecto, adjunto un recibo de pago correspondiente a la tasa por solicitud de modificación para solicitud de patente en trámite. De igual manera, adjuntamos un capítulo reivindicatorio con las modificaciones visibles, para facilidad del examen.

2. NOVEDAD

En la búsqueda de documentos realizada por el Examinador para determinar si la solicitud cumple con los requisitos de patentabilidad, encontró que la patente US 5834472 (D1) "Composición antifungal con biodisponibilidad aumentada" afecta la novedad de las reivindicaciones 1 a 4 y 18 a 20.

En particular, el Examinador establece que: "*La patente US 5834472 revela una composición farmacéutica que comprende:*

- *Posaconazol*
- *Un surfactante iónico*
- *Un diluyente como azúcares*"

Luego el Examinador compara la composición divulgada en US 5834472 y las composiciones de la presente solicitud y concluye que las composiciones de la presente invención, contenidas en las reivindicaciones 1 a 4 y 18 a 20, no son novedosas.

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3. NIVEL INVENTIVO

En cuanto al nivel inventivo el Examinador establece que los documentos US 5834472 (D1) "Composición antifungal con biodisponibilidad aumentada" y el EP 0499299 (D2) "Nanopartículas de droga modificada de superficie modificada" afectan el nivel inventivo de la presente solicitud.

3.1. El Examinador considera que:

"El documento US 5834472 revela una composición farmacéutica de posaconazol que comprende un surfactante iónico y un diluyente como azúcares. Las partículas pueden ser micronizadas a un nivel menor de 100 micrones, la composición puede ser formulada suspendida en agua.

La publicación EP 0499299 enseña que la biodisponibilidad de un principio activo es fuertemente influenciada por su tasa de disolución y que la tasa de disolución puede ser aumentada por una disminución del tamaño de partícula.

Teniendo en cuenta que el objeto de la solicitud, el cual es proveer suspensiones de posaconazol de bajo tamaño de partícula que tienen una biodisponibilidad aumentada, es una derivación evidente del estado de la técnica ya que una persona versada en la materia tomaría las composiciones de posaconazol reveladas en US 5834472 y disminuiría el tamaño de partícula del principio activo para aumentar su biodisponibilidad, tal y como se enseña en EP 0499299 y llegar a las composiciones que se desean proteger."

4. ARGUMENTOS A FAVOR DE LA PATENTABILIDAD DE LA PRESENTE INVENCION

4.1. Novedad

La novedad de una solicitud está legalmente definida en el Artículo 16 de la Decisión 486 así:
"una invención se considerará nueva, cuando no está comprendida en el estado de la técnica. El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de patente o, en su caso de la prioridad reconocida..."

En virtud de la anterior definición, consideramos que el documento US 5,834,472 (D1) no constituye arte previo que afecte la novedad de la presente solicitud por cuanto en D1 se describe en general formaciones no acuosas, secas, sólidas de posaconazol, como cápsulas, tabletas y polvos; solamente se revela un ejemplo de una formulación líquida. Esta formulación líquida contiene posaconazol, un surfactante no iónico (Pluronic F68) y agua exclusivamente. Sin embargo, D1 no provee ninguna enseñanza con respecto al tamaño medio de la partícula de posaconazole en la formulación líquida revelada. Por el contrario, D1 revela posaconazol micronizado de varios tamaños de partículas, pero no describe el rango de tamaño de partícula medio que se reclama en la presente invención (tamaño de partícula media de 1000 nm a 1800 nm). Específicamente, D1, en la columna 3, líneas 60 a 67, dice lo siguiente:

“Cerca del 99% de las partículas micronizadas antifungales son menores o iguales a 100 micrones de longitud, de las cuales 95% son menores o iguales a 90 micrones. Preferiblemente, cerca del 99% de las partículas son menores o iguales a 50 micrones, de las cuales 95% son menores o iguales a 40 micrones. Mas preferiblemente, 99% de las partículas micronizadas son menores o iguales a 20 micrones, de las cuales 95% son menores o iguales a 10 micrones.”

Sin embargo, la solicitante presenta que en el rango de micro-tamaño de partículas reclamado, la mejora en la biodisponibilidad no es predecible (por ejemplo, basado en la ley de Fick). Para demostrar lo impredecible de la mayor biodisponibilidad, la solicitante pone de manifiesto dos artículos ejemplares de la literatura científica que se adjuntan al presente escrito como Anexo A y Anexo B. La solicitante muestra que la biodisponibilidad medida (Área bajo la curva -AUC- por su sigla en inglés area under the curve) de partículas de micro-tamaño usando los dos compuestos descritos en estos artículos no reflejan la biodisponibilidad (es decir AUC) pronosticada basado en la ley de Fick. Por el contrario, la biodisponibilidad medida es menor que la pronosticada basada en la ley de Fick.

El Anexo A corresponde a: Antal *et al.*, “Biodisponibilidad comparativa de dos suspensiones de acetato de medroxiprogesterona” *International Journal of Pharmaceutics*, 54:33-39 (1989). En resumen, se compararon las características de medicamento a nivel de partícula de dos suspensiones estériles de MPA comercializadas como Depo-Provera (DP) y Farlutal Depot (FD). Notablemente, las partículas MPA en DP tuvieron tamaños entre 0.5 y 4 micras mientras que aquellas en FD tuvieron diámetros entre 1.0 y 47 micras (ver Antal *et al.* página 37, 1er párrafo, 2a frase). El AUC medido usando estas partículas se resume en la Tabla 1 en página 36 (última

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entrada para DP y FD). Las AUC medidas, sin embargo, entran en conflicto con la AUC pronosticada usando la ley de Fick como se muestra en detalle abajo.

Producto	Tamaño de Partícula Medio (micras)	AUC	AUC Normalizado	AUC Normalizado Pronosticado usando la ley de Fick
DP	1.4 ^(a)	5733	1	1
FD	7 ^(b)	4675	0.82	0.28

- (a) Rango de Tamaño de Partícula 0.5-4.0 micras; registro medio del tamaño de partícula $\approx$ 1.4 micras
- (b) Rango de Tamaño de Partícula 1.0-47 micras; registro medio del tamaño de partícula $\approx$ 7 micras

En el anexo B se muestra Timming *et al.*, “Desarrollo de una tableta con combinación fija de gliburido-metformin con tamaño de partícula gliburido optimizado” *Drug Development Research*, 66:25-35 (2006). Timmins *et al.* describen cuatro estudios farmacocinéticos separados usando tabletas con formulas de combinación fija de hidroclorito metformin y gliburido que tienen diferentes propiedades de tamaño de partícula. Notablemente, la distribución de tamaño de partícula de gliburido usado en las tabletas con formulas de combinación fija se describe en detalle en Tabla 2 página 29. La AUC medida usando estas partículas se resume en Tabla 4 página 30 (Formulaciones 1 y 2); en Tabla 6 página 31 (Formulaciones 3 y 4); y en Tablas 7 y 8 página 32 (Formulaciones 6, 7, y 8). La AUC medida sin embargo, no corresponde con la AUC pronosticada usando ley de Fick como se describe abajo.

Formulación	Tamaño de Partícula Medio (micras)	AUC	AUC Normalizado	AUC Normalizado Pronosticado usando Ley de Fick
1	33	494	0.72	0.24
2	58	339	0.49	0.14
3	25	531	0.77	0.32
4	11	716	1.04	0.72
6	9	971 ^(a)	1.41	0.89
6	9	757 ^(b)	1.10	0.89
7	14	633	0.92	0.57
8	8	689	1	1

- (a) Studio # 3
- (b) Studio # 4

En resumen, aunque el estudio de biodisponibilidad en humanos muestra que la AUC depende del tamaño de la partícula:

- MPA BA relativo 0.82-1.00 sobre el mismo rango de tamaño de partícula (7-1.4 micras)
- Gliburido BA relativo 0.49-1.41 sobre el mismo rango de tamaño de partícula (58-8 micras)

La ley de Fick pronostica una dependencia más pendiente de AUC con el tamaño de partícula.

- MPA BA relativo 0.28-1.00 sobre el mismo rango de tamaño de partícula (7-1.4 micras)
- Gliburido BA relativo 0.14-1.00 sobre el mismo rango de tamaño de partícula (58-8 micras)

En otras palabras, la biodisponibilidad medida en humanos no es afectada tan fuertemente por el tamaño de partícula como se pronosticaría basado en la ley de Fick. Como estos dos medicamentos lo ilustran, la biodisponibilidad medida es menor que la pronosticada basado en la ley de Fick. Ambos medicamentos ilustran que la biodisponibilidad pronosticada basada en la ley de Fick no es precisa.

Una persona con conocimiento en el arte por lo tanto, no esperaría que la ley de Fick fuera precisa para el posaconazol tampoco.

De la misma manera, aunque D1 revela que partículas micronizadas donde “*mas preferiblemente, 99% son menores o iguales a 20 micras, de las cuales 95% son menores o iguales a 10 micras,*” basado en la ley de Fick, una persona con conocimiento en la técnica esperaría que posaconazol fuera menos biodisponible sobre el rango de 1.7-2.3 micras de lo que fue medido. El punto al cual la biodisponibilidad es aumentada en humanos con posaconazol teniendo un tamaño de partícula media de 1.7 micras cuando se compara con 2.3 micras es bastante inesperado. De hecho, la biodisponibilidad de tandas de producto del medicamento de posaconazol con tamaño de partícula media de 1.7 micras y 2.3 micras se encontró sorprendentemente no ser bioequivalente (es decir dentro del rango de 80-125% de niveles de suero en sangre) como se evidencia en las solicitudes NDA (por su sigla en inglés para New Drug application) mostradas aquí como Anexo C.

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Las composiciones de la presente invención también proveen sorprendentemente mejor biodisponibilidad aumentada que la formulación sólida y la formulación líquida revelada en D1. Como se demostró en la presente especificación, comparaciones de biodisponibilidad entre un ejemplar de formulación líquida de la presente invención (es decir una suspensión oral) y un ejemplar de formulación sólida de D1 (es decir una tableta) demuestra que:

*“En promedio, la formulación en suspensión de la presente invención **resultó en un incremento de 23% en Cmax y un incremento de 36% en AUC(tf) comparado con la tableta de la anterior técnica.**”* (Énfasis fuera de texto)

La anterior afirmación se encuentra en la publicación PCT para esta invención WO 02/080678 A1, pagina 23, líneas 9-11. Ver también, WO 02/080678 A1, pagina 20, líneas 6-10, formulación líquida – Tratamiento B; formulación sólida – Tratamiento D; datos comparativos – pagina 22, líneas 12-15, Tabla 3 y pagina 22, línea 16 a pagina 23, línea 8, Tabla 4.

Ciertamente, estos resultados son particularmente sorprendentes a la luz de que D1 se aparta de las formulaciones líquidas, con formulaciones sólidas -Ejemplos 1 y 2 tuvieron biodisponibilidad aumentada cuando se comparan con las formulaciones líquidas allí reveladas. Es importante tener en cuenta que, el Ejemplo Comparativo de una formulación sólida (es decir una capsula) consta de posaconazol, lauril sulfato de sodio como surfactante (un surfactante aniónico), celulosa microcristalina, glicolato de almidón de sodio, y estearato de magnesio. (Ver, D1, formulaciones sólidas de D1 – Ejemplos 1 y 2 en la columna 7, líneas 32-62; formulación líquida – Ejemplo 3 en columnas 8, líneas 15-23, Ejemplo comparativo en columna 8, líneas 24-35; datos comparativos – columna 8, líneas 55-63). Basado en la comparación de biodisponibilidad, D1 concluye que

“Las cápsulas de los Ejemplos 1 y 2 exhiben biodisponibilidad realzada sobre la de los de suspensión acuosa del Ejemplo 3 y especialmente sobre las capsulas de los Ejemplos Comparativos” (Énfasis fuera de texto).

La anterior afirmación se encuentra en D1, columna 8, líneas 64-67. Esto está en completo contraste con las formulaciones de suspensión oral de la presente invención las cuales tienen sorprendentemente mayor biodisponibilidad de posaconazol comparadas con las formulaciones sólidas de D1.

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Adicionalmente, la presente invención provee numerosas ventajas. Por ejemplo, como claramente se mostró en la presente solicitud, las formulaciones líquidas de la presente invención tienen una vida de almacenamiento más larga, mejor homogeneidad y facilidad de dispersión. Los sólidos que se sedimentan no forman una pasta sólida difícil de dispersar. De hecho no ocurre virtualmente ninguna sedimentación de los sólidos en la formulación líquida no reconstituida por un período de al menos tres días. Esta característica sorprendente asegura que un paciente tratado con formulaciones líquidas recibirá una cantidad efectiva de posaconazol antifungal. Estas características son benéficas para las farmacias, farmacólogos, doctores, y pacientes. Ver, WO 02/080678 A1, página 2, línea 12 a página 3, línea 5.

Las ventajas de las formulaciones líquidas reivindicadas aquí ha llevado a que tengan alta demanda comercial. De hecho, pruebas clínicas conducidas usando formulaciones al incluidas en las presentes reivindicaciones han llevado a la aprobación regulatoria por parte de la *US Food and Drug Administration* así como la *European Commission* para la suspensión oral de posaconazol en el tratamiento de infecciones fungales invasivas en adultos. La eficacia de la formulación líquida actualmente reivindicada ha sido también demostrada en muchas publicaciones. Ver, por ejemplo, Pitisuttithum *et al.*, "Actividad de posaconazol en el tratamiento de infecciones fungales del sistema nervioso central," *J Antimicrob Chemother*, **56(4)**:745-755 (octubre 2005); Epub 2005 agosto 31 (una copia de la cual se adjunta aquí en el Anexo D).

Basado en la comparación de biodisponibilidad anteriormente mencionada y ventajas sorprendentes de la suspensión reivindicada, que ha llevado a su comercialización, proponemos que la presente solicitud sea novedosa a la luz de las enseñanzas contenidas en D1.

4.2. Nivel Inventivo

Tal y como lo establece el artículo 18 de la Decisión 486, una invención cumple con el requisito de nivel inventivo cuando "*si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica*".

Después de analizar las objeciones planteadas por el Examinador sobre la base de las enseñanzas reportadas en US 5834472 (D1) y EP 0499299 (D2), considero que la presente invención goza

del requisito de nivel inventivo según lo establece el artículo 18 por cuanto esta invención no es obvia ni evidente a la luz de las enseñanzas contenidas en el arte previo citado por el Examinador.

Siguiendo con la argumentación que presente en el punto anterior, confirmo que la presente invención cuenta con nivel inventivo a partir de lo divulgado en D1 ya que las formulaciones que ahora se reclaman tienen biodisponibilidad incrementada en comparación con las formulaciones de D1.

Con referencia a EP 0 499 299 (D2), se tiene que el mismo describe partículas de medicamento con un tamaño de partícula promedio de menos de 400 nm y métodos para su preparación. Ninguna de las preparaciones de partículas en este documento se refieren a posaconazol, tampoco a un tamaño de partícula media de cerca de 1000 nm a cerca de 1800 nm como está reclamado en las reivindicaciones actualmente solicitadas. De hecho, basados en lo revelado en este documento, un tamaño de partícula menor (menor de 400 nm) parece ser favorable sobre un tamaño de partícula mayor para alcanzar una incrementada biodisponibilidad del medicamento.

Por lo tanto, sostengo que la presente solicitud tiene nivel inventivo a partir de las divulgaciones hechas en D1 y D2; particularmente no hay sugerencia en D2 del uso de un tamaño de partícula promedio mayor a 400 nm.

5. SOLICITUDES DE PATENTE OTORGADAS EN OTROS PAÍSES PARA LA PRESENTE INVENCION

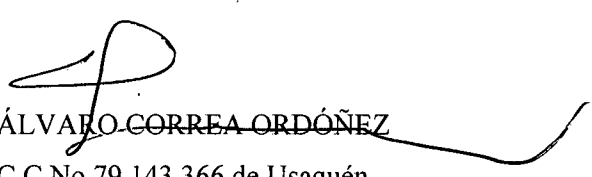
A manera informativa, pongo en conocimiento del Examinador que las patentes de Australia (2002257104) (que tiene la divisional pendiente), India, (203528), Corea del Sur (10-0607742), Nueva Zelanda (528363), Perú (4424), Singapur (99676) y Sudáfrica (2003/7684) equivalentes a la presente solicitud colombiana ha sido otorgadas. Copias de las patentes de Perú, Australia, Nueva Zelanda e India se adjuntan en el Anexo E, aún no tenemos copias disponibles de las otras patentes. Aún cuando entiendo que estas decisiones de ninguna manera obligan al Despacho para decidir de igual forma, considero que por tratarse de una pregunta fáctica la determinación de la existencia de novedad y nivel inventivo, las decisiones de otras oficinas de patentes constituyen un antecedente persuasivo en la materia.

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En virtud de lo anterior, solicito que se acepten las razones expuestas en el presente documento y en consecuencia, se otorgue el privilegio de patente de invención para el invento que se tramita en este expediente.

Atentamente,



ÁLVARO CORREA ORDÓÑEZ

C.C.No.79.143.366 de Usaquén

T.P.20.281

Anexos:

- Capítulo reivindicatorio con las modificaciones visibles
- Texto de la solicitud y reivindicaciones modificadas
- Formulario de modificación
- Recibo
- Copia de la solicitud US 5,661,151
- Anexo A. Artículo "Biodisponibilidad comparativa de dos suspensiones de acetato de medroxiprogesterona"
- Anexo B. Artículo "Desarrollo de una tableta con combinación fija de gliburido-metformin con tamaño de partícula gliburido optimizado".
- Anexo C. Solicitud NDA
- Anexo D. Artículo "Actividad de posaconazol en el tratamiento de infecciones fungales del sistema nervioso central,"
- Anexo E. Copias de las patentes de Perú, Australia, Nueva Zelanda e India.



Industria y Comercio
SUPERINTENDENCIA

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FORMULARIO UNICO DE CORRECCIONES Y MODIFICACIONES

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SOLICITUD DE

☐

Corrección de Error Material

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Modificación de una solicitud

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SOLICITANTE

Nombre: SCHERING CORPORATION

Dirección: 2000 Galloping Hill Road, Kenilworth, New Jersey 07033-0530, Estados Unidos de América

Nacionalidad o Domicilio: Estadounidense

Lugar de Constitución: New Jersey, Estados Unidos de América

Teléfono:

Fax:

E-Mail

IDENTIFICACION

C.C.

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Otro

☐

Cual

Número

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REPRESENTANTE O APODERADO

Nombre: ALVARO CORREA ORDÓÑEZ

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IDENTIFICACION

C.C.

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Cual

Número 79.143.366

TP 20.281

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Numero de expediente

03.088.515

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Descripción de la corrección o modificación solicitada

El solicitante presenta nuevo texto de la solicitud y nuevo capítulo reivindicatorio para dar respuesta a las objeciones del examinador de fijación en lista del 25 de mayo de 2007.

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Comprobante de Pago No.

07-57809

Fecha

16-JUL-07

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Anexos

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Comprobante de pago de la tasa.

☐

Poderes, si fuere el caso.

☐

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

⑧

NOMBRE

ALVARO CORREA ORDÓÑEZ

FIRMA

C.C. 79.143.366

TP. 20.281

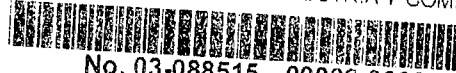
Instrucciones para el diligenciamiento del presente formulario, ver reverso de esta página.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 03-088515- -00006-0000

Fecha: 2007-10-08 15:58:22 Dep. 2020 NUECREACIÓN
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 Act. 444 RESPUESTAREQUE Folios: 186

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 07 - 57,809
 FECHA : JULIO 16 DE 2007

***** CONSIGNACION *****

DEBITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
RUIZ BECK LARA RODRIGUEZ	CONSIGNACION	BANCO POPULAR	050-00110-6	51180926	16/07/2007	42,977,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-03	CAMBIO DE MODALIDAD Y MODIFICA	
		12 MARCAS Y LEMAS COMERCIALES	99,000.00
		TOTAL :	\$ 99,000.00

SDN: NOVENTA Y NUEVE MIL PESOS

RESPONSABLE :

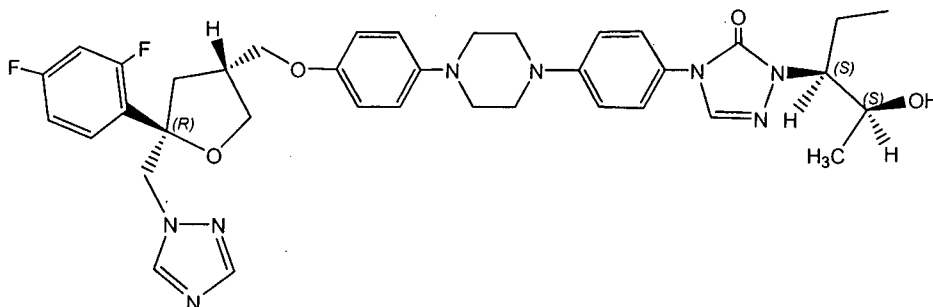
REIVINDICACIONES

00015

1. Una suspensión líquida que comprende una cantidad antifungal eficaz del
 5 compuesto micronizado representado por la fórmula estructural química I,

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- 10 en donde el compuesto micronizado de la fórmula I tiene un tamaño de partícula medio de 1.000 nm a 1.800 nm, un agente espesador, un surfactante no iónico y un portador líquido farmacéuticamente aceptable.

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2. La suspensión líquida de la reivindicación 1, en donde el un surfactante no iónico es un copolímero de bloque de óxido de etileno y óxido de propileno, glicol o
 15 gliceril ésteres de ácidos C₈ a C₂₀ saturados o insaturados, ésteres de polioxietileno de ácidos C₈ a C₂₀ saturados o insaturados, éteres de polioxietileno de ácidos C₈ a C₂₀ saturados o insaturados, polivinilalcoholes o ésteres de sorbitán de ácidos C₁₀ a C₂₀ saturados o insaturados.

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- 20 3. La suspensión líquida de la reivindicación 1, en donde el un surfactante no iónico es un derivado de polioxietileno de un éster de sorbitán de un ácido C₁₀ a C₂₀ saturado o insaturado.

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4. La suspensión líquida de la reivindicación 1, en donde el un agente espesador se selecciona entre goma de xantano, azúcares líquidos, almidones, celulosas y mezclas de los mismos.

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5. La suspensión líquida de la reivindicación 1, en donde se usa una combinación de goma de xantano y un azúcar líquido, como el un agente espesador.

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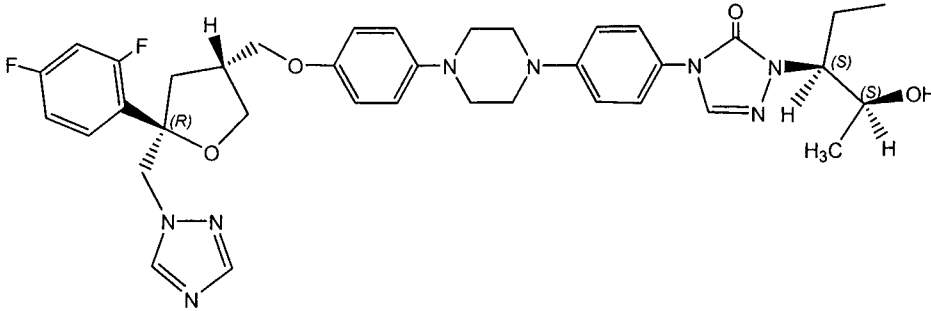
6. La suspensión líquida de la reivindicación 1, en donde el compuesto micronizado de fórmula I tiene un tamaño de partícula medio de 1200 a 1600 nm.

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7. Una suspensión líquida que comprende:

(a) una cantidad antifungal eficaz del compuesto micronizado representado por la fórmula estructural química I:



en donde el compuesto micronizado de la fórmula I tiene un tamaño de partícula medio de 1.000 nm a 1.800 nm.

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(b) una cantidad eficaz de un agente espesador;

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(c) una cantidad de un sistema buffer eficaz para mantener el pH del sistema en el rango de 4,0 a 6,0;

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(d) una cantidad eficaz de un surfactante no iónico; y

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(e) un portador líquido farmacéuticamente aceptable.

8. La suspensión líquida de la reivindicación 7, en donde el un surfactante no iónico es un derivado de polioxietileno de un éster de sorbitán de un ácido C₁₀ a C₂₀ saturado o insaturado.

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5 9. La suspensión líquida de la reivindicación 8, en donde el éster de sorbitán es un éster de ácido graso de sorbitán seleccionado entre sorbitán monolaurato, sorbitán monooleato, sorbitán sesquioleato, sorbitán trioleato, sorbitán monopalmitato, sorbitán monoestearato y sorbitán triestearato, o mezclas de los mismos.

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10. La suspensión líquida de la reivindicación 7, en donde el un agente espesador se selecciona entre gomas, azúcares líquidos, almidones, celulosas y mezclas de dos o más de los mismos.

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15 11. La suspensión líquida de la reivindicación 7, en donde se usa una combinación de una goma de xantano y un azúcar líquido, como el un agente espesador.

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20 12. La suspensión líquida de la reivindicación 7, en donde se usa una combinación de una goma de xantano y una glucosa líquida, como el agente espesador.

25 13. La suspensión líquida de la reivindicación 7, en donde el sistema buffer comprende citrato sódico y ácido cítrico.

14. La suspensión líquida de la reivindicación 7, en donde el portador líquido farmacéuticamente aceptable es una combinación de agua purificada, glucosa y glicerina.

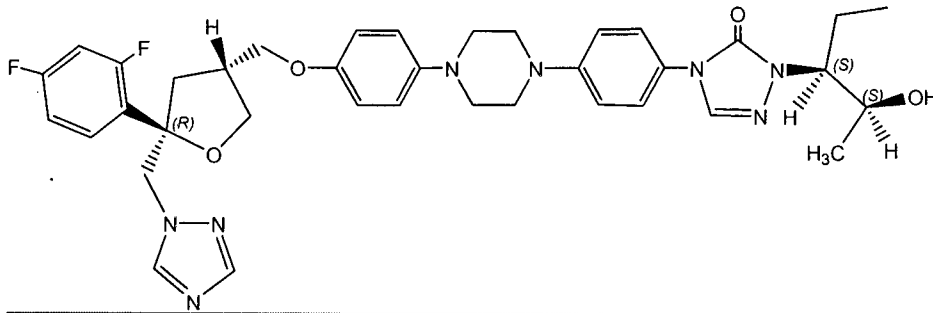
15. La suspensión líquida de la reivindicación 7, en donde el compuesto micronizado de fórmula I tiene un tamaño de partícula medio de 1200 nm a 1600 nm.

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16. Una suspensión líquida que comprende:

- 5 (a) una cantidad antifungal eficaz del compuesto micronizado que comprende la fórmula estructural química I:



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10 en donde el compuesto micronizado de la fórmula I tiene un tamaño de partícula medio de 1.000 nm a 1.800 nm.

(b) una cantidad eficaz de un derivado de polioxietileno de ésteres de sorbitán de ácidos C₁₂ a C₁₈ saturados o insaturados;

15 (c) una cantidad eficaz de un sistema buffer suficiente para mantener un pH en el rango de 4,0 a 6,0;

(d) una cantidad eficaz de una combinación de dos agentes espesadores, en donde uno es un azúcar líquido; y

(e) un portador líquido farmacéuticamente aceptable.

20 17. La suspensión de la reivindicación 16, en donde el compuesto micronizado de la fórmula I tiene un tamaño de partícula medio de 1.200 nm a 1.600 nm.

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18. Una suspensión que comprende una cantidad eficaz antifungal de las partículas micronizadas del compuesto que comprende la fórmula estructural química I: ¶

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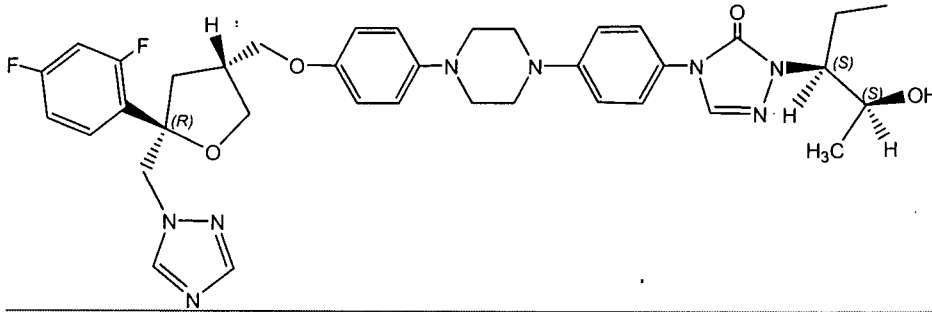
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por lo menos un agente espesador y un surfactante no iónico en un portador líquido farmacéuticamente aceptable.

18. Una composición de material que comprende partículas micronizadas con un tamaño de partícula medio de 1.000 nm a 1.800 nm del compuesto representado por la fórmula estructural I

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y un surfactante no iónico.

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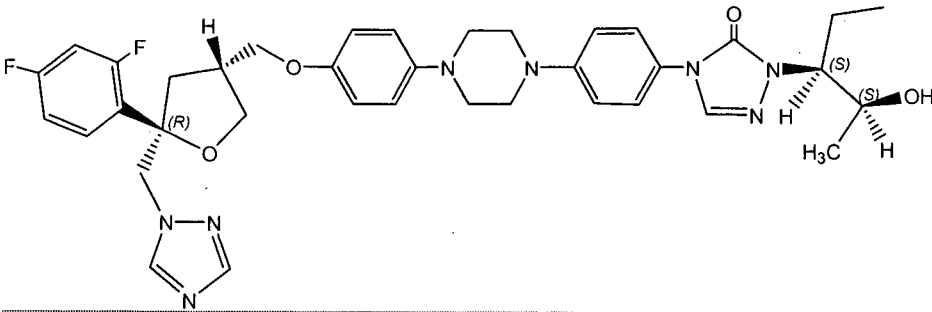
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19. Una composición de material que comprende partículas micronizadas con un tamaño de partícula medio de 1.000 nm a 1.800 nm del compuesto representado por la fórmula estructural química I:



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20. La suspensión de la reivindicación 1, en donde el agente espesante es goma de xantana.

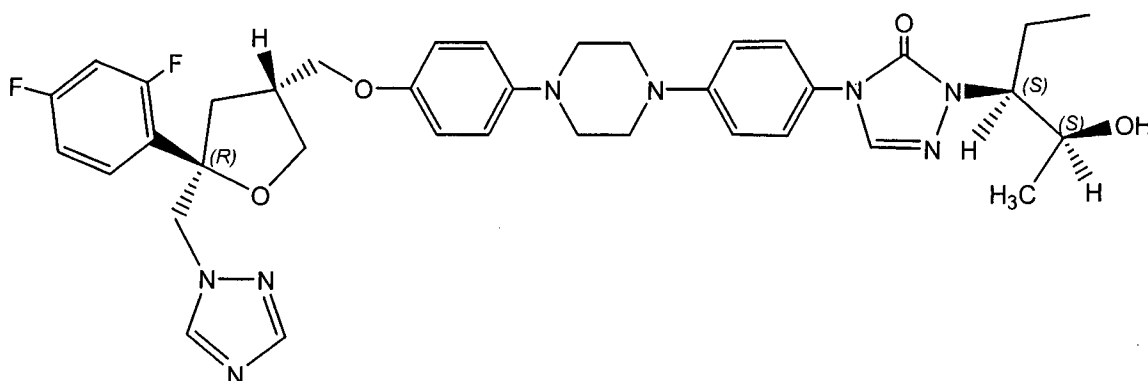
	21. <u>La suspensión líquida de la reivindicación 20, en donde el surfactante no-iónico es monooleato de polioxietileno de sorbitán.</u>	Formatted: Indent: Left: 0 cm, First line: 0 cm, Tabs: 1,25 cm, Left Formatted: Bullets and Numbering
5	22. <u>La suspensión de la reivindicación 1 en donde el agente espesante es goma de xantana y azúcar líquida, y el surfactante no-iónico es monooleato de polioxietileno de sorbitán.</u>	Formatted: Bullets and Numbering
10	23. <u>La suspensión de la reivindicación 22 que además comprende un sistema efectivo de búfer para mantener el pH del sistema en el rango de 4.0 a 6.0.</u>	Formatted: Bullets and Numbering
	24. <u>La suspensión líquida de la reivindicación 23, en donde el sistema búfer comprende citrato de sodio y ácido cítrico.</u>	Formatted: Bullets and Numbering
15	25. <u>La suspensión de la reivindicación 1, en donde el portador líquido farmacéuticamente aceptable es una combinación de agua purificada, glucosa y glicerina.</u>	Formatted: Bullets and Numbering
20	26. <u>La suspensión de la reivindicación 1, en donde el portador líquido farmacéuticamente aceptable es una combinación de agua purificada, glucosa y glicerina.</u>	Formatted: Bullets and Numbering
	27. <u>La suspensión de la reivindicación 1, en donde la cantidad del compuesto micronizado varía desde 10 mg/ml a 100 mg/ml.</u>	Deleted: <#>¶ Formatted: Bullets and Numbering
25	28. <u>La suspensión de la reivindicación 7, en donde la cantidad del compuesto micronizado varía desde 10 mg/ml a 100 mg/ml.</u>	Formatted: Bullets and Numbering
30	29. <u>La suspensión de la reivindicación 16, en donde la cantidad del compuesto micronizado varía desde 10 mg/ml a 100 mg/ml.</u>	Formatted: Bullets and Numbering Deleted: ¶ <#>¶ <#>¶ ¶ Formatted: Justified Formatted: Font: (Default) Courier New, 6 pt

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SUSPENSIÓN DE PARTÍCULAS MICRONIZADAS DE UN COMPUESTO ANTIFUGAL

ANTECEDENTES DE LA INVENCION

Esta invención se relaciona con suspensiones líquidas, estables, que
5 contienen una cantidad antifungal eficaz del compuesto micronizado representado
por la fórmula estructural química I:



por lo menos un agente espesador, un surfactante no iónico y un portador líquido
10 farmacéuticamente aceptable, y con métodos de uso de las suspensiones para tratar
o prevenir infecciones fúngicas.

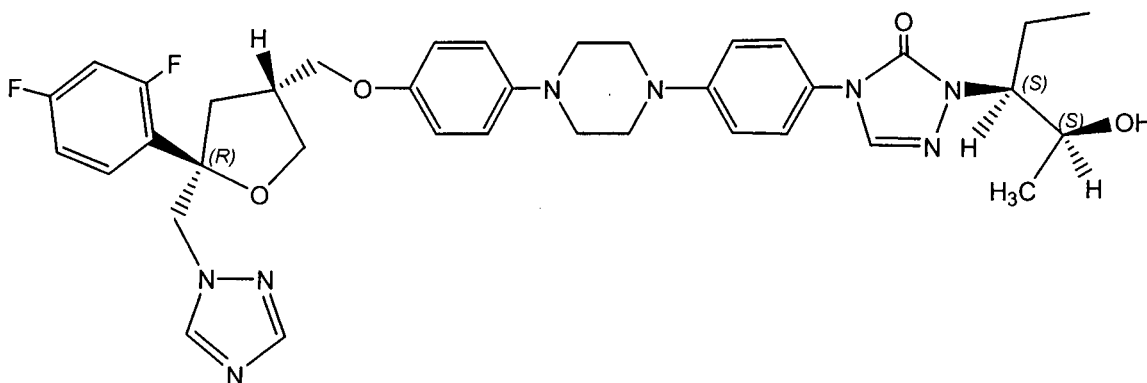
La Patente Norteamericana No. 5.661.151 describe el compuesto de fórmula I
y su potente actividad antifungal contra un amplio rango de hongos, tales como
Aspergillus, *Candida*, *Cryptococcus*, *Fusarium* y otros hongos oportunistas.

15 Las Patentes Norteamericanas No. 5.834.472 y 5.846.971 describen
composiciones de cápsulas farmacéuticas orales del compuesto de fórmula
estructural I, de revestimiento sobre cuentas inertes junto con un aglutinante. Sin
embargo, el compuesto de fórmula estructural I es altamente lipofílico, y tiene una
solubilidad en agua extremadamente baja. En consecuencia, se halló que las
20 composiciones acuosas del compuesto de fórmula estructural I tienen reducida
actividad antifungal y/o biodisponibilidad, supuestamente debido a la
extremadamente baja solubilidad en agua del compuesto. Por lo tanto, existe una
necesidad de contar con una composición farmacéutica oral del compuesto de

fórmula estructural I, que tenga enriquecida biodisponibilidad y perfeccionadas características de estabilidad.

SUMARIO DE LA INVENCION

- 5 Hemos hallado composiciones farmacéuticas en la forma de suspensión líquida, adecuadas para administración oral, que comprenden partículas micronizadas de posaconazol, el compuesto que tiene la fórmula estructural química I:



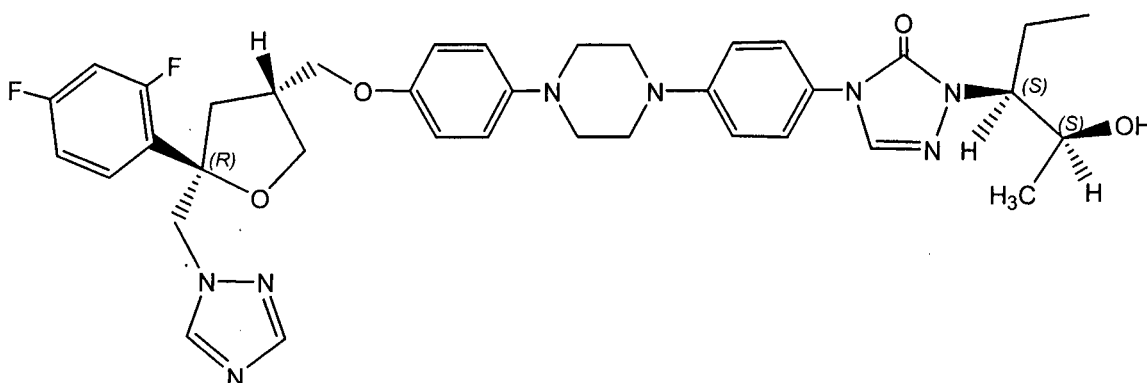
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por lo menos un agente espesador, un surfactante no iónico y un portador líquido farmacéuticamente aceptable, que proporciona significativas ventajas sobre el arte previo.

- Las ventajas de las suspensiones líquidas de la presente invención incluyen
- 15 perfeccionada homogeneidad de la suspensión y facilidad de dispersabilidad de la suspensión. Los sólidos que se establecen en la suspensión líquida de la presente invención no forman una torta sólida que es difícil de dispersar nuevamente. Prácticamente no hay sedimentación de los sólidos en la suspensión líquida no reconstituida de esta invención, durante un período de por lo menos tres días. Este
- 20 sorprendente rasgo asegura que un paciente que tiene una infección fungal que toma las suspensiones líquidas de la presente invención recibirá una cantidad antifungal eficaz de posaconazol. Las suspensiones líquidas de la presente invención poseen un período de vida útil más largo. Adicionalmente, la suspensión líquida, con la reconstitución, proporciona sustancialmente la misma cantidad

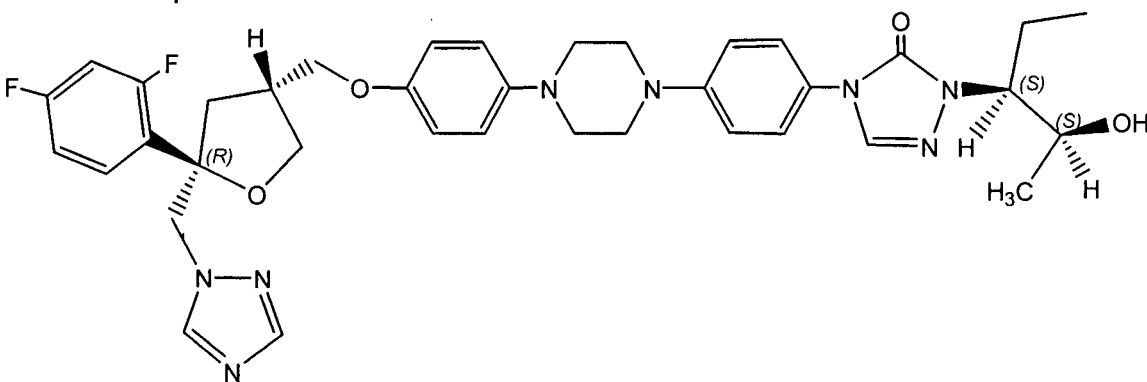
antifungal eficaz de posaconazol que la suspensión inicialmente preparada. Estas características de las suspensiones líquidas de la presente invención proporcionan beneficios para las farmacias, farmacéuticos, médicos y pacientes que tienen infecciones fungales.

- 5 Por lo tanto, la presente invención proporciona una suspensión líquida que comprende posaconazol micronizado, que tiene la fórmula estructural química I:



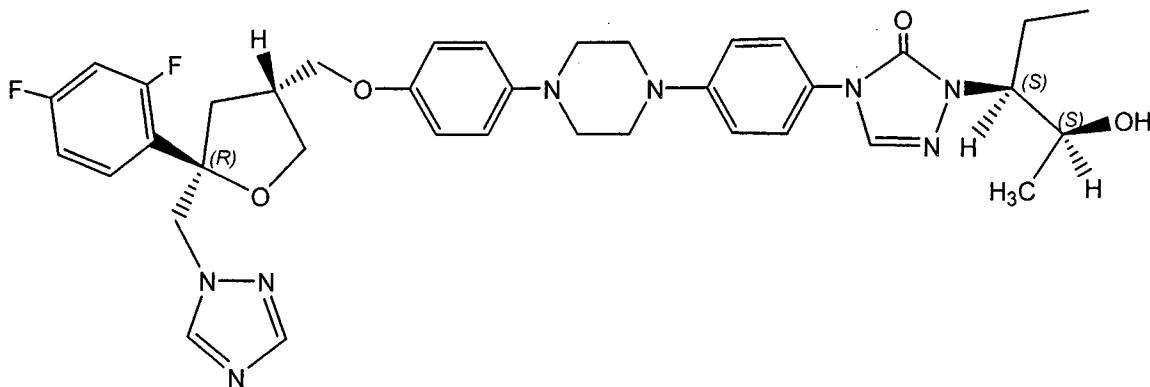
- 10 por lo menos un agente espesador, un surfactante no iónico y un portador líquido farmacéuticamente aceptable.

Esta invención además proporciona una suspensión líquida que comprende una cantidad antifungal eficaz de posaconazol micronizado que tiene la fórmula estructural química I:



- 15 una cantidad eficaz de por lo menos un agente espesador, una cantidad eficaz de un sistema buffer eficaz para mantener un pH del sistema en el rango de aproximadamente 4,0 a aproximadamente 6,0, una cantidad eficaz de un surfactante no iónico y un portador líquido farmacéuticamente aceptable.

Esta invención también proporciona una suspensión líquida que comprende una cantidad antifungal eficaz de posaconazol micronizado que tiene la fórmula estructural química I:



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en donde el compuesto micronizado tiene un tamaño de partícula medio en el rango de aproximadamente 1.200 nm a aproximadamente 1.600 nm, una cantidad eficaz de derivados de polioxietileno de ésteres de sorbitán de ácidos C₁₂ a C₁₈ saturados o insaturados, una cantidad eficaz de un sistema buffer suficiente para mantener un pH en el rango de aproximadamente 4,0 a aproximadamente 6,0, una cantidad eficaz de una combinación de dos agentes espesadores, en donde uno es un azúcar líquido, y un portador líquido farmacéuticamente aceptable.

BREVE DESCRIPCION DE LAS FIGURAS

Las Figuras 1 y 2 exhiben gráficamente los perfiles de tiempo de concentración en plasma medios de tabletas de posaconazol y de la suspensión líquida del Ejemplo 1 de la presente invención. La Figura 1 es un perfil gráfico lineal:lineal de la concentración en plasma (ng/ml) del compuesto de fórmula I contra tiempo (horas) luego de la administración de los siguientes cuatro **Tratamientos A-D**: una simple de 2 x 100 mg del compuesto de fórmula I en la formulación de coprecipitado de tableta de la Patente Norteamericana 5.834.472 con un desayuno alto en grasas estandarizado - **Tratamiento D** y símbolo -●-; una de 200 mg del compuesto de fórmula I en la suspensión oral de esta invención (5 ml) luego de 10 h de ayuno - **Tratamiento A** y símbolo -○-; una de 200 mg del compuesto de fórmula I

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en la suspensión oral de esta invención (5 ml) con un desayuno alto en grasas estandarizado - **Tratamiento B** y símbolo - Δ -; y una de 200 mg del compuesto de fórmula I en la suspensión oral de esta invención (5 ml) con un desayuno sin grasas estandarizado - **Tratamiento C** y símbolo - $\square$ -.

- 5 La Figura 2 es un perfil gráfico log:lineal de la concentración en plasma (ng/ml) del compuesto de fórmula I contra tiempo en horas para la información que se presenta en la Figura 1.

DESCRIPCION DETALLADA DE LA INVENCION

- 10 La presente invención proporciona una suspensión estable de partículas micronizadas del compuesto antifungal posaconazol en un portador líquido farmacéuticamente aceptable. La suspensión de la presente invención es estable al estancamiento, sin sedimentación, cuando se almacena sin interrupción durante más de tres días a 25°C (ver Tabla 1 a continuación).

- 15 La Tabla 2 a continuación muestra que las formulaciones de suspensiones líquidas de esta invención son estables en cuanto a que la concentración de posaconazol en la suspensión es sustancialmente la misma ($\pm 2\%$), comparada con la concentración inicial (medida por HPLC) durante períodos de hasta 12 meses.

- Además hemos hallado que la suspensión estable de la presente invención
20 tiene una biodisponibilidad notablemente superior (23-36%) comparada con una tableta oral optimizada de partículas micronizadas de posaconazol cuando cada una se administra a individuos en forma concurrente con un desayuno alto en grasas. Ver Tablas 3 y 4 y Figuras 1 y 2.

- Un aspecto de la presente invención es proporcionar una composición
25 farmacéutica que contiene partículas micronizadas de posaconazol en combinación con un surfactante no iónico, tal como un éster de sorbitán, y por lo menos un agente espesador, con preferencia una combinación de goma de xantano y un azúcar líquido, que son fácilmente dispersables en un portador líquido farmacéuticamente aceptable, tal como agua purificada. La composición farmacéutica proporciona una
30 suspensión estabilizada que no se estanca durante por lo menos tres días,

asegurando así que los pacientes obtendrán una dosis eficaz de posaconazol. Otro rasgo de la suspensión estabilizada de la presente invención es que es útil en el tratamiento de pacientes con infección de HIV-1 con aftas orales, sin que posaconazol se precipite de la solución. Otro aspecto de la presente invención es
5 que la suspensión de la presente invención evita la formación de tortas sólidas que son difíciles de dispersar.

El compuesto de fórmula I utilizado en las suspensiones de la presente invención se encuentra disponible de Schering Corporation, Kenilworth, New Jersey, y se ha preparado de acuerdo con los Ejemplos 24 y 32 de la Patente de los Estados
10 Unidos No. 5.661.151 y WO 95/17407.

Las partículas micronizadas de posaconazol con preferencia tienen un tamaño de partícula medio en el rango de aproximadamente 1.000 nanómetros (nm) a aproximadamente 1.800 nm, con preferencia aproximadamente 1.200 nm a aproximadamente 1.600 nm, y con más preferencia, aproximadamente 1.400 nm.
15 Este tamaño de partícula se puede obtener ya sea por medio de la etapa final durante la fabricación del compuesto antifungal de fórmula I, o por medio del uso de técnicas de micronización convencionales luego del o los procedimientos de cristalización convencionales.

La técnica de micronización preferida que se emplea para micronizar el
20 posaconazol al rango de tamaño de partícula medio deseado es la microfluidización. La microfluidización es una alternativa para la homogeneización tradicional que utiliza la colisión de dos corrientes de producto a altas presiones, para producir una distribución de tamaño de partícula mucho más uniforme (de acuerdo con Microfluidics International Co.) y tamaño de partícula promedio menor de
25 aproximadamente 1.200 nm a 1.600 nm. El proceso y equipo utilizado en la microfluidización se describen en la Patente Norteamericana No. 4.533.254.

El posaconazol micronizado de la presente invención también puede estar presente en forma cristalina. Con preferencia es sustancialmente química y ópticamente puro, y contiene menos de aproximadamente 10 % de sus isómeros
30 ópticos, enantiómeros u otros diastereómeros. Puede ser 99 % del isómero levógiro

o dextrógiro ópticamente puro. Este compuesto ópticamente puro de estructura química I debería evitar muchos de los efectos colaterales indeseados de una mezcla de otros isómeros ópticos.

La suspensión líquida de posaconazol se emplea en la composición en
5 cantidades antifungales eficaces para controlar los hongos de interés. Dichas cantidades eficaces antifungales pueden variar desde aproximadamente 10 mg/ml a aproximadamente 100 mg/ml de concentración de las formulaciones de suspensión líquidas de la presente invención, con más preferencia, desde aproximadamente 20 mg/ml a aproximadamente 60 mg/ml, y con mayor preferencia, aproximadamente 40
10 mg/ml del compuesto de fórmula I.

La presente invención además proporciona un método de tratamiento y/o prevención de infección fungal en un mamífero, que comprende administrar al mamífero una cantidad de la suspensión líquida que contiene el posaconazol micronizado en una cantidad eficaz para el tratamiento y/o prevención de dicha infección fungal. Las
15 cantidades antifungales eficaces de las suspensiones líquidas de la presente invención que contienen 40 mg/ml del compuesto de fórmula I se administran oralmente en las dosis de 5 ml - que contienen 200 mg de fórmula I - tres veces por día (TID) o cuatro veces por día (QID) - ó 10 ml - que contienen 400 mg del compuesto de fórmula I - una vez por día (BID). Naturalmente, el médico en atención
20 puede cambiar la dosis y el régimen de dosificación en vista de la edad, la salud y el sexo del paciente, así como también de la gravedad de la infección fungal.

Los siguientes términos se usan para describir las presentes composiciones farmacéuticas, ingredientes que pueden emplearse en su formulación y métodos para la evaluación de la bioactividad o biodisponibilidad del compuesto.

25 El surfactante no iónico se refiere a un surfactante que carece de una carga iónica neta y no se disocia a un alcance apreciable en medio acuoso. Las propiedades de los surfactantes no iónicos dependen en gran medida de las proporciones de los grupos hidrofílicos e hidrofóbicos en la molécula. Los grupos hidrofílicos incluyen el grupo oxietileno (-OCH₂CH₂-) y el grupo hidroxilo. Variando el
30 número de estos grupos en una molécula hidrofóbica, tal como un éster de un ácido

graso, se obtienen sustancias que varían desde compuestos fuertemente hidrofóbicos e insolubles en agua, tales como gliceril monoestearato, a compuestos fuertemente hidrofílicos y solubles en agua, tales como los macrogol. Entre estos dos tipos extremos se incluyen aquéllos en los que las proporciones de los grupos

5 hidrofílicos e hidrofóbicos son equilibrados en forma más pareja, tales como los éteres y ésteres de macrogol y derivados de sorbitán. Los surfactantes no iónicos adecuados pueden hallarse en Martindale, The Extra Pharmacopoeia, 28^o edición, 1982, The Pharmaceutical Press, Londres, Gran Bretaña, páginas 370 a 379.

Dichos surfactantes no iónicos adecuados incluyen copolímeros de bloque de

10 óxido de etileno y óxido de propileno, glicol o gliceril ésteres de ácidos C₈ a C₂₀ saturados o insaturados, con preferencia, ésteres de polioxietileno de ácidos C₈ a C₂₀ saturados o insaturados, éteres de polioxietileno de ácidos C₈ a C₂₀ saturados o insaturados y polivinilalcoholes o ésteres de sorbitán de ácidos C₁₀ a C₂₀ saturados o insaturados. Con preferencia, el surfactante no iónico es un éster de sorbitán de un

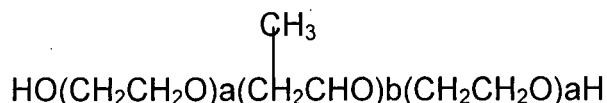
15 ácido C₁₀ a C₂₀ saturado o insaturado, y con más preferencia, el éster de sorbitán es un éster de ácido graso de sorbitán seleccionado entre sorbitán monolaurato, sorbitán monooleato, sorbitán sesquioleato, sorbitán trioleato, sorbitán monopalmitato, sorbitán monoestearato y sorbitán triestearato, o mezclas de los mismos.

20 Los ésteres de sorbitán adecuados incluyen, por ejemplo, Polisorbato 20, Polisorbato 40, Polisorbato 60, Polisorbato 65, Polisorbato 80, Polisorbato 85, Sorbitán Monolaurato, Sorbitán Mono-oleato, Sorbitán Monopalmitato, Sorbitán Monoestearato, Sorbitán Sesquioleato, Sorbitán Trioleato y Sorbitán Triestearato. El surfactante no iónico más preferido es Polysorbato 80, disponible de ICI Americas

25 bajo el nombre comercial Tween 80, que es una mezcla de ésteres de oleato de sorbitol y anhídridos de sorbitol, que consiste principalmente en el monoéster, condensado con aproximadamente 20 moles de óxido de etileno.

Los copolímeros de bloque adecuados de óxido de etileno y óxido de propileno genéricamente denominados "Poloxámeros" incluyen aquéllos

30 representados por la siguiente estructura química I:



5 en donde a es un entero que varía desde aproximadamente 10 a aproximadamente 110, con preferencia, desde aproximadamente 12 a 101; con más preferencia, desde aproximadamente 12 a 80; y

b es un entero que varía desde aproximadamente 20 a aproximadamente 60, con más preferencia, desde aproximadamente 20 a aproximadamente 56; también
10 desde aproximadamente 20 a 27.

Los ésteres de glicol y glicerilo adecuados de ácidos grasos incluyen gliceril monooleato y derivados solubles en agua similares.

Los ésteres de polioxietileno adecuados de ácidos grasos (macrogl ésteres) incluyen aceite de ricino de polioxietileno y derivados de aceite de ricino
15 hidrogenados.

Los éteres de polioxietileno adecuados de ácidos grasos (macrogl éteres) incluyen Cetomacrogel 1000, Lauromacrogles (una serie de lauril éteres de macrogles de diferentes longitudes de cadena), por ejemplo, Laureth 4, Laureth 9 y Lauromacrogol 400.

20 La cantidad eficaz de surfactante no iónico en la composición puede variar desde aproximadamente 5 mg/ml a aproximadamente 50 mg/ml de concentración de la formulación, con más preferencia, desde aproximadamente 5 mg/ml a aproximadamente 25 mg/ml, y más preferentemente 10 mg/ml.

Los agentes espesadores hallados adecuados en la presente invención
25 incluyen cualquier agente comercialmente disponible útil para dicho propósito. Goma de xantano, azúcares líquidos, almidones, celulosas y mezclas de los mismos son agentes espesadores preferidos. Es más preferida una combinación de goma de xantano y un azúcar líquido, y es más preferida una combinación de goma de xantano, NF y glucosa, NF. Con preferencia, la goma de xantano está presente en
30 una cantidad de aproximadamente 1 mg/ml a aproximadamente 5 mg/ml, y con más

preferencia, la goma de xantano está presente en una cantidad de aproximadamente 3 mg/ml. Con preferencia, la glucosa NF está presente en una cantidad de aproximadamente 200 a aproximadamente 500 mg/ml, y con más preferencia aproximadamente 350 mg/ml. La cantidad eficaz de agente espesador de la presente invención puede ser aproximadamente 1 a aproximadamente 500 mg/ml, con más preferencia aproximadamente 200 a aproximadamente 500 mg/ml, con mayor preferencia aproximadamente 353 mg/ml. Los agentes espesadores de la presente invención facilitan la suspensión de la formulación luego de la constitución con mínima agitación, y previenen el rápido estancamiento y formación de torta de la suspensión con el transcurso del tiempo.

Los portadores farmacéuticamente aceptables incluyen aquéllos bien conocidos en el arte, incluyendo agua purificada USP, glucosa líquida, NF y glicerol anhidro. Son más preferidos agua purificada USP y glucosa líquida, NF. El portador farmacéuticamente aceptable puede estar presente en una cantidad de aproximadamente 10 a aproximadamente 500 mg/ml, con más preferencia, aproximadamente 200 mg/ml a aproximadamente 400 mg/ml, más preferentemente aproximadamente 350 mg/ml.

Los sistemas buffer adecuados para las formulaciones de la presente invención son aquéllas que mantienen el pH de la suspensión líquida en el rango de aproximadamente 4 a aproximadamente 6, con preferencia en el rango de 4,5 a 5,0, y con mayor preferencia aproximadamente 4,5. Se prefiere el uso de un sistema buffer de citrato de sodio, USP y ácido cítrico, USP. Otros sistemas buffer adecuados pueden utilizarse para mantener el rango de pH deseado de 4 a 6. Los agentes buffer pueden estar presentes en una concentración de aproximadamente 0,4 a aproximadamente 1,5 mg/ml, con más preferencia, aproximadamente 0,7 a aproximadamente 1,5 mg/ml, con mayor preferencia, aproximadamente 1,1 mg/ml.

Los agentes antiespumantes hallados adecuados en la presente invención incluyen cualquier agente comercialmente disponible útil para tal propósito, incluyendo los polímeros de siloxano lineales metilados de terminación bloque con unidades de trimetilsiloxilo, tales como dimeticona y simeticona, así como también

mezclas de dimeticona con una longitud promedio de cadena de 200 a 250 unidades de dimetilsiloxano y gel de sílice. La cantidad eficaz de agentes antiespumantes es una cantidad suficiente para proporcionar una concentración de aproximadamente 2 mg/ml a aproximadamente 4 mg/ml, con preferencia, aproximadamente 3 mg/ml.

5 Los preservadores solubles en agua hallados útiles en la presente invención incluyen benzoato sódico, citrato sódico y cloruro de benzalconio, así como también otros preservadores solubles en agua farmacéuticamente aceptables. El uso de benzoato sódico como un preservador es preferido. La cantidad eficaz del preservador soluble en agua es una cantidad suficiente para proporcionar una
10 concentración de aproximadamente 0,5 mg/ml a aproximadamente 3 mg/ml, con mayor preferencia aproximadamente 2 mg/ml.

 Los agentes opacadores hallados útiles en la presente invención incluyen óxidos metálicos farmacéuticamente aceptables, especialmente dióxido de titanio. La cantidad eficaz del agente opacador es una cantidad suficiente para proporcionar
15 una concentración de aproximadamente 2 mg/ml a aproximadamente 6 mg/ml, con mayor preferencia aproximadamente 4 mg/ml.

 Los agentes saborizantes típicos son aquéllos aprobados por la FDA (Administración de Medicamentos y Alimentos) para el uso en productos farmacéuticos endulzados, alimentos, dulces, bebidas y similares; estos materiales
20 imparten sabores tales como uva, cereza, cítrico, durazno, frutilla, goma de mascar, menta y muchos otros. La cantidad eficaz de los agentes saborizantes es una cantidad suficiente para proporcionar una concentración de aproximadamente 0,01 mg/ml a aproximadamente 6 mg/ml, con mayor preferencia aproximadamente 5 mg/ml.

25 Los siguientes ejemplos describen composiciones de la presente invención que contienen posaconazol, pero no deben ser interpretados como un límite al alcance de las reivindicaciones.

Ingrediente	Rango de Concentración (mg/ml)
Posaconazol (micronizado)	10-100
Polisorbato 80	5-50

Citrato Sódico, USP, Monohidrato	0,4-0,8
Acido Cítrico, USP, Monohidrato	0,36-0,6
Simeticona, USP	2-4
Goma de Xantano, NF	1-5
Benzoato Sódico, NF	0,5-3
Glucosa Líquida, NF	200-500
Glicerina, USP	50-150
Sabor de Cereza Artificial	2-10
Dióxido de Titanio	2-6
Agua Purificada, USP q. s. ad	---

Los rangos de ingredientes proporcionados anteriormente pueden variarse, como es evidente para una persona experta en el arte.

Ejemplos específicos de una composición dentro del alcance de la invención se exponen a continuación.

5

Ejemplo 1

Ingrediente	Concentración (mg/ml)
Posaconazol (micronizado)	40
Polisorbato 80	10
Citrato Sódico, USP, Monohidrato	0,6
Acido Cítrico, USP, Monohidrato	0,48
Simeticona, USP	3
Goma de Xantano, NF	3
Benzoato Sódico, NF	2
Glucosa Líquida, NF	350
Glicerina, USP	100
Sabor de Cereza Artificial	5
Dióxido de Titanio	4
Agua Purificada, USP q. s. ad	1 ml

Este ejemplo se prepara de la siguiente manera: cargar aproximadamente 5 % del volumen de lote final de agua purificada a 20 ± 3°C a un recipiente adecuado equipado con un propulsor de mezcladora. Agregar 40 % del polisorbato 80 al agua purificada en la etapa 1, y mezclar hasta disolver. Agregar 40 % de la simeticona y mezclar hasta que se dispersa. Hacer recircular la mezcla en la etapa 3 a través de un microfluidizador, operando a aproximadamente 30.000 ± 5.000 psi (206,84 ± 34,47 MPa) durante aproximadamente 5 pasadas. Agregar adicionalmente 7 % del volumen de lote final de agua purificada a aproximadamente 20 ± 3°C, y mezclar durante aproximadamente cinco minutos. Agregar el posaconazol al recipiente en la etapa 5 con mezcla constante. Continuar la mezcla hasta que se dispersa por

completo. Hacer recircular la porción de la suspensión de la etapa 6 a través de un microfluidizador, operando a una presión de aproximadamente 30.000 ± 5.000 psi ($206,84 \pm 34,47$ MPa). Procesar el concentrado hasta que la media de las partículas muestra un tamaño de partícula de aproximadamente $1,4 \pm 0,2$ μm , cuando se mide
5 por medio de técnicas de difracción que son conocidas en el arte.

Cuando el tamaño de partícula ha sido logrado, pasar la suspensión a través del microfluidizador y recolectar en un recipiente de tamaño adecuado. Agregar aproximadamente 8-10 % del volumen de lote final de agua purificada (a $20 \pm 3^\circ\text{C}$) al recipiente de la alimentación, y pasar a través del microfluidizador operando a
10 aproximadamente 30.000 psi (206,84 MPa). Recoger el enjuague en el recipiente que contiene el concentrado. Agregar aproximadamente 22 % del volumen de lote final de agua purificada ($20 \pm 3^\circ\text{C}$) al recipiente con el concentrado, y mezclar durante aproximadamente cinco (5) minutos. Agregar el resto del polisorbato 80 y simeticona, y mezclar durante aproximadamente cinco (5) minutos. Agregar el resto
15 del polisorbato 80 y simeticona, y mezclar durante aproximadamente cinco (5) minutos.

Agregar el benzoato sódico, citrato sódico y ácido cítrico y mezclar durante aproximadamente cinco (5) minutos. Agregar la goma de xantano lentamente con mezcla constante. Continuar la mezcla luego de la adición de la goma de xantano.
20 Dejar que la goma de xantano se hidrate sin mezcla durante 30 minutos. Agregar la glicerina con mezcla constante. Agregar la glucosa líquida lentamente con mezcla constante. Mezclar durante cinco minutos o hasta que se disuelve. Agregar el dióxido de titanio y mezclar usando un homogeneizador adecuado hasta que el ingrediente está completamente disperso. Agregar el sabor de cereza artificial y mezclar durante
25 aproximadamente cinco minutos. Agregar agua purificada a $20 \pm 3^\circ\text{C}$, y qs hasta un volumen final, y mezclar hasta que se logra una suspensión uniforme. Recoger aproximadamente 20 ml de muestra para medición de pH y observación física de la suspensión. El pH de la suspensión del Ejemplo 1 fue 5,0.

El Ejemplo 2 es otro ejemplo de una formulación dentro del alcance de la presente invención, preparada utilizando el procedimiento del Ejemplo 1, y tiene un pH de 4,5.

Ingrediente	Concentración (mg/ml)
Posaconazol (micronizado)	40
Polisorbato 80	10
Citrato Sódico, USP, Monohidrato	0,6
Acido Cítrico, USP, Monohidrato	1,5
Simeticona, USP	3
Goma de Xantano, NF	3
Benzoato Sódico, NF	2
Glucosa Líquida, NF	350
Glicerina, USP	100
Sabor de Cereza Artificial	5
Dióxido de Titanio	4
Agua Purificada, USP q. s. ad	1 ml

5

El índice de sedimentación de la suspensión líquida de la presente invención se determinó como se expone a continuación.

TABLA 1

Suspensión Oral de Posaconazol 40 mg/ml

Índice de Sedimentación

Botella Número	Tiempo	Posaconazol mg/ml	% Fuerza de Rótulo	Benzoato sódico mg/ml	% Fuerza de Rótulo
1	0 min.	39,9	99,8	2,00	100
1	5 min.	40,0	100	1,99	99,5
1	10 min.	40,0	100	2,00	100
1	30 min.	40,0	100	2,00	100
1	60 min.	40,2	101	2,01	101
1	3 días	40,2	101	2,02	101
2	0 min.	39,8	99,5	2,01	101
2	5 min.	39,9	99,8	2,00	100
2	10 min.	40,2	101	2,01	101
2	30 min.	39,8	99,5	1,99	99,5
2	60 min.	40,2	101	2,02	101
2	3 días	40,1	100	2,01	101

Dos botellas que contenían la suspensión de la presente invención se sacudieron y se dejaron reposar. Las botellas luego se muestrearon de inmediato

00005 165

(tiempo cero), luego después de 5 minutos, 10 minutos, 30 minutos, 60 minutos y después de 72 horas (tres días) post sacudido. Se ensayaron los niveles de posaconazol y del preservador (Benzoato sódico) en estas muestras, por HPLC. Los métodos de HPLC de detección son bien conocidos para una persona experta en el arte.

Los resultados del ensayo del preservador y del posaconazol permanecieron constantes, y no cambiaron. Estos variaron desde 39,8 a 40,2 mg/ml (99,5 a 101 %) para el activo, y 1,99 a 2,02 mg/m: (99,5 a 101 %) para el preservador, respectivamente. Los resultados de este experimento se muestran en la Tabla 1 anterior.

No se esperaba que el benzoato sódico sedimentara. Sorprendentemente, el posaconazol no sedimentó después de 3 días.

Por lo tanto, las composiciones de la presente invención poseen tanto facilidad de dispersabilidad como homogeneidad, como queda probado por la estabilidad de las muestras en la Tabla 1.

Luego, se efectuó evaluación de homogeneidad acelerada en la suspensión líquida de la presente invención.

TABLA 2
Suspensión Oral de Posaconazol 40 mg/ml

Acelerada		Posaconazol	Benzoato Sódico
Condición	Punto de Tiempo	% Fuerza de Rótulo	% Fuerza de Rótulo
Inicial	Inicial	103; 102; 104	105; 102; 103
30 H (30°C/60%HR)	3 Meses	103; 105; 104	103; 107; 105
HR4 (40°C/75%HR)	3 Meses	102; 104; 103	104; 106; 106
30 H	6 Meses	102; 101; 102	103; 101; 102
HR4	6 Meses	102; 102; 102	101; 101; 102
25 H	12 Meses	104; 104; 104	101; 100; 100
25 H	24 Meses	104; 104; 104	101; 101; 101

* Sacudiendo y recibiendo la dosis por instrucción de paciente.

Estos datos (experimento de índice de sedimentación) concordaron con los datos de estabilidad de tiempo real (hasta 6 meses a 40°C/75%HR y hasta 24 meses a 25°C/60 %HR) que se muestran en la Tabla 2. Los resultados de homogeneidad del ensayo, sorprendentemente, permanecieron consistentemente homogéneos y
5 prácticamente sin cambiar.

Después de 6 meses a 40°C/75 % HR, los resultados de homogeneidad variaron desde 40,7 a 40,8 mg/ml (101 %) para el activo y 2,01 a 2,03 mg/ml (101 a 102 %) para el preservador, respectivamente. Estos resultados se obtuvieron sin consideración de la porción de la botella ensayada, es decir, parte superior, o inferior
10 de la botella. Por lo tanto, puede concluirse que la suspensión fue homogénea en toda la botella, aún luego de la exposición relativamente larga a condiciones de estabilidad acelerada.

Luego de 24 meses a 25°C/60 % HR, los resultados de homogeneidad variaron desde 41,5 a 41,6 mg/ml (104 %) para el activo y 2,01 mg/ml (101 %) para
15 el preservador, respectivamente. Estos resultados se obtuvieron sin consideración de la porción de la botella ensayada, es decir, parte superior, o inferior de la botella. Por lo tanto, puede concluirse que la suspensión fue homogénea en toda la botella, aún luego de la exposición de largo plazo (24 meses) a 25°C/60 % HR.

La biodisponibilidad se define como el índice y el alcance que el ingrediente
20 de droga activa o porción terapéutica es absorbida por la circulación sistémica desde una forma de dosificación administrada, comparada con un estándar o control.

El valor C_{max} se define como la concentración máxima del compuesto antifungal medida (es decir, "pico") en el suero de plasma.

Las formulaciones de la presente invención poseen la ventaja de que tienen
25 una incrementada biodisponibilidad y menor variabilidad que las formulaciones previas.

La relativa biodisponibilidad de la suspensión oral de posaconazol fue comparada con una forma de dosificación sólida de posaconazol en individuos sanos.

El primer objetivo fue determinar la relativa biodisponibilidad de posaconazol suministrado como una suspensión oral, comparada con una formulación sólida oral cuando es administrada con un desayuno alto en grasas. El segundo objetivo fue determinar el efecto de alimentos altos en grasas y sin grasas en relación a las condiciones de ayuno sobre la biodisponibilidad oral del compuesto de fórmula I, cuando se suministró como una suspensión oral.

Veinte individuos sanos completaron este estudio aleatorizado, de rótulo abierto, de cruce de 4 sentidos, de biodisponibilidad de dosis individual y efecto de alimento, de posaconazol. Los individuos recibieron cada uno de los siguientes cuatro tratamientos separados por al menos un período de aclaramiento de 7 días:

Tratamiento A:	200 mg del compuesto de fórmula I en la suspensión oral de esta invención (5 ml) luego de un ayuno de 10 horas.
Tratamiento B:	200 mg del compuesto de fórmula I en la suspensión oral de esta invención (5 ml) con un desayuno alto en grasas estandarizado.
Tratamiento C:	200 mg del compuesto de fórmula I en la suspensión oral de esta invención (5 ml) con un desayuno sin grasas estandarizado.
Tratamiento D:	2 x 100 mg del compuesto de fórmula I en las tabletas (formulación de co-precipitado de la Patente Norteamericana No. 5.834.472), con un desayuno alto en grasas estandarizado.

Los individuos se aleatorizaron para o bien permanecer en ayuno (Tratamiento A), para recibir un desayuno alto en grasas estandarizado (Tratamiento B o D), o un desayuno sin grasas estandarizado (Tratamiento C). Las comidas se consumieron en un período de 20 minutos antes de la administración de droga de la mañana, y los individuos recibieron el tratamiento apropiado dentro de 5 minutos de la finalización del desayuno.

Se recogieron muestras de sangre (6 ml) en tubos heparinizados para cada tratamiento, inmediatamente antes de la dosificación (0 hora), y a 0,5, 1, 1,5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, 48 y 72 horas luego de la dosificación. La sangre se centrifugó a 4°C, y se almacenó el plasma a -20°C o temperatura inferior hasta el ensayo. Se ensayaron las concentraciones de plasma de posaconazol utilizando un ensayo cromatográfico líquido de alto rendimiento validado, con un LOQ de ng/ml.

Se utilizaron los datos de concentración de plasma-tiempo individuales para el análisis farmacocinético, utilizando métodos independientes de modelo. La concentración máxima (Cmax) y tiempo de concentración máxima (Tmax) fueron los valores observados. El área bajo la curva de concentración de plasma-tiempo desde tiempo cero al tiempo de muestreo cuantificable final [AUC(tf)] se calculó utilizando el método trapezoide lineal, y fue extrapolado a infinitad (I), de la siguiente manera:

$$AUC(I) = AUC(tf) + \frac{C(tf)}{K}$$

donde C(tf) es la concentración estimada determinada desde regresión lineal a tiempo, tf.

El aclaramiento del cuerpo total se calculó por lo siguiente:

$$CL = Dosis / AUC(I)$$

El volumen evidente de distribución (Vdarea/F) se calculó a partir de la relación del aclaramiento de cuerpo total a la constante de índice de fase terminal (K).

Se calcularon estadísticas de resumen para la formulación de suspensión de plasma de la presente invención, comparada con una información de concentración de formulación-tiempo de tableta del arte previo, en cada punto de tiempo, y los parámetros farmacocinéticos derivados. La escala original y valores Cmax y AUC log-transformados se analizaron usando un análisis de variación (ANOVA). Los efectos debidos a individuo, fase y tratamiento fueron extraídos.

La información de concentración de plasma-tiempo y los parámetros farmacocinéticos para el compuesto de fórmula I se citan en las Tablas 3 y 4, y se representan gráficamente en las Figuras 1 y 2.

Todos los individuos tuvieron concentraciones de hora 0 el Día 1 informadas como a continuación el LOQ (5 ng/ml), excepto el Individuo 20 en las Fases 3 y 4, que tuvo niveles cuantificables de posaconazol a 0 hora para los Tratamientos B y A de 8,5 y 22,5 ng/ml, respectivamente. Estos niveles se deben con mayor probabilidad a un efecto residual de la acumulación de dosis previas.

42

Se proporciona un resumen de los parámetros farmacocinéticos medios^a, en la tabla a continuación:

Tabla 3

Parámetro	Unidad	Tableta		Suspensión					
		Alta Grasa D		10 h Ayuno A		Alta Grasa B		Sin Grasa C	
		Media	% CV	Media	% CV	Media	% CV	Media	% CV
Cmax	ng/ml	413	33	132	50	512	34	378	43
Tmax	Hr	5,5	32	5,01	49	4,8	9	4,1	21
AUC(tf)	ng-hr/ml	10304	41	3553	36	13885	41	9511	38
AUC(l)	ng-hr/ml	11832	39	4179	31	15059	26	10753	35
t ½	hr	21,0	15	23,5	25	23,0	19	22,2	18

5 a: medias balanceadas, n = 20 excepto para AUC(l) y t ½ , n = 15

Posaconazol fue lentamente absorbido; los valores Tmax medios variaron desde 4,1 a 5,5 horas. Posaconazol fue lentamente eliminado con un t ½ terminal medio de aproximadamente 22 horas, que fue independiente del tratamiento. Este estudio fue conducido para evaluar la biodisponibilidad de la suspensión oral de posaconazol (Tratamiento B), comparada con una formulación de tableta (Tratamiento D), ambas suministradas con un alimento alto en grasas. Los resultados, basados en información log-transformada, se muestran a continuación:

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Tabla 4

Parámetro	Tratamientos suministrados luego de desayuno alto en grasas	Media geométrica	Biodisponibilidad relativa (%) ^b	90 % Intervalo Confianza
Cmax	Suspensión Tableta	485 ng/ml 394 ng/ml	123,3	104-106
AUC(tf) ^a	Suspensión Tableta	13141 ng.hr/ml 9624 ng.hr/ml	136,5	119-156

a: AUC(tf) se usó para comparaciones estadísticas debido a que pudo ser calculado para todos los tratamientos para todos los individuos.

00010 120

b: Suspensión en relación a tableta.

En promedio, la formulación de suspensión de la presente invención produjo un incremento del 23 % en Cmax y un incremento del 36 % en AUC(tf), comparado con la tableta del arte previo.

El objetivo secundario del estudio fue evaluar el efecto del alimento alto en grasas (Tratamiento B) y del alimento sin grasas (Tratamiento C) comparados con el ayuno (Tratamiento A), sobre la biodisponibilidad oral de posaconazol administrado como una suspensión oral. Los resultados, en base a información log-transformada, se muestran a continuación:

Tabla 5

Parámetro	Tratamientos de suspensión	Media geométrica	Biodisponibilidad relativa (%) ^a	90 % Intervalo Confianza
Cmax (ng/ml)	Alto en grasas	485	417	352-493
	Sin grasas	345	296	250-350
	Ayuno	116	--	--
AUC(tf) (ng.hr/ml)	Alto en grasas	13141	392	343-448
	Sin grasas	8857	264	231-302
	Ayuno	3352	--	--

a: Expresado como un porcentaje de Tratamiento A - Suspensión/Ayuno.

Un desayuno alto en grasas produjo un incremento de 4 veces en la biodisponibilidad de posaconazol suministrado en una suspensión. Esto fue consistente con los resultados de un estudio previo donde el alimento incrementó significativamente la biodisponibilidad de posaconazol 3-5 veces, tanto para formulaciones de tabletas como de cápsulas. El efecto de un desayuno sin grasas (Tratamiento C) comparado con el ayuno (Tratamiento A) fue menor, con un incremento de 2,5-3 veces en la biodisponibilidad.

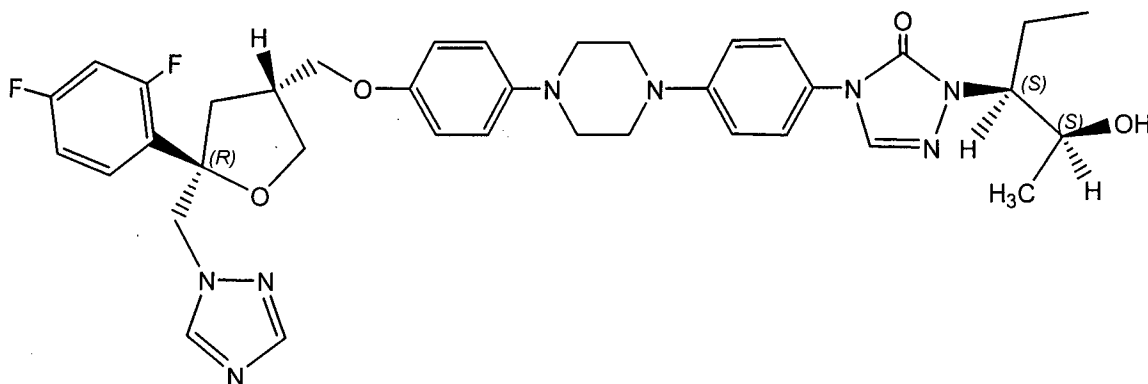
Pueden hacerse muchas modificaciones y variaciones de esta invención sin alejarse de su espíritu y alcance, como será evidente para el experto en el arte. Las

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realizaciones específicas que se describen en la presente se ofrecen a modo de ejemplo solamente, y la invención sólo debe ser limitada por los términos de las reivindicaciones adjuntas junto con el alcance completo de equivalentes a los cuales dichas reivindicaciones tienen derecho.

REIVINDICACIONES

1. Una suspensión líquida que comprende una cantidad antifungal eficaz del
5 compuesto micronizado representado por la fórmula estructural química I,



- en donde el compuesto micronizado de la fórmula I tiene un tamaño de partícula
10 medio de 1.000 nm a 1.800 nm, un agente espesador, un surfactante no iónico y un portador líquido farmacéuticamente aceptable.

2. La suspensión líquida de la reivindicación 1, en donde el un surfactante no
15 iónico es un copolímero de bloque de óxido de etileno y óxido de propileno, glicol o gliceril ésteres de ácidos C₈ a C₂₀ saturados o insaturados, ésteres de polioxietileno de ácidos C₈ a C₂₀ saturados o insaturados, éteres de polioxietileno de ácidos C₈ a C₂₀ saturados o insaturados, polivinilalcoholes o ésteres de sorbitán de ácidos C₁₀ a C₂₀ saturados o insaturados.

- 20 3. La suspensión líquida de la reivindicación 1, en donde el un surfactante no iónico es un derivado de polioxietileno de un éster de sorbitán de un ácido C₁₀ a C₂₀ saturado o insaturado.

4. La suspensión líquida de la reivindicación 1, en donde el un agente espesador se selecciona entre goma de xantano, azúcares líquidos, almidones, celulosas y mezclas de los mismos.

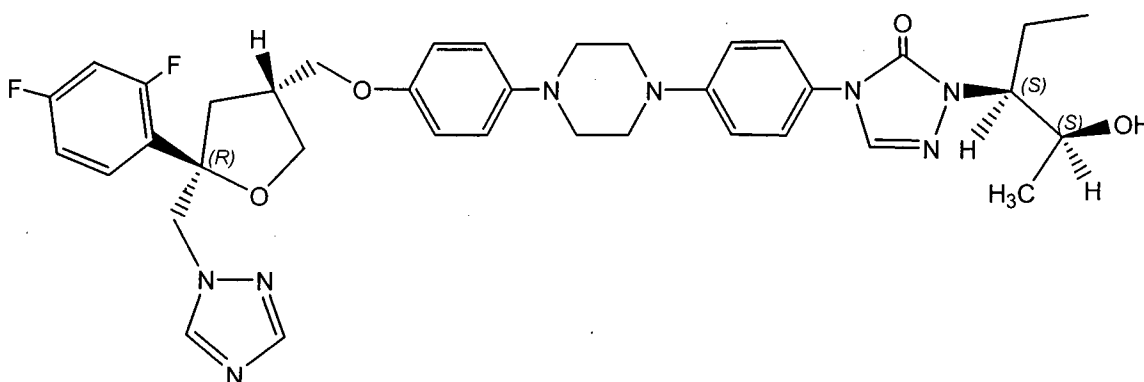
5. La suspensión líquida de la reivindicación 1, en donde se usa una combinación de goma de xantano y un azúcar líquido, como el un agente espesador.

6. La suspensión líquida de la reivindicación 1, en donde el compuesto micronizado de fórmula I tiene un tamaño de partícula medio de 1200 a 1600 nm.

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7. Una suspensión líquida que comprende:

(a) una cantidad antifungal eficaz del compuesto micronizado representado por la fórmula estructural química I:



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en donde el compuesto micronizado de la fórmula I tiene un tamaño de partícula medio de 1.000 nm a 1.800 nm,

(b) una cantidad eficaz de un agente espesador;

(c) una cantidad de un sistema buffer eficaz para mantener el pH del sistema

20 en el rango de 4,0 a 6,0;

(d) una cantidad eficaz de un surfactante no iónico; y

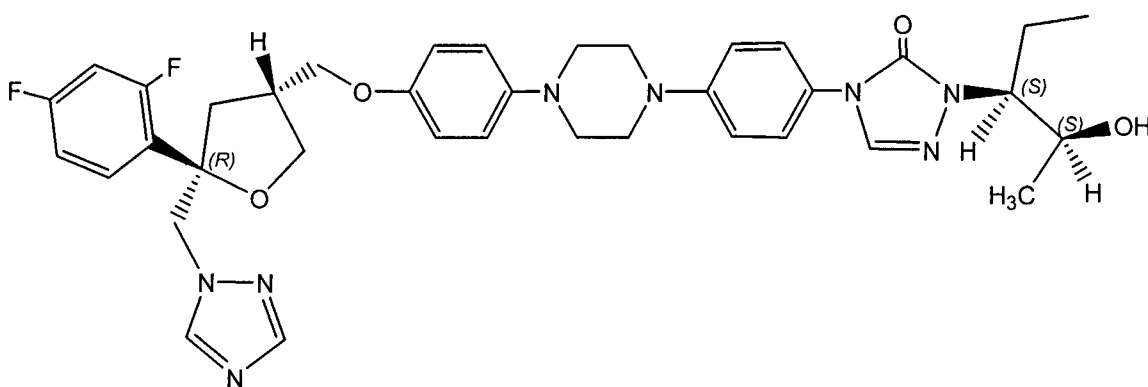
(e) un portador líquido farmacéuticamente aceptable.

8. La suspensión líquida de la reivindicación 7, en donde el un surfactante no iónico es un derivado de polioxietileno de un éster de sorbitán de un ácido C₁₀ a C₂₀ saturado o insaturado.
- 5 9. La suspensión líquida de la reivindicación 8, en donde el éster de sorbitán es un éster de ácido graso de sorbitán seleccionado entre sorbitán monolaurato, sorbitán monooleato, sorbitán sesquioleato, sorbitán trioleato, sorbitán monopalmitato, sorbitán monoestearato y sorbitán triestearato, o mezclas de los mismos.
- 10 10. La suspensión líquida de la reivindicación 7, en donde el un agente espesador se selecciona entre gomas, azúcares líquidos, almidones, celulosas y mezclas de dos o más de los mismos.
- 15 11. La suspensión líquida de la reivindicación 7, en donde se usa una combinación de una goma de xantano y un azúcar líquido, como el un agente espesador.
- 20 12. La suspensión líquida de la reivindicación 7, en donde se usa una combinación de una goma de xantano y una glucosa líquida, como el agente espesador.
- 25 13. La suspensión líquida de la reivindicación 7, en donde el sistema buffer comprende citrato sódico y ácido cítrico.
14. La suspensión líquida de la reivindicación 7, en donde el portador líquido farmacéuticamente aceptable es una combinación de agua purificada, glucosa y glicerina.

15. La suspensión líquida de la reivindicación 7, en donde el compuesto micronizado de fórmula I tiene un tamaño de partícula medio de 1200 nm a 1600 nm.

16. Una suspensión líquida que comprende:

- 5 (a) una cantidad antifungal eficaz del compuesto micronizado que comprende la fórmula estructural química I:



- 10 en donde el compuesto micronizado de la fórmula I tiene un tamaño de partícula medio de 1.000 nm a 1.800 nm,

(b) una cantidad eficaz de un derivado de polioxietileno de ésteres de sorbitán de ácidos C₁₂ a C₁₈ saturados o insaturados;

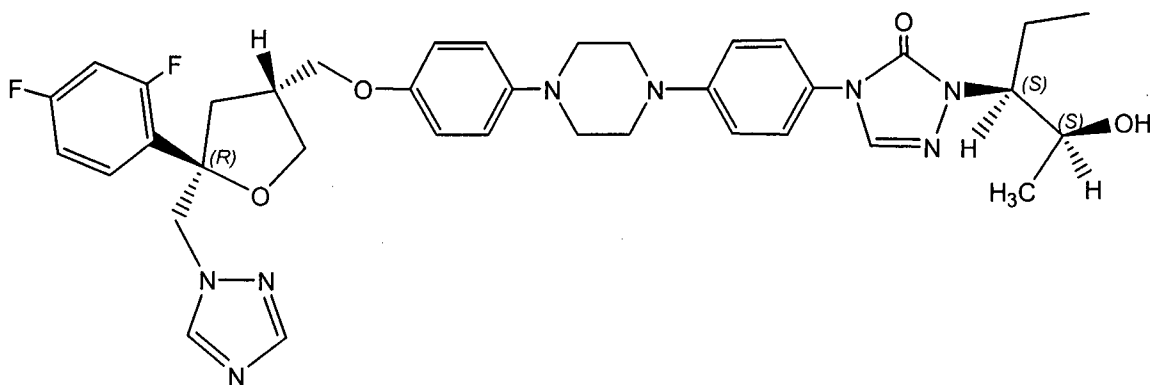
- (c) una cantidad eficaz de un sistema buffer suficiente para mantener un pH
15 en el rango de 4,0 a 6,0;

(d) una cantidad eficaz de una combinación de dos agentes espesadores, en donde uno es un azúcar líquido; y

(e) un portador líquido farmacéuticamente aceptable.

- 20 17. La suspensión de la reivindicación 16, en donde el compuesto micronizado de la fórmula I tiene un tamaño de partícula medio de 1.200 nm a 1.600 nm.

18. Una composición de material que comprende partículas micronizadas con un tamaño de partícula medio de 1.000 nm a 1.800 nm del compuesto representado por la fórmula estructural I

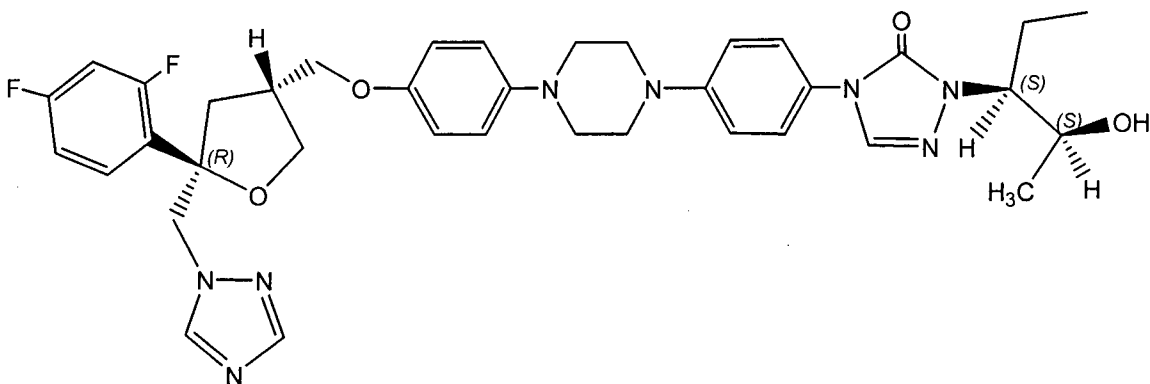


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y un surfactante no iónico.

19. Una composición de material que comprende partículas micronizadas con un tamaño de partícula medio de 1.000 nm a 1.800 nm del compuesto representado por la fórmula estructural química I:

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en suspensión.

20. La suspensión de la reivindicación 1, en donde el agente espesante es goma de xantana.

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21. La suspensión líquida de la reivindicación 20, en donde el surfactante no-iónico es monooleato de polioxietileno de sorbitán.

22. La suspensión de la reivindicación 1 en donde el agente espesante es goma de xantana, y azúcar líquida, y el surfactante no-iónico es monooleato de polioxietileno de sorbitán.

23. La suspensión de la reivindicación 22 que además comprende un sistema efectivo de búfer para mantener el pH del sistema en el rango de 4.0 a 6.0.

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24. La suspensión líquida de la reivindicación 23, en donde el sistema búfer comprende citrato de sodio y ácido cítrico.

25. La suspensión de la reivindicación 1, en donde el portador líquido farmacéuticamente aceptable es una combinación de agua purificada, glucosa y glicerina.

26. La suspensión de la reivindicación 1, en donde el portador líquido farmacéuticamente aceptable es una combinación de agua purificada, glucosa y glicerina.

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27. La suspensión de la reivindicación 1, en donde la cantidad del compuesto micronizado varía desde 10 mg/ml a 100 mg/ml.

28. La suspensión de la reivindicación 7, en donde la cantidad del compuesto micronizado varía desde 10 mg/ml a 100 mg/ml.

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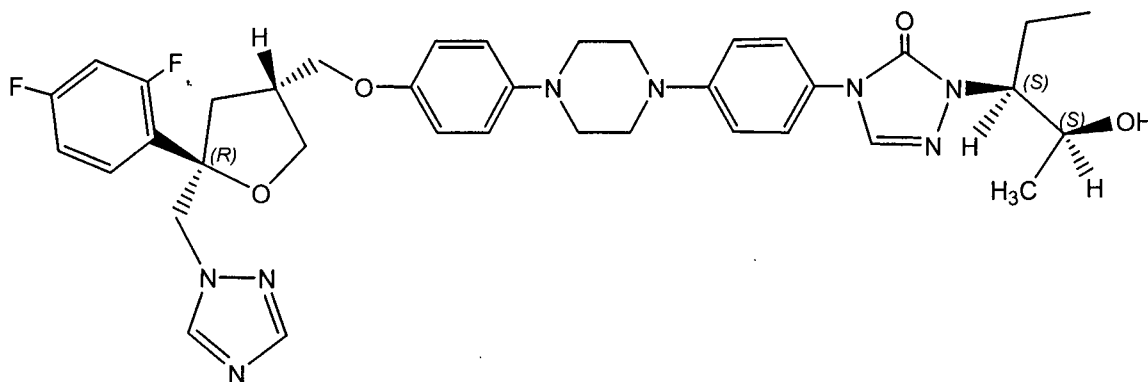
29. La suspensión de la reivindicación 16, en donde la cantidad del compuesto micronizado varía desde 10 mg/ml a 100 mg/ml.

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RESUMEN

Se describe una suspensión líquida que comprende una cantidad antifungal eficaz del compuesto micronizado representado por la fórmula estructural química I:

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por lo menos un agente espesador, un surfactante no iónico y un portador líquido farmacéuticamente aceptable.

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United States Patent [19]

Saksena et al.

US005661151A

[11] Patent Number: 5,661,151

[45] Date of Patent: Aug. 26, 1997

[54] TETRAHYDROFURAN ANTIFUNGALS

[75] Inventors: Anil K. Saksena, Upper Montclair; Vyyoor M. Girijavallabhan, Parsippany; Raymond G. Lovey, West Caldwell; Russell E. Pike, Stanhope; Haiyan Wang, Dayton; Yi-Tsung Liu, Morris Township; Ashit K. Ganguly, Upper Montclair; Frank Bennett, Piscataway, all of N.J.

[73] Assignee: Schering Corporation, Kenilworth, N.J.

[21] Appl. No.: 460,752

[22] Filed: Jun. 2, 1995

Related U.S. Application Data

[63] Continuation-in-part of PCT/US94/14236, Dec. 20, 1994, which is a continuation-in-part of Ser. No. 171,083, Dec. 21, 1993, abandoned.

[51] Int. Cl.⁶ A61K 31/495; C07D 405/14; C07D 413/14; C07F 9/6518

[52] U.S. Cl. 514/252; 514/85; 514/227.8; 514/236.2; 544/60; 544/121; 544/337; 544/357; 544/364; 544/366; 548/112; 548/268.6; 548/268.8; 549/559; 560/254; 562/840

[58] Field of Search 544/366, 60, 121, 544/337, 357, 364; 514/252, 85, 227.8, 236.2

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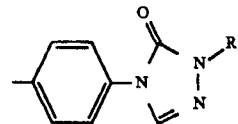
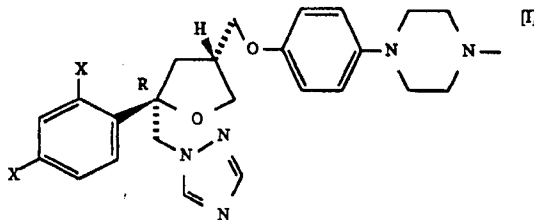
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Primary Examiner—Emily Bernhardt
Attorney, Agent, or Firm—Thomas D. Hoffman

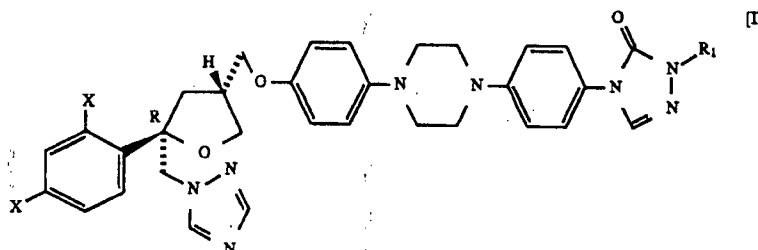
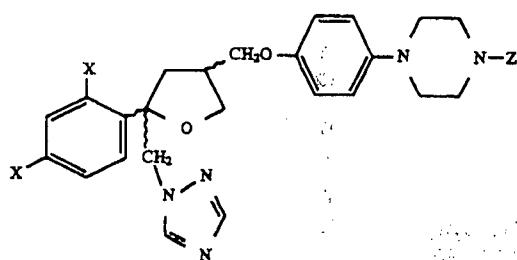
[57] ABSTRACT

A compound represented by the formula I



wherein X is independently both F or both Cl or one X is independently F and the other is independently Cl; R₁ is a straight or branched chain (C₃ to C₈) alkyl group substituted by one or two hydroxy moieties, an ether or ester thereof (e.g., a polyether ester amino acid ester or phosphate ester) thereof or a pharmaceutically acceptable salt thereof and pharmaceutical compositions thereof useful for treating and/or preventing fungal infections are disclosed.

13 Claims, No Drawings



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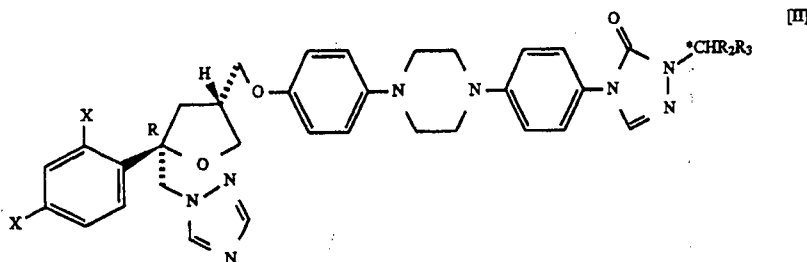
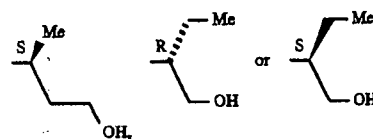
wherein X is independently both F or both Cl or one X is independently F and the other is independently Cl;

R₁ is a straight or branched chain (C₃ to C₈) alkyl group substituted by one or two hydroxy moieties or stereoisomers thereof or by one or two groups convertible in vivo into hydroxy moieties or an ester or ether thereof, or a pharmaceutically acceptable salt thereof.

In a preferred aspect of the present invention, there is provided compounds represented by formula II

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-continued



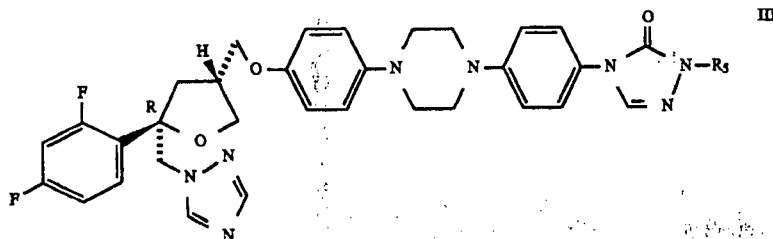
wherein X is independently both F or both Cl or one X is independently F and the other is independently Cl;

wherein R₂ is H or (C₁-C₃) alkyl and R₃ is (C₁-C₃) alkyl substituted by one hydroxy moiety or by a group convertible in vivo into a hydroxy moiety and the carbon with the asterisk (*) has the R or S absolute configuration; an ester or ether thereof or a pharmaceutically acceptable salt thereof.

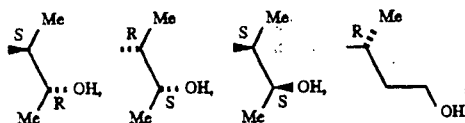
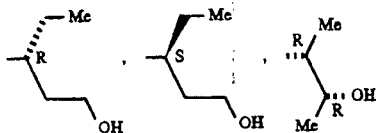
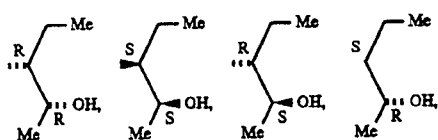
an ester or ether thereof or a pharmaceutically acceptable salt thereof.

Preferably the ester or ether is a group convertible in vivo into OH e.g. a polyether ester, phosphate ester or an amino acid ester.

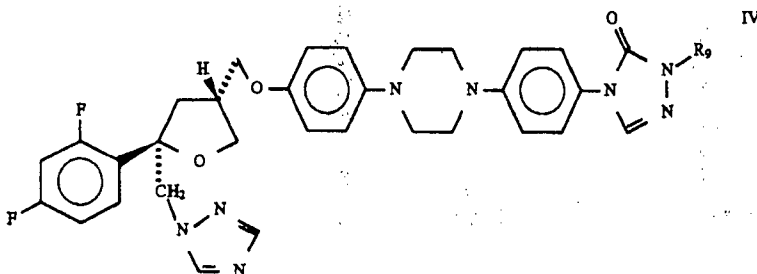
In another preferred aspect, the present invention provides a compound represented by formula III



wherein R₃ is



In another aspect of the present invention there is provided a compound represented by the formula IV



wherein $R_9 = \text{---}\overset{*}{\text{C}}\text{H}(\text{C}_2\text{H}_5)\text{CH}(\text{R}_6)\text{CH}_3$ or $\text{---}\overset{*}{\text{C}}\text{H}(\text{CH}_3)\text{CH}(\text{R}_6)\text{CH}_3$

wherein R_6 is OH, or a group convertible in vivo into OH or a pharmaceutically acceptable salt thereof.

DETAILED DESCRIPTION OF THE INVENTION AND OF THE PREFERRED EMBODIMENTS

The term " $(\text{C}_3\text{--}\text{C}_8)$ alkyl group substituted by one or two hydroxy moieties", as used herein means straight and branched chain alkyl groups of three to eight carbons including but not limited to methyl, ethyl, n- and iso-propyl, n-, sec-, iso- and tert-butyl, n-, sec-, iso-, tert and neo-pentyl, n-, sec-, iso-, tert- and neo-hexyl, n-, sec-, iso-, tert- and neo-heptyl, n-, sec-iso, tert- and neo-octyl, substituted by one or two hydroxy moieties and includes R and S stereoisomers of such $(\text{C}_3\text{--}\text{C}_8)$ alkyl groups.

The term " $(\text{C}_1\text{--}\text{C}_3)$ alkyl substituted by one hydroxy moiety" means $\text{---}\text{CH}_2\text{OH}$, $\text{---}\overset{*}{\text{C}}\text{H}(\text{OH})\text{CH}_3$, $\text{---}\text{CH}_2\text{CH}_2\text{OH}$, $\text{---}\overset{*}{\text{C}}\text{H}(\text{OH})\text{C}_2\text{H}_5$, $\text{---}\overset{*}{\text{C}}\text{H}_2\text{CH}(\text{OH})\text{CH}_3$, and $\text{---}(\text{CH}_2)_3\text{---}\text{OH}$ wherein the carbons with the asterisk(*) have the R or S absolute configuration.

The term "hydroxy-substituted C_4 or C_5 alkyl group" means $\text{---}\overset{*}{\text{C}}\text{H}(\text{C}_2\text{H}_5)\overset{*}{\text{C}}\text{H}(\text{OH})\text{CH}_3$, $\text{---}\overset{*}{\text{C}}\text{H}(\text{C}_2\text{H}_5)\text{CH}_2\text{CH}_2\text{OH}$, $\text{---}(\text{CH}_2)_2\text{---}\overset{*}{\text{C}}\text{H}(\text{OH})\text{C}_2\text{H}_5$, $\text{---}\overset{*}{\text{C}}\text{H}(\text{CH}_3)\overset{*}{\text{C}}\text{H}(\text{OH})\text{CH}_3$, $\text{---}\overset{*}{\text{C}}\text{H}(\text{CH}_3)\overset{*}{\text{C}}\text{H}(\text{OH})\text{CH}_3$ or $\text{---}\overset{*}{\text{C}}\text{H}(\text{C}_2\text{H}_5)\text{CH}_2\text{OH}$ wherein each carbon with the asterisk (*) has the R or S absolute configuration.

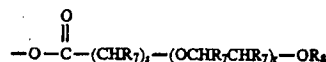
The term "group convertible in vivo into OH" means a group transformable in vivo by e.g. hydrolysis and/or by an enzyme, e.g. an esterase into a hydroxyl group. Such groups include polyether esters, phosphate esters, sulfate esters, heterocyclic esters, alkanate esters, alkenate esters, amino acid esters and acid esters. Preferred groups convertible in vivo into a hydroxyl group are the polyether esters, phosphate esters and amino acid esters.

The term "ethers" means (a) straight and branched chain alkyloxy groups of one to twenty carbons, preferably of one to eight carbons, more preferably one to six carbons and (b) polyethylene glycols, e.g. PEG200 to PEG 10000, preferably PEG200 to 5000 or (c) aryl $(\text{C}_1\text{--}\text{C}_6)$ alkyloxy groups of the formula $\text{---}\text{O}(\text{CHR}_7)_n\text{---}\text{Ar}$ wherein R_7 is $(\text{C}_1\text{--}\text{C}_6)$ straight and branched chain alkyl and $n=0$ to 6 preferably 1 to 3 and Ar is phenyl, phenyl substituted by halo, especially chloro and fluoro, or by nitro, cyano and trihalomethyl

especially trifluoromethyl. Most preferred ether groups include methoxy and benzyloxy.

The term "esters" means (a) polyether esters (b) phosphate esters (c) heterocyclic esters (d) alkanate and alkenate esters (e) amino-acid esters (f) acid esters and (g) sulfate esters.

The term "polyether esters" as used herein means those polyether esters represented by the formula

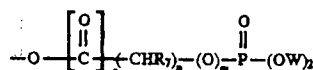


wherein

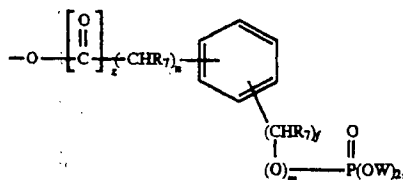
R_7 is as defined herein and s is an integer from 1 to 6, preferably $s=1$ to 3 and more preferably $s=1$; t is an integer from 1 to 6; preferably t is 1 to 3, more preferably t is 2 or 3.

R_8 is R_7 or $\text{---}(\text{CHR}_7)_s\text{---}\text{CO}_2R_7$; preferably R_8 is CH_3 or C_2H_5 or $\text{---}\text{CH}_2\text{CO}_2\text{H}$ or $\text{---}\text{CH}_2\text{CO}_2\text{CH}_3$. Typically suitable polyether esters include $\text{---}\text{COCH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_1\text{CH}_3$; $\text{---}\text{COCH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_2\text{CH}_3$, and $\text{---}\text{COCH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_3\text{CH}_3$.

The term "phosphate esters" as used herein means those phosphate acids esters represented by the formula



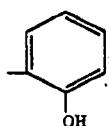
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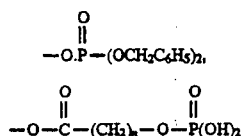
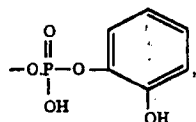
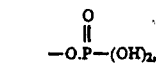
wherein z is 0 or 1;

R_7 is as defined herein above and preferably is H; n and f are independently an integer from 0 to 6, m is 0 or 1 and W is H, CH_2Ar or

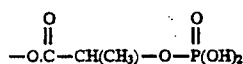
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and wherein Ar is as defined herein above. Typically suitable phosphate acids and esters include

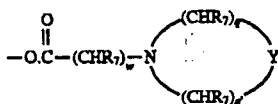


wherein m=n=1 to 4; or



and pharmaceutically acceptable salts thereof.

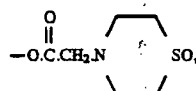
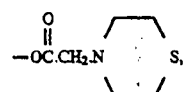
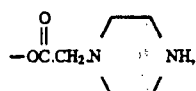
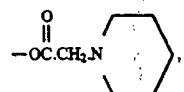
The term "heterocyclic ester" as used herein means heterocyclic esters represented by the formula



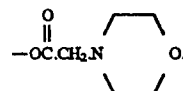
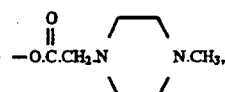
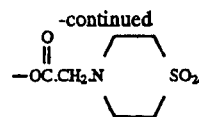
wherein

R_7 is as defined herein above, W is an integer of from 1 to 5 preferably W is 1 to 3; q and q' are independently 1 to 4, and q+q' are preferably equal to 2, 3, 4, or 5, and Y is CHR_7 , $-O-$, NH , NR_7 , S, SO or SO_2

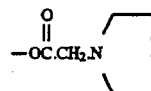
Typically suitable heterocyclic esters include



8



and

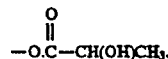


The term "alkanoate and alkenoate esters" as used herein means straight or branched chain alkanoate or alkenoate groups optionally substituted by a hydroxy or ether moiety or mixtures of such alkanoates or alkenoates.

Preferred alkanoate esters include acetate to decanoate, especially acetate to butanoate. Preferred hydroxy substituted alkanoate ester include C_1 to C_8 alkanoate substituted one hydroxy moiety or one C_1 - C_6 alkoxy group, especially

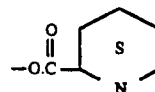


and

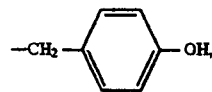


Preferred alkenoate esters are the C_{10} - C_{20} alkenoates and include C_{14} to C_{18} alkenoates, such as cis-7-hexadecenoate.

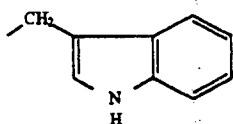
The term "amino acid ester" as used herein includes α -aminoalkanoxyloxy, natural i.e., (L)- α -amino acid ester groups, e.g. the ester of glycine, i.e. $OCOCH_2NH_2$, peptides esters thereof, unnatural α -amino acid ester groups such as $O-CO-CH(NH_2)(CH_2)_3CO_2H$, $OCOCH(NH_2)(CH_2)_2NH_2$, $OCOCH(NH_2)(CH_2)_3NH_2$ and



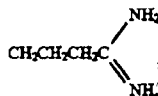
α -amino alkanoates represented by the formula $-OCOCH(NR_{20}R_{21})R_{22}$ wherein R_{20} and R_{21} are independently hydrogen or (C_1-C_8) straight or branched chain alkyl groups or R_{20} and R_{21} together with N form a 4, 5 or 6 membered ring optionally substituted with NR_{21} , $-O-$ or $-S-$ and R_{22} is H, CH_3 , CH_2OH , $CH(OH)CH_3$, CH_2SH ,



-continued

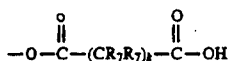


CH₂ CONH₂, -(CH₂)₂CONH₂, CH(CH₃)₂, CH(CH₃)₂,
CH(CH₃)C₂H₅, (CH₂)₂SCH₃, CH₂, CO₂H, (CH₂)₂CO₂H,
(CH₂)₄NH₂, -CH₂C₆H₅,



and pharmaceutically acceptable acid addition salts thereof, or (C₁-C₆) straight and branched chain alkyl groups optionally substituted by hydroxyl or NR₂₀R₂₁. Preferred amino acid acids are the natural α amino acid groups, dipeptides and α-amino alkanooates wherein R₂₀ and R₂₁ are each CH₃. The most preferred amino acid esters are those derived from alanine, phenylalanine, glycine, leucine, isoleucine and valine.

The term "acid ester" as used herein means those acid esters represented by the formula



wherein

R₇ is as defined herein above and k is an integer of from 1 to 8. Typically suitable acid esters include oxalic, malonic, succinic, glutaric and adipic acids as well as branched chain diacids such as



The term "ether" as used herein means (C₁-C₆) alkoxy or aryl (C₁-C₆) alkoxy which are conveniently made by the well known Williamson Synthesis of ethers. Typically suitable ether groups include methoxy and benzyloxy.

The compounds of the present invention as well as the esters and ethers thereof exhibit broad spectrum antifungal activity in various in vitro assays against *Candida*, other yeasts, dermatophytes, *Aspergillus* and opportunistic fungi. The in vitro antifungal activity tests were performed via conventional broth dilution methods in Sabouraud dextrose broth ("SDB") medium and Eagles Minimum Essential Medium ("EMEM") against a large number of fungi. Minimum Inhibitory Concentrations ("MICs") were measured after 24, 48 and 72 hour tests. In many cases, Minimum Fungicidal Concentrations ("MFCs") were measured after 48 and 72 hours.

The term "opportunistic fungi" include *Cryptococcus*, *Histoplasma*, *Blastomyces*, *Coccidioides*, *Fusarium*, *Mucor*, *Paracoccidioides*, *Fonsecaea*, *Wangiella*, *Sporothrix*, *Pneumocystis*, *Trichosporon* as shown by in vivo activity in an appropriate animal species e.g. mouse, rat or rabbit. The compounds of the inventions are expected to exhibit activity against many genera and species of protozoa, bacteria, gram negatives, gram positives, anaerobes, including *Legionella*, *Borrelia*, *Mycoplasma*, *Treponema*, *Gardnerella*, *Trichomonas* and *Trypanosoma*.

The preferred compounds of formula III wherein R₃=hydroxy-substituted C₄ and C₅ alkyl groups, exhibited

the following in vitro antifungal activity in SDB against 37 species of *Aspergillus*, *flavus*, *fumigatus* and other species: geometric mean MICs were in the range of ≤0.05 to ≤0.81 (mcg/ml) and geometric mean MFCs were in the range of 0.42 to ≥3.78 mcg/ml.

The preferred compounds of formula III wherein R₃ is a hydroxy-substituted C₅ alkyl group exhibited (1) superior antifungal activity as measured by geometric mean MICs and MFCs in various in vitro assays against *C. albicans* (N=26), *C. krusei* (N=16), *C. glabrata* (N=9), *C. tropicalis* (N=4), *C. stellatoidea* (N=1), *C. neoformans* (N=3), and of the dermatophytes, *T. rubrum*, *T. mentagrophytes*, and *T. tonsurans* (N=6) (after 48 or 78 hours) compared to fluconazole as well as (2) superior anti-fungal activity in the following in vivo models: an *Aspergillus flavus* and *fumigatus* (four strains) in a pulmonary immuno-compromised mouse model (PO-1XDX4D) compared to other azoles e.g. itraconazole, and in a *Candida albicans* (four strains) systemic model with normal and compromised mice (PO-1XDX4D) compared to other azoles, e.g. fluconazole. The preferred compounds of formula IV wherein R₃ is a

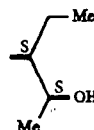
-CH(C₂H₅)CH(R₄)CH₃, wherein R₄ is a group convertible in vivo into OH exhibited superior antifungal activity comparable to the above listed preferred compounds of formula III wherein R₃ is a hydroxy-substituted C₅ alkyl group.

The in vivo oral antifungal activity of the compounds of the present invention were compared to azole antifungals, e.g., fluconazole in an *Aspergillus* pulmonary infection model in mice. The procedure of David Loeberberg et al. entitled "In vitro and In vivo Activity of Sch 42427, The Active Enantiomer of the Antifungal Agent Sch 39304", *Antimicrobial Agents and Chemotherapy*, (1992), 36, 498-501 was used. The *Aspergillus flavus* pulmonary model is also described in European Patent Application No. 0539, 938 A1 published on 5 May 1993.

The preferred compounds of formula III exhibited superior antifungal in vitro activity in SDB against 37 species of *Aspergillus* with (a) geometric mean MICs of ≤0.05 to ≤0.81 compared to fluconazole (geometric mean MIC≥32 and (b) with geometric mean MFCs of ≤0.42 to ≥3.78 compared to fluconazole (geometric mean MFC≥32).

The Tables Q, R, and S hereinbelow display the superior in vitro antifungal activity of three preferred compounds of formula III compared to fluconazole. Table Q displays such antifungal activity as the percentage of strains of various fungi with MICs≤1 mcg/ml for the three preferred compounds of formula III compared to fluconazole. Table R displays the antifungal activity as the percentage of the same strains with MFCs≤1 mcg/ml. Table S displays the in vitro MIC 90 values for the three preferred compounds of formula III against the same organisms listed in Tables Q and R.

The most preferred compound of formula III where R₃=



showed consistently higher serum levels in mice, rats, dogs and monkeys following oral dosing with a methyl cellulose formulation compared to azoles of similar structure and also exhibited very long serum half lives following O.D. dosing with good tissue distribution. The above listed most preferred compound of formula III are not inducers of various

cytochrome P-450 liver drug metabolizing enzymes after oral administration in an in vivo rat model.

TABLE Q

IN VITRO ANTI-FUNGAL ACTIVITY FOR SELECTED COMPOUNDS OF FORMULA III ¹					
PERCENTAGE OF STRAINS WITH MICs ≤ 1 MCG/ML (MCG/ML)					
R ₄ =					
 Me S OH Me S OH Me S OH					
ORGANISM	STRAINS				FLZ ²
<i>Aspergillus</i>	37	100	100	100	0
<i>Candida albicans</i>	26	100	100	100	100
<i>Candida krusei</i>	16	100	100	100	0
<i>Candida tropicalis</i>	5	100	100	100	100
<i>C. stellatoidea</i>					
<i>Candida glabrata</i>	9	22	22	33	0
<i>Cryptococcus neoformans</i>	3	100	100	100	0
Dermatophytes	6	100	100	100	100

1.

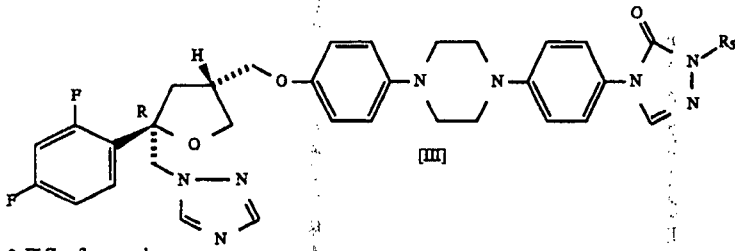


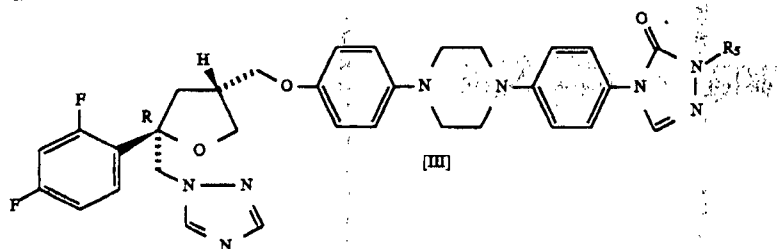
TABLE R

IN VITRO ANTI-FUNGAL ACTIVITY FOR SELECTED COMPOUNDS OF FORMULA III ¹					
PERCENTAGE OF STRAINS WITH MFCs ≤1 MCG/ML					
ORGANISM	STRAINS	(MCG/ML)			
		R ₅ =			
					FLZ ²
<i>Aspergillus</i>	37	50	62	89	0
<i>Candida albicans</i>	26	100	100	100	100
<i>Candida krusei</i>	16	88	94	100	0
<i>Candida tropicalis</i>	5	100	100	100	100
<i>C. stellatoidea</i>					
<i>Candida glabrata</i>	9	22	22	22	0

TABLE R-continued

IN VITRO ANTIFUNGAL ACTIVITY FOR SELECTED COMPOUNDS OF FORMULA III ¹					
PERCENTAGE OF STRAINS WITH MPCs ≤ 1 MCG/ML					
ORGANISM	STRAINS	(MCG/ML)			FLZ ²
		R ₁ =	R ₁ =	R ₁ =	
<i>Cryptococcus neoformans</i>	3	100	100	100	0
Dermatophytes	6	67	83	100	0

1.

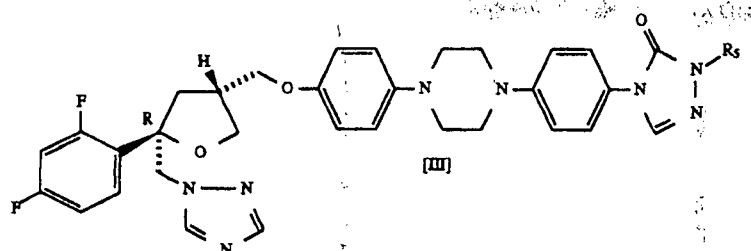


2. FLZ = fluconazole

TABLE S

IN VITRO ANTIFUNGAL ACTIVITY FOR SELECTED COMPOUNDS OF FORMULA III ¹					
MIC 90 Values ^a					
ORGANISM	STRAINS	(MCG/ML)			FLZ ²
		R ₁ =	R ₁ =	R ₁ =	
<i>Aspergillus</i>	37	.122	.096	.112	29.9
<i>Candida albicans</i>	26	.274	.174	.139	.887
<i>Candida krusei</i>	16	.058	.014	.12	29.9
<i>Candida tropicalis</i>	5	.117	.117	.354	.917
<i>C. stellatoidea</i>					
<i>Candida glabrata</i>	9	28.8	17.1	28.8	29.3
<i>Cryptococcus neoformans</i>	3	.05	.007	.101	25.9
Dermatophytes	6	.165	.101	.707	29.4

1.



2. FLZ = fluconazole

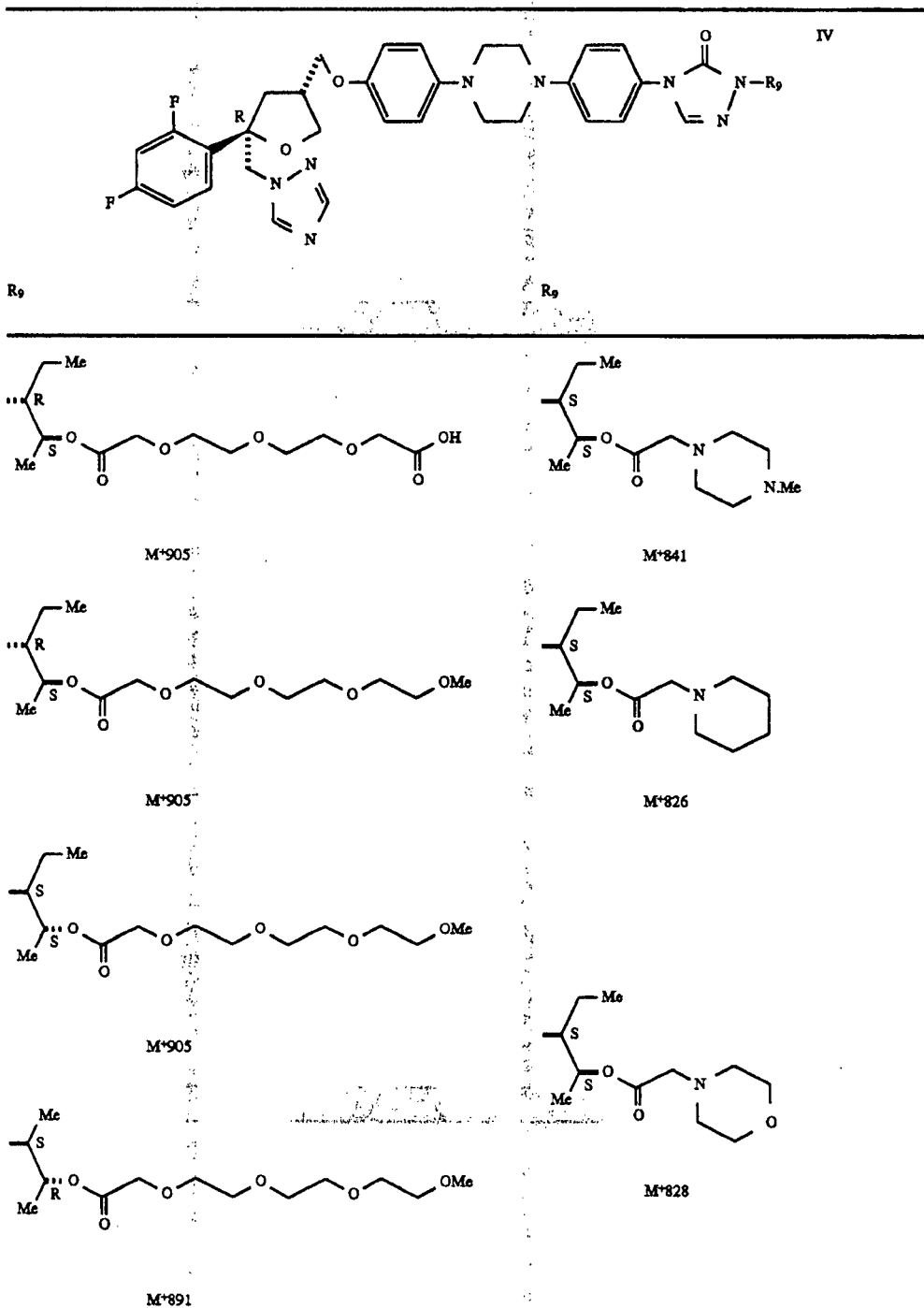
^aMinimum Inhibitory Concentrations for 90% of the strains

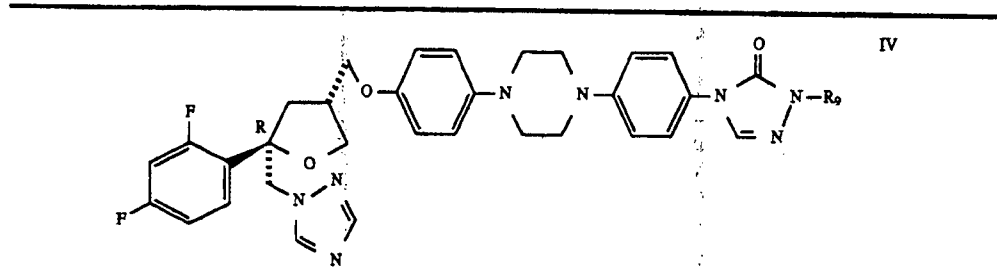
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The preferred esters and ethers of the compounds of the present invention of formula IV are soluble and/or suspendible in an aqueous medium suitable for IV or oral administration and also exhibit superior in vivo antifungal activity against a broad range of fungi after oral and parenteral e.g. IV administration in mice, rats, dogs and monkeys. The preferred esters and ethers of the compounds of the present

16

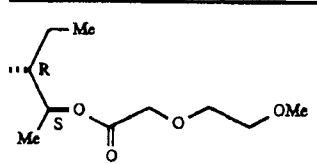
invention of formula IV which are convertible in vivo into the corresponding alcohols have a solubility in aqueous medium of at least about 1 to 50 mg/ml, preferably greater than or equal to about 10 mg/ml and more preferably about 20 to about 50 mg/ml. The preferred esters and ethers of formula IV listed below wherein R_9 is:



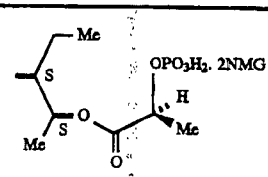


R₉

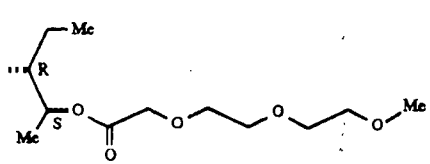
R₉



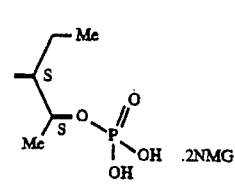
M*817



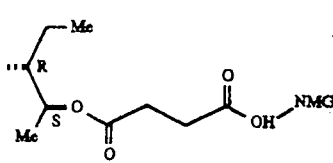
M*853.2



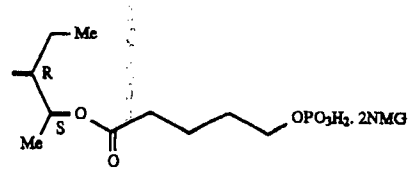
M*861



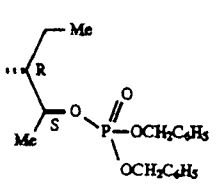
M*781.7



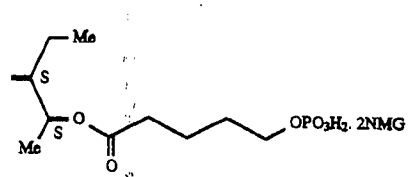
M*801



M*881.3

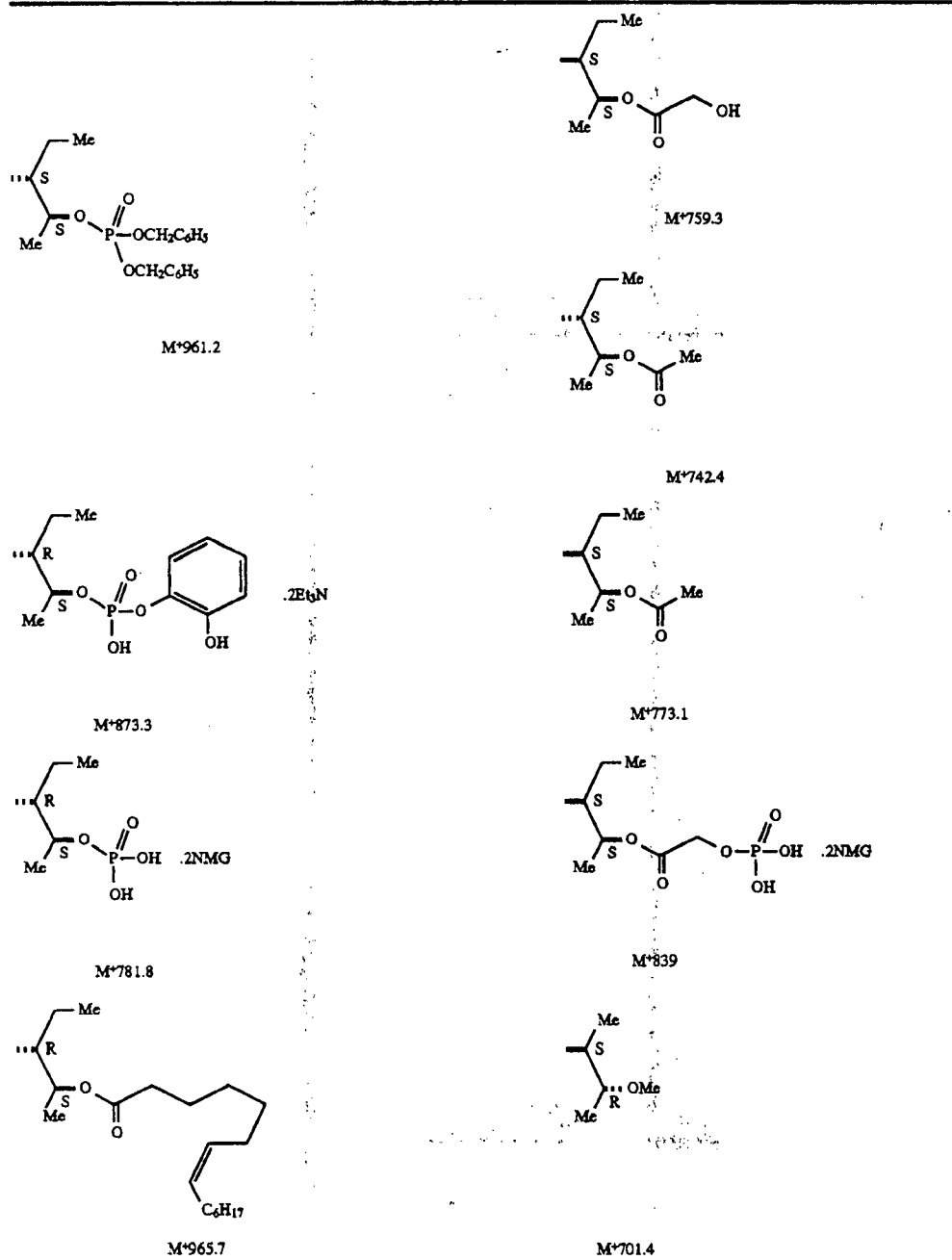
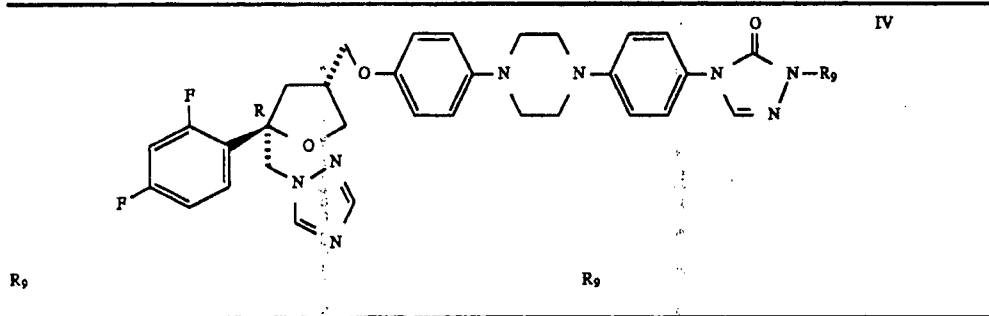


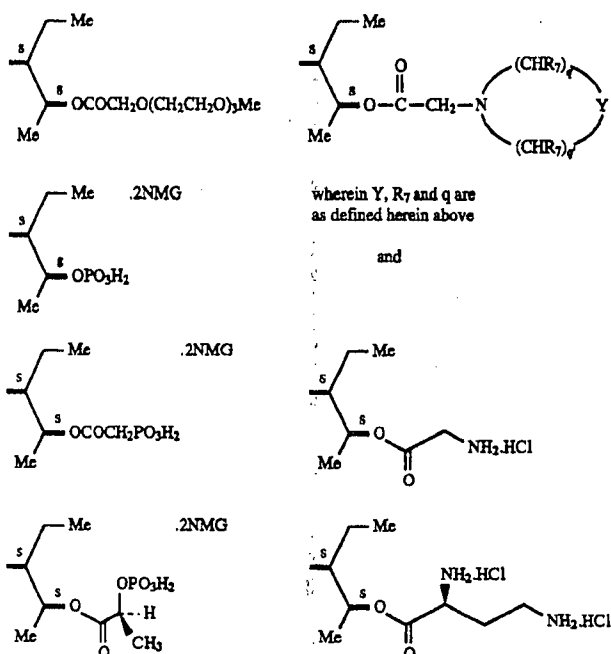
M*961.4



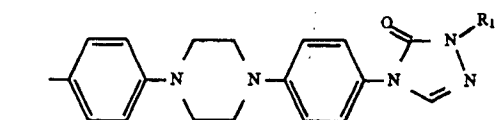
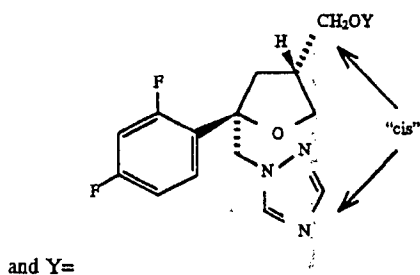
M*881.3

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The antifungal compounds of this invention represented by formula I have the R absolute stereochemical configuration at the carbon in the tetrahydrofuran ring bearing the di-halophenyl and 1H, 1,2,4-triazol-1-ylmethyl moieties, and the CH₂OY moiety has the "cis" stereochemical configuration relative to the 1H, 1,2,4-triazol-1-ylmethyl moiety. See the formula I hereinbelow.



wherein R₁ is a straight or branched chain (C₃-C₈) alkyl group substituted by one or two hydroxy groups, which preferably exists as a single stereoisomer, but mixtures of stereoisomers are also contemplated as within the scope of this invention.

The compounds of formula I are generically but not specifically disclosed as the "cis" series, type ii, at col. 9 lines 59-68 of Saksena et al. U.S. Pat. No. 5,039,676 and Example 68 at Col. 5, line 16 to col. 52, line 44.

GENERAL SYNTHETIC PREPARATIONS

The compounds of this invention may be prepared by use of the sequence of steps illustrated in the following Schemes

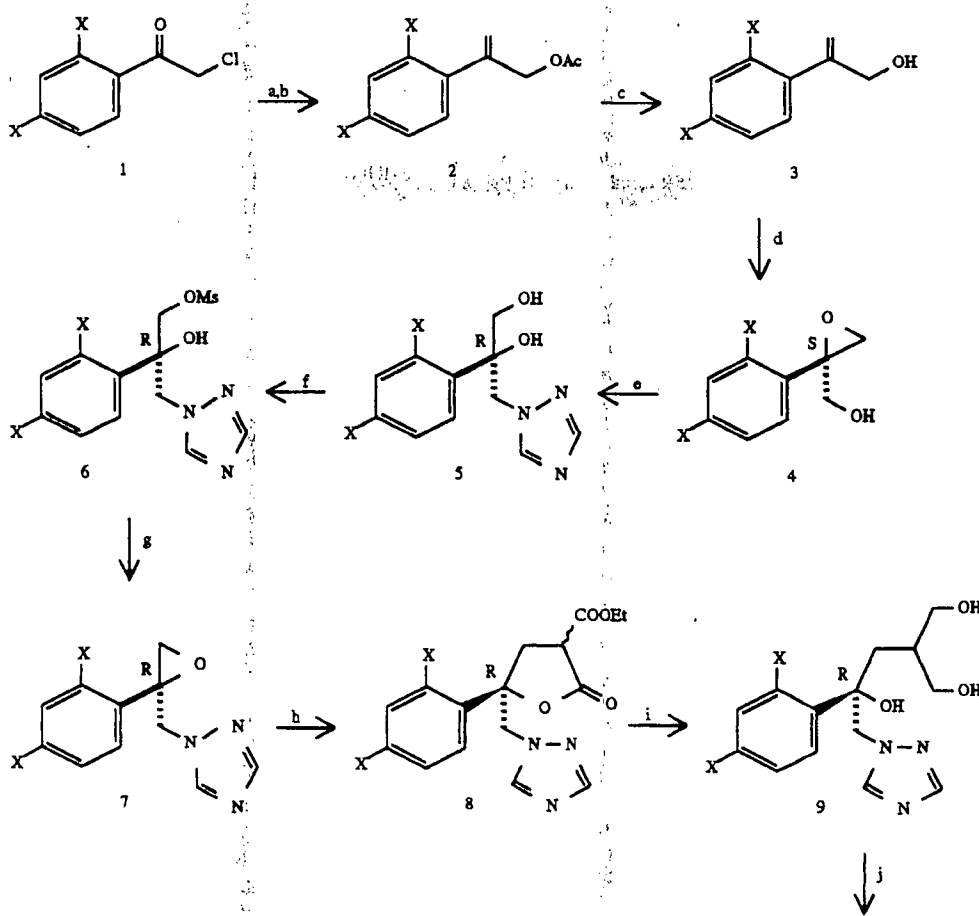
I-V. In Scheme I, compound 3 is readily prepared from commercially available compound 1 according to Examples 1a, 1b and 1c. Compound 4 is prepared by reaction of L(+)-diethyl tartarate ("L-DET") and molecular sieves in the presence of titanium tetra-isopropoxide (i-PrO)₄Ti in an aprotic solvent, such as methylene chloride, at a temperature 0° to -35° C. See for Example, T. Katsuki, K. B. Sharpless, *J. Am. Chem. Soc.*, 102, 5974 (1980); and 103, 464 (1981). An oxidizing agent, e.g. tert-butylhydroperoxide ("TBHP") is added to this reaction mixture (step d of Scheme I). Compound 3 is added and the compound of formula 4 (when L(+)-diethyl tartarate is used) is produced. Reaction of compound 4 with 1H-1,2,4-triazole in the presence of strong base, e.g., NaH in an aprotic solvent, such as DMF, at 0°-80° C. provides the diol compound of formula 5. The primary hydroxy group in compound 5 is converted into a leaving group, e.g., mesylate or tosylate (compound 6) by reaction of 5 with, for example, mesyl chloride ("MsCl"), in an aprotic solvent, e.g., methylene chloride in the presence of base, e.g., triethylamine ("Et₃N"). Compound 6 is treated with strong base, e.g., sodium hydride (NaH) in an aprotic solvent, e.g., DMF at room temperature to give oxirane compound 7. Reaction of 7 with diethyl malonate in the presence of strong base, e.g., sodium hydride in an aprotic solvent, e.g., DMSO at 25°-75° C. provides the lactone 8. Reduction of 8 with a metal hydride, e.g., lithium borohydride (LiBH₄) in an alcohol, e.g., ethanol (EtOH), provides the triol 9. Conversion of the two primary alcohols of 9 into leaving groups (mesylates or tosylates) by reaction of 9 with excess tosyl chloride in an aprotic solvent, e.g., THF, in the presence of base, e.g., Et₃N, provides ditosylate 10. Compound 10 is contacted with strong base, e.g., NaH, in an aprotic solvent such as toluene at elevated temperatures of 100°-120° C. to provide a mixture of two tosylates (cis and trans) which are separated by chromatography to yield to the cis-tosylate 11. Reaction of compound 11 with alcohols HOY in the presence of strong base, such as NaH in an aprotic solvent, such as DMSO at a temperature of 25°-75° C. provides compounds of formula I.

Scheme II provides an alternative reaction sequence to obtain compounds of the present invention. Reaction of compound 11 with the commercially available compound 12 in the presence of NaH gives compound 13. Hydrolysis of N-acetyl group in 13 is accomplished with a strong base such as NaOH in the presence of n-BuOH to provide compound 14. It should be made clear that instead of N-acetyl group in compound 12, any other base labile groups such as N-formyl, N-benzoyl, etc., can also be used to provide corresponding N-formyl and N-benzoyl derivatives of compound 13. Reaction of 13 with p-chloronitrobenzene in the presence of a hydrochloric acid scavenger such as K_2CO_3 provides the nitro compound 15. Catalytic reduction of 15 in the presence of a platinum or palladium catalyst yields the amine 16. Treatment of 16 with phenylchloroformate in the presence of pyridine gives the urethane intermediate 17. Reaction of 17 with hydrazine yields the semicarbazide 18 which is cyclized in the presence of formamidinium acetate to furnish the key triazolone 19. Alkylation of 19 according to Examples 19 and 20 provides the compounds of structure 20 including compounds of formula I wherein R_1 is defined as hereinabove.

Scheme III provides a stereospecific access to the cis-alcohol 26 and cis-tosylate 11 by application of enzyme chemistry. For Example, reaction of the triol 9 with ethyl acetate in the presence of porcine pancreatic lipase gives a single monoacetate 21. The remaining primary hydroxy group in 21 is protected by an acid labile group such as tetrahydropyranyl group to give a compound such as 22. Hydrolysis of the acetoxy group in 22 is accomplished with a base such as KOH which provides 23. The remaining steps are: (i) tosylation of compound 23 to provide 24; (ii) cyclization of 24 in the presence of NaH to provide 25; (iii) deprotection of THP ether in 25 using an acid catalyst such as p-toluene sulfonic acid (to give 26) followed by tosylation of the resulting 26 to furnish the key intermediate 11.

A detailed description of a preferred preparation of key intermediate is disclosed in commonly owned U.S. patent application Ser. No. 08/055,268, filed Apr. 30, 1993, now U.S. Pat. No. 5,403,937 which is hereby incorporated by reference.

SCHEME I

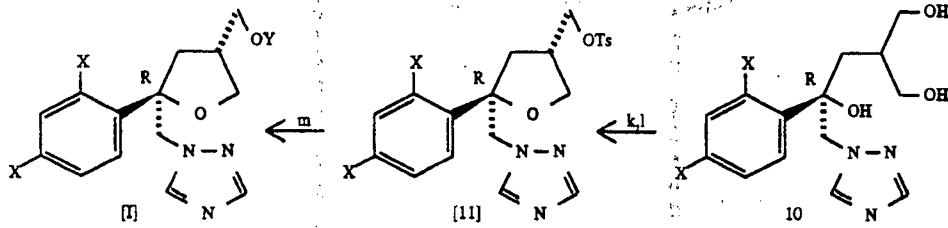


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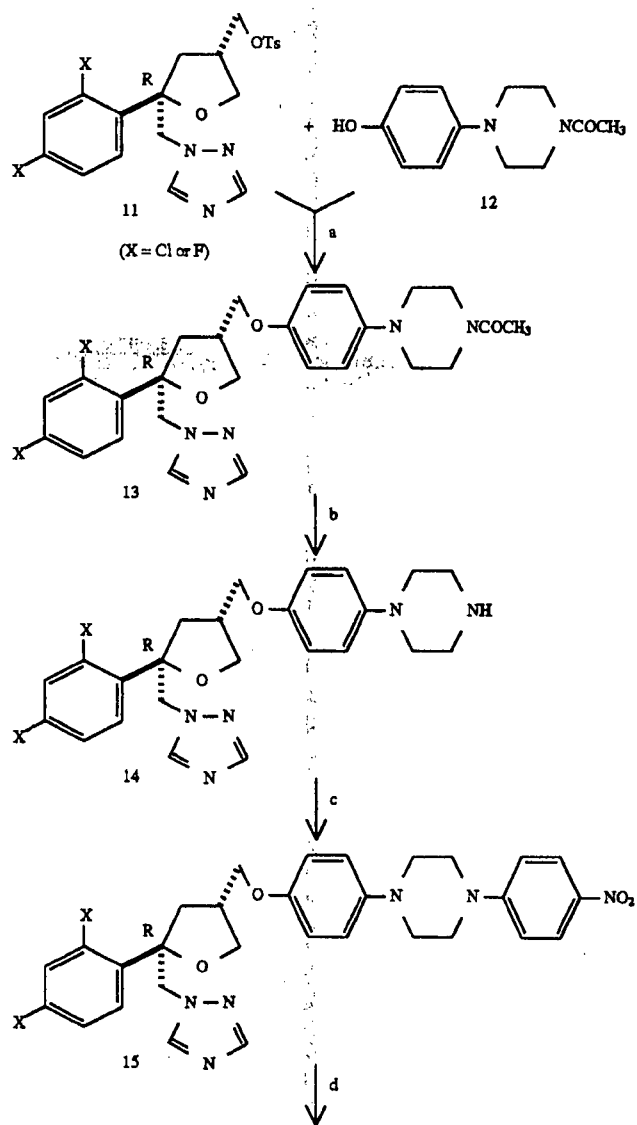
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SCHEME I

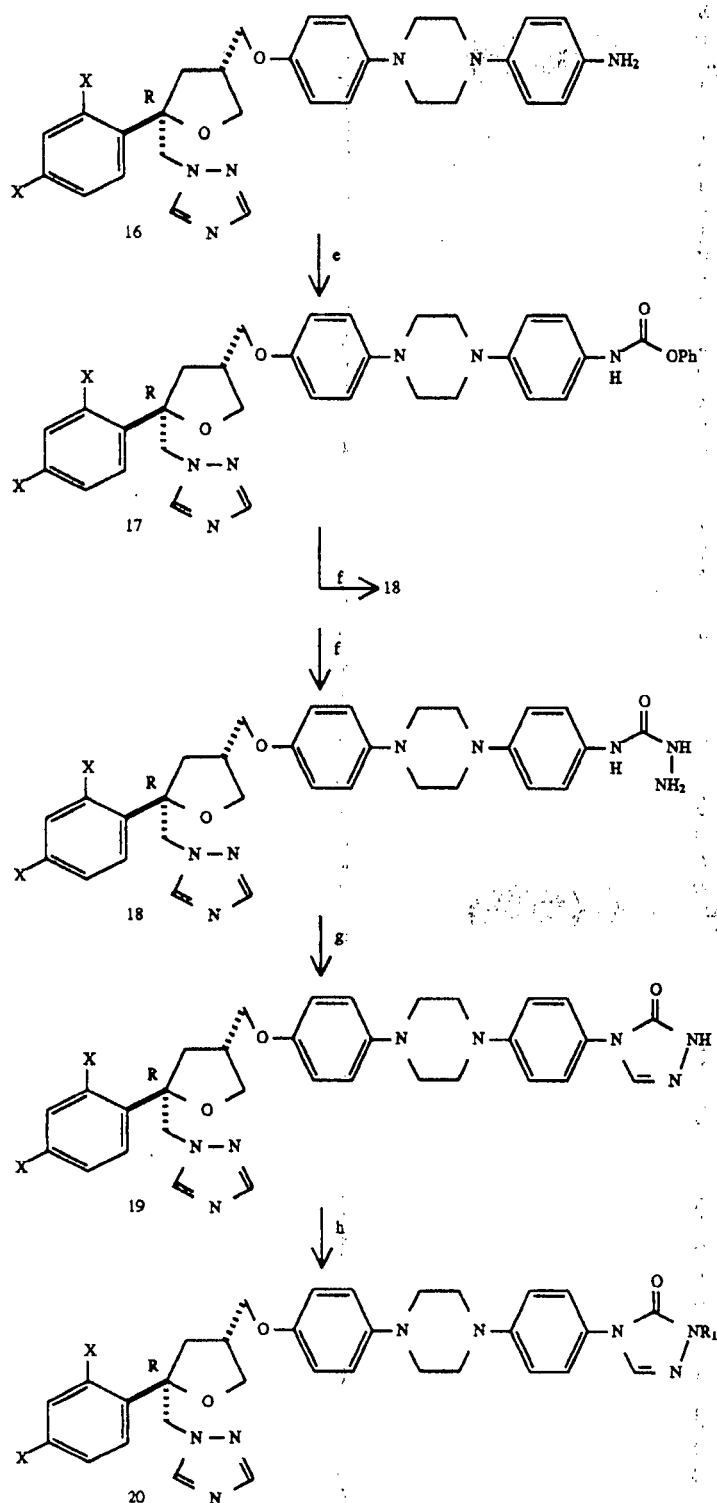


X=F or Cl

Reagents: (a) NaOAc; (b) Wittig Reaction; (c) KOH; (d) L-DET, TBHP, (i-Pr)₄Ti; (e) NaH, 1,2,4-triazole, DMF; (f) MsCl, Et₃N, CH₂Cl₂, 0°-5° C.; (g) NaH, DMF; (h) NaH, diethylmalonate, DMSO, 50°-55° C.; (i) LiBH₄, EtOH; (j) TsCl, Et₃N, DMAP, CH₂Cl₂-THF (1:1, v/v); (k) NaH, toluene, 100° C.; (l) Chromatography; (m) NaOY, DMSO.

SCHEME II



-continued
SCHEME II

Reagents: (a) NaH, DMF; (b) NaOH, n-BuOH; (c) p-Cl—
 C₆H₄NO₂, K₂CO₃, DMSO; (d) H₂, Pt-C; (e) C₆H₅OCOCl, 65
 pyridine, CH₂Cl₂; (f) NH₂NH₂·H₂O, H₂O, dioxane; (g)
 formamidine acetate, DMF, 80° C.; (h) according to
 Examples 19 and 20.

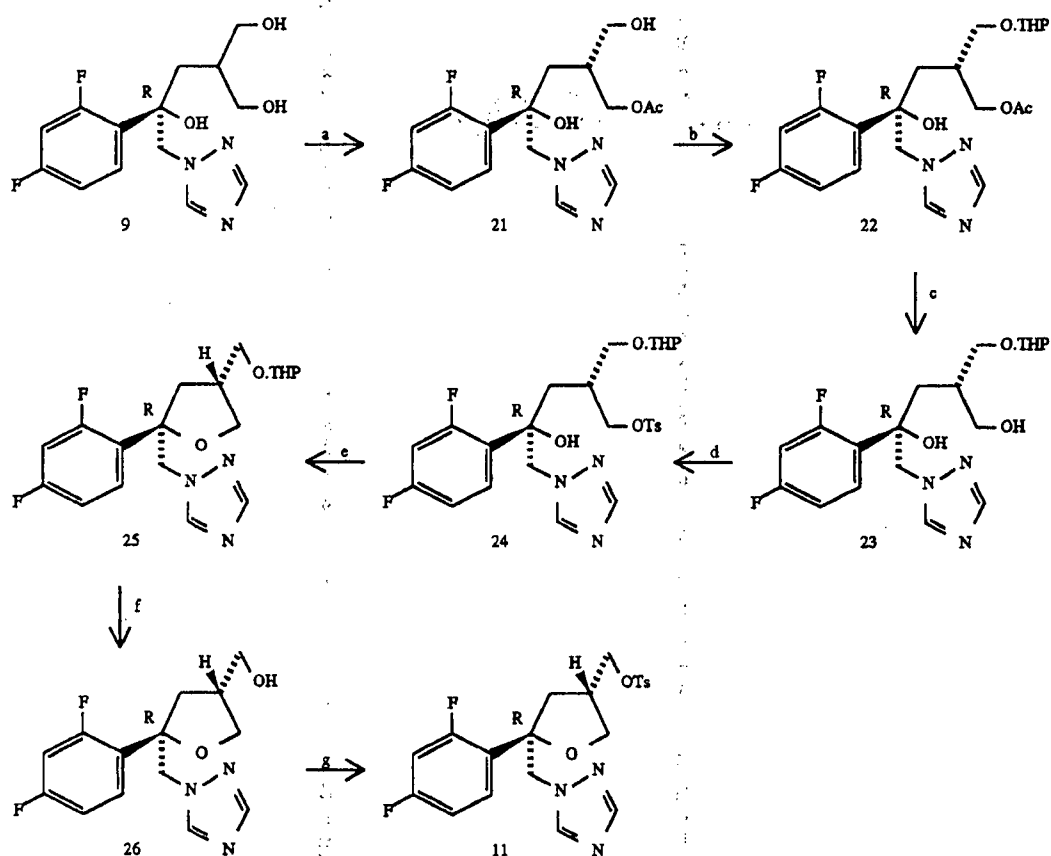
1a6

5,661,151

31

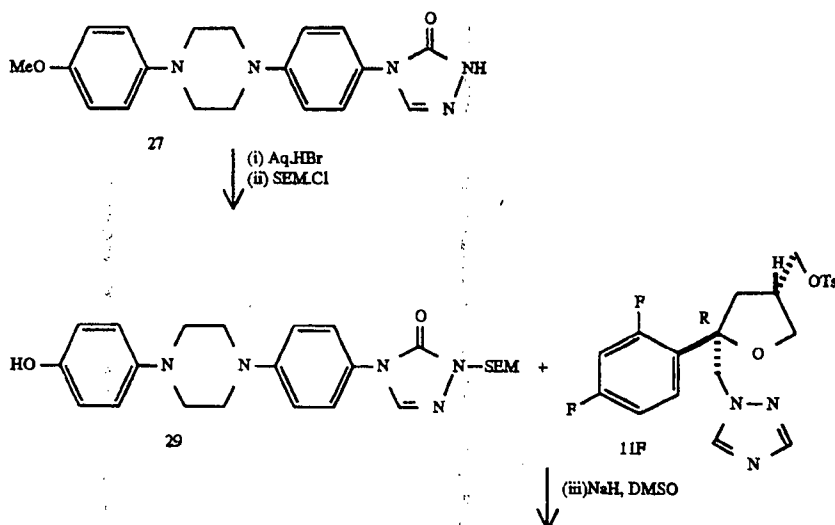
32

SCHEME III



Reagents: (a) Porcine pancreatic lipase, EtOAc; (b) pyridine; (c) NaH, toluene; (d) H₂O, methanol, H⁺; (e) tosyl chloride, pyridine; (f) H₂O, methanol, H⁺; (g) tosyl chloride, pyridine.

SCHEME IV



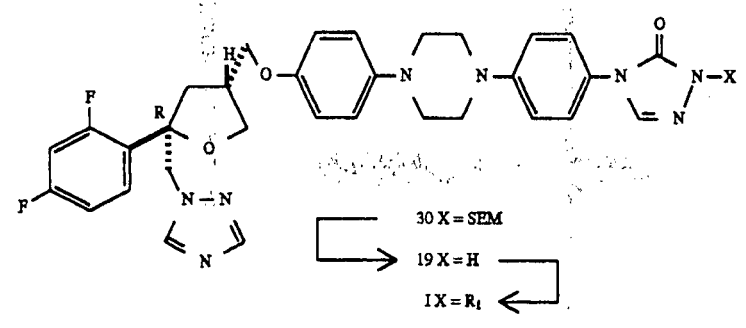
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5,661,151

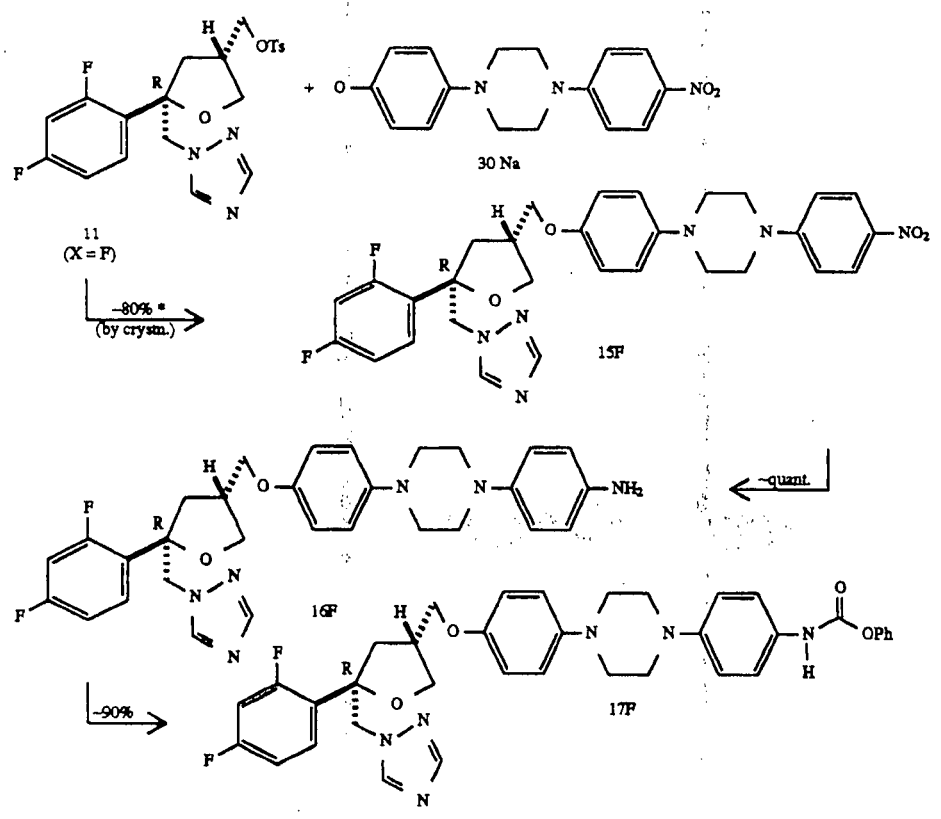
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34

-continued
SCHEME IV



SCHEME V



71

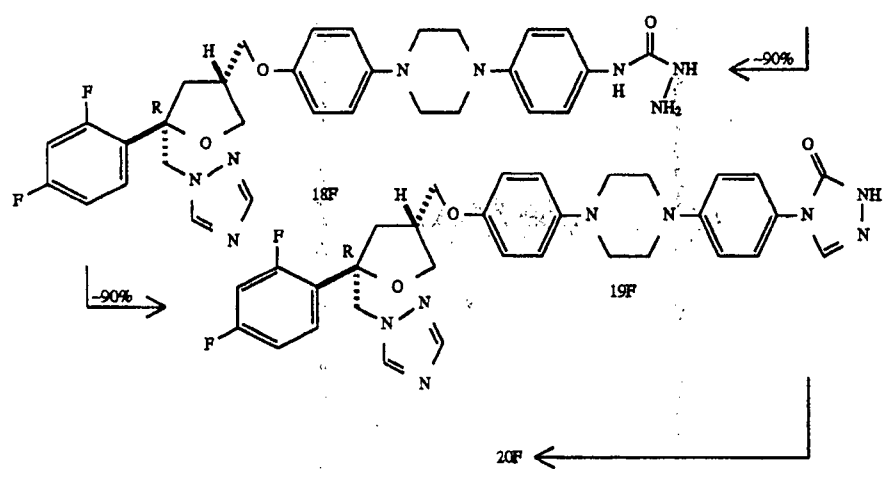
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5,661,151

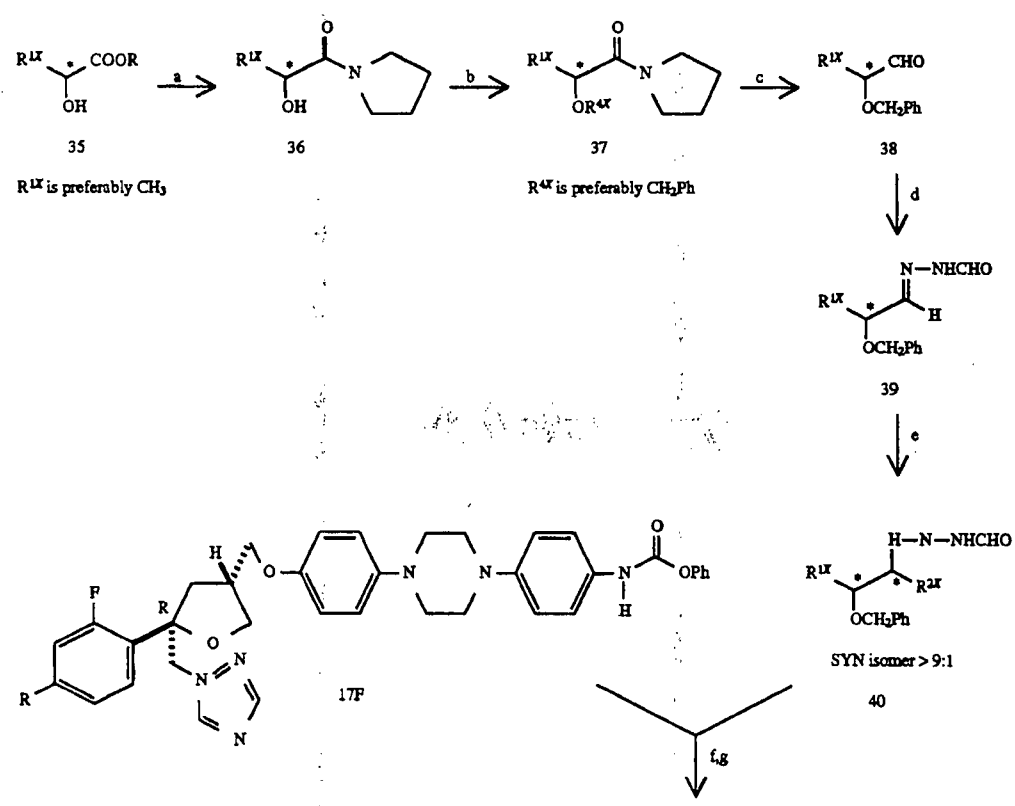
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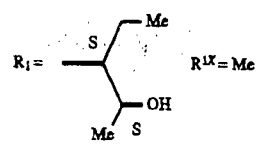
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SCHEME V



SCHEME VI



1999



Scheme VII



45X

700

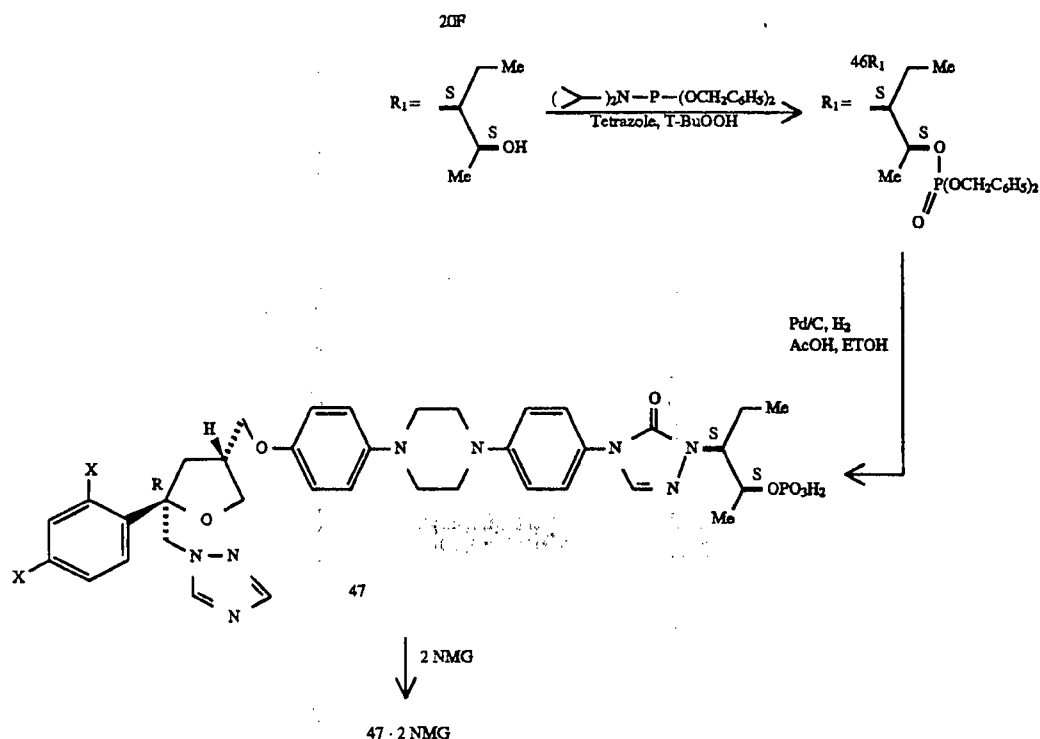
Table for Scheme VII

42	43	45	M.S.
—	PGOCH ₂ CO ₂ H PG = Protecting Group, e.g., CH ₂ Ph	X COCH ₂ OH	M+ 759.3
CH ₃ (OCH ₂ CH ₂) ₃ OH	ClCH ₂ CO ₂ H	COCH ₂ O(CH ₂ CH ₂ O) ₃ Me	905
CH ₃ (OCH ₂ CH ₂) ₂ OH	ClCH ₂ CO ₂ Na	—COCH ₂ O(CH ₂ CH ₂ O) ₃ Me	817
CH ₃ (OCH ₂ CH ₂)OH	ClCH ₂ CO ₂ Na	—COCH ₂ O(CH ₂ CH ₂ O) ₂ Me	861
CH ₃ (OCH ₂ CH ₂) ₂ OH	ClCH ₂ CO ₂ Na	—COCH ₂ O(CH ₂ CH ₂ O) ₃ Me	903
HO ₂ C(OCH ₂ CH ₂) ₂ OH	ClCH ₂ CO ₂ Na	—COCH ₂ O(CH ₂ CH ₂ O) ₂ CO ₂ H	905
PEG2000	ClCH ₂ CO ₂ Na	—COCH ₂ OPEG2000	*
PEG5000	ClCH ₂ CO ₂ Na	—COCH ₂ OPEG5000	*

*A range in MS values were observed which corresponded to the molecular weight range of the PEG2000 and PEG5000 used as starting materials.

Phosphate Esters

Scheme VIIIA



72

SCHEME VIII B

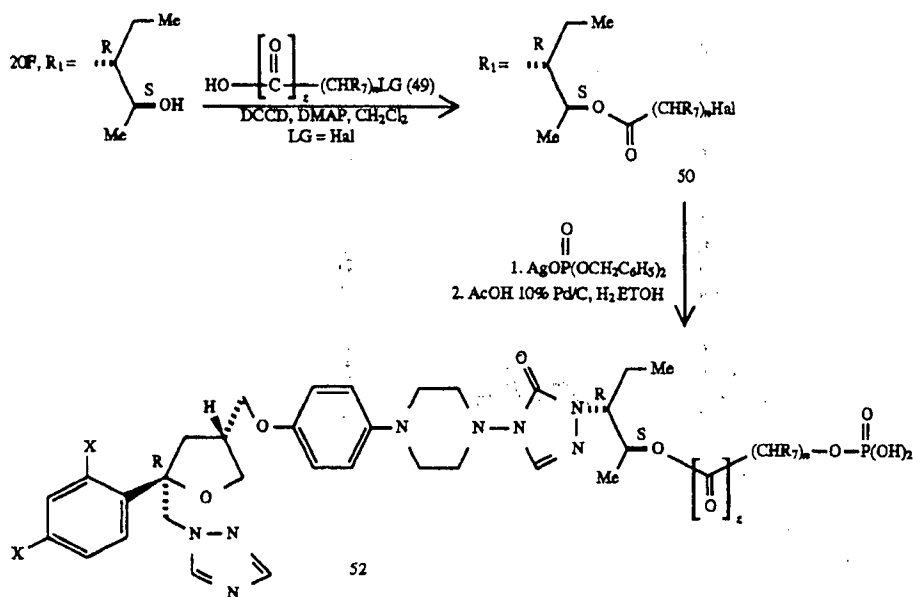


Table for Scheme VIIIA
X = F

47 R ₁	M.S. (M ⁺)
	781.8
	781.7
	873.3
	961.4
	961.2

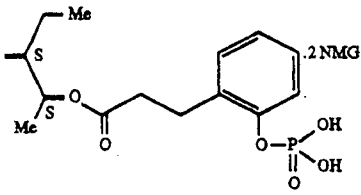
Table for Scheme VIIIB

49	52R ₁	M.S. (M ⁺)
35 HOCOCH ₂ Cl		839
40 HOCO(CH ₂) ₄ OH		881.3
45 HOCO(CH ₂) ₄ OH		881.3
50 HOCO(CH ₂) ₄ OH		853.2
55 S-HOCOCH(OH)CH ₃		
60		

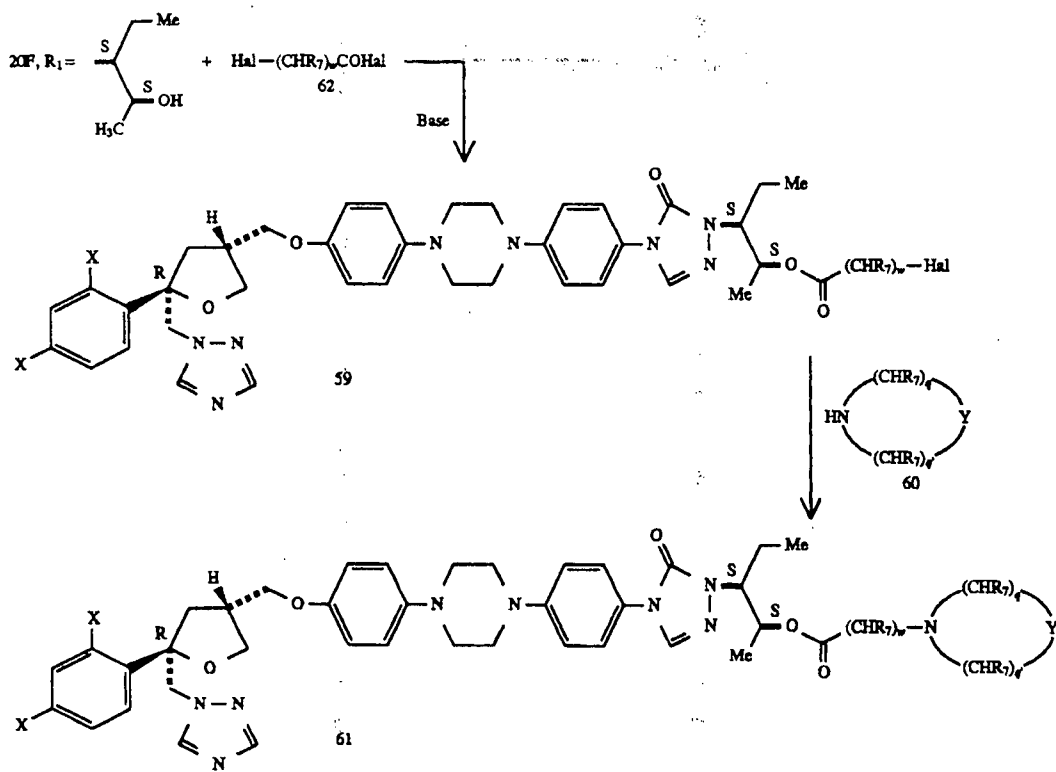
65

-continued

Table for Scheme VIII C

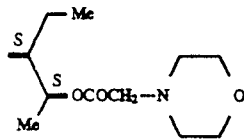
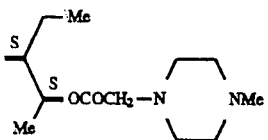
54a	58 R ₁	M.S. (M ⁺)
o-(HO ₂ C(CH ₂) ₂ C ₆ H ₄ OCH ₂ Ph		929.9

Scheme IX
Preparation of Heterocyclic Esters



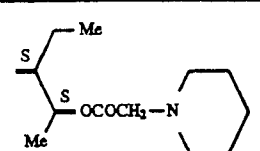
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Table for Scheme IX

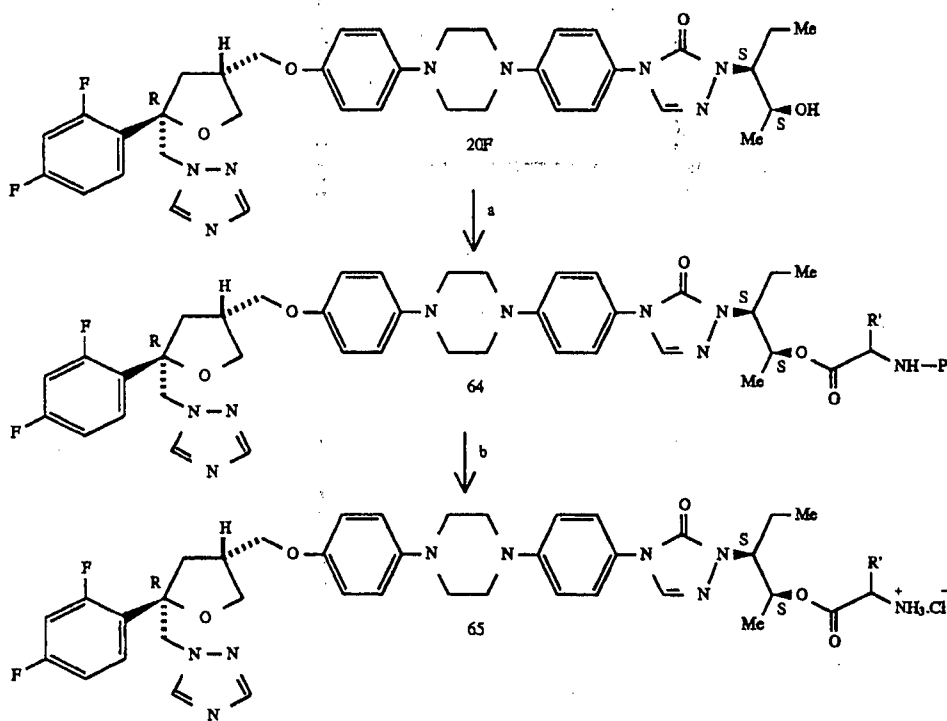
61 R ₁	M.S. (M ⁺)	61 R ₁	M.S. (M ⁺)
	828		841

204

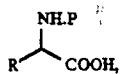
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Table for Scheme IX	
61 R ₁	M.S. (M ⁺)
	826
	10

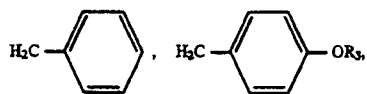
SCHEME X



Reagents: (a)

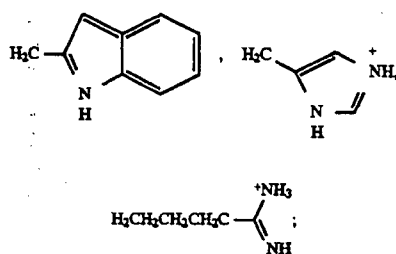


DCCD, DMAP, CH₂Cl₂; (b) HCl in dioxane Where
 R'=CH₃, C₂H₅, H; R₂=H, CH₃, CH₂OH, CH(OH)CH₃,
 CH₂SH, CH₂CONH₂, CH₂CH₂CONH₂, CH(CH₃)₂,
 CH(CH₃)CH₂CH₃, CH₂CH₂SMe, CH₂COO⁻,
 CH₂CH₂COO⁻, (CH₂)₄NH₃⁺,



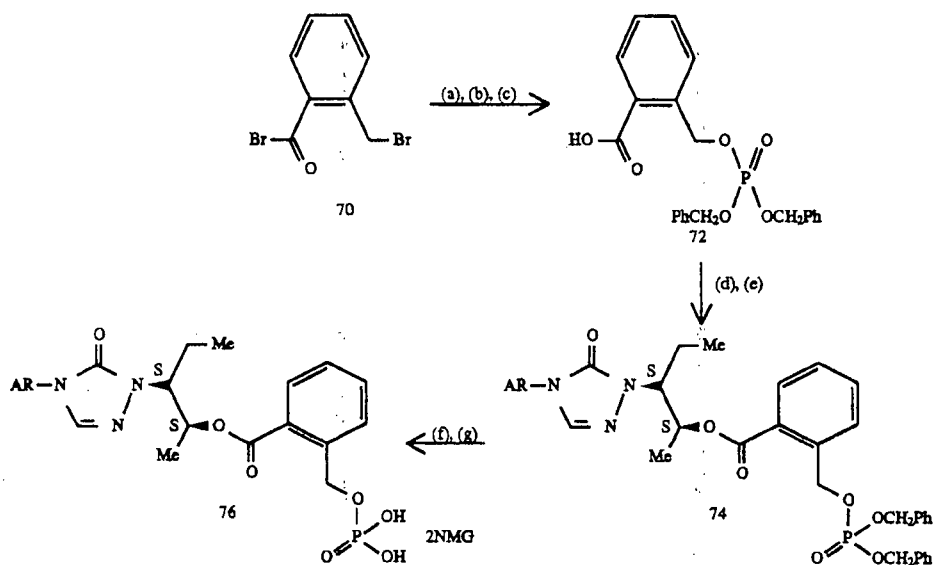
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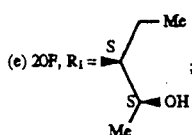


65 R₃=H, Phosphate ester, Sulphate ester; and P=N-tert-butoxycarbonyl (N-t-Boc), or N-Carbobenzyloxy (N-Cbz)

SCHEME XIA

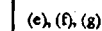
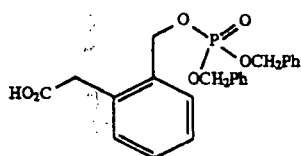
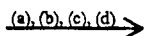
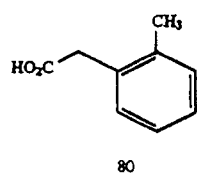
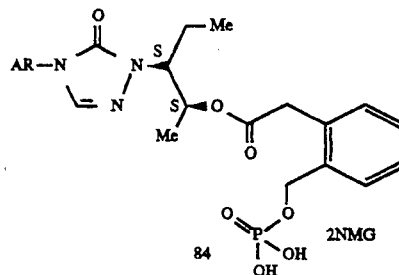


Reagents: (a) 2,2,2-trichloroethanol; (b) silver dibenzyl phosphate; (c) Zn, HOAc-THF; (d) SOCl₂;

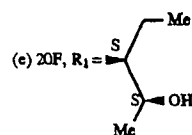


(f) H₂, 10% Pd-C; (g) 2eq. N-methyl glucamine.

SCHEME XIB

-continued
SCHEME XIB

Reagents: (a) 2,2,2-trichloroethanol, DCCD, DMAP; (b) N-bromosuccinimide; (c) silver dibenzyl phosphate; (d) Zn, HOAc-THF;



DCCD, DMAP; (f) H₂, 10% Pd-C; (g) 2eq. N-methyl glucamine

Scheme IV provides an additional reaction sequence to obtain the compounds of the present invention. Compound 27 is prepared from the methyl ether of compound 12 in Scheme II by subjecting the methyl ether of 12 to the reactions of steps a to g of Scheme II. Reaction of compound 27 with aqueous HBr or BBr₃ gives phenolic compound 28. Reaction of compound 28 with one equivalent of NaH and subsequent treatment with, for example, 2-(trimethyl)silyloxyethyl chloride ("SEM-Cl") and DMF at ambient

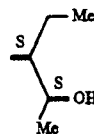
51

temperatures produces SEM-protected compound 29. Deprotonation of compound 29 with NaH followed by reaction of the so-formed anion with tosylate 11 in DMF or DMSO at elevated temperatures produces compound 30. The nitrogen protecting group of 30, e.g., SEM is removed by treatment with, for example, 6NHCl in methanol at ambient temperatures for 3 hr to produce compound 19. Compound 19 is treated with NaH and DMSO at 20° C. for ¼ hr. and thereafter alkylated with R1X to produce compound I. In R1X, R1 is a C3–C8 alkyl group having at least one protected hydroxy moiety, e.g., O-SEM and X is a leaving group, for example, brosylate. Removal of the hydroxy protecting group from compound 31, e.g., O-SEM is accomplished by, for example, 6NHCl in methanol to give compounds of this invention of formula I.

Scheme V provides a preferred route for preparation of the compounds of this invention set forth in Scheme II. The sodium salt of compound 31 prepared by reaction of (4-[4-(4-nitrophenyl)-1-piperazinyl]phenol with NaH in anhydrous DMSO at 50°–60° C. for 30 minutes is reacted with the 2,4-difluorophenyl tosylate 11F (compound 11 in Scheme II wherein X=F) for 1 h. at 50°–70° C. to provide, after flash silica chromatography or crystallization, compound 15F (compound 15 in Scheme II wherein X=F). Reduction of 15F by hydrogenation in the presence of 5% Pd/C in ethanol containing 1NHCl provided amino compound 16F (compound 16 in Scheme II wherein X=F). Reaction of 16F with phenylchloroformate in anhydrous pyridine at 0°–5° C. for 2 h. provided phenylcarbamate 17F (compound 17 of Scheme II wherein X=F). Reaction of 17F with hydrazine hydrate in 1,2-dimethoxyethane at 80° C. for 4 h. provided the semicarbazide 18F (compound 18 of Scheme II wherein X=F). Reaction of 18F with formamidine acetate and Et₃N in 2-methoxyethanol under dry argon in stirred reactor at 80° C. overnight provided 3H-1,2,4-triazol-3-one 19F (compound 19 in Scheme II wherein X=F). Reaction of compound 19(f) with R₁X in accordance with the procedure of Scheme IV produced compounds of formula I.

Scheme VI provides an alternative, stereoselective route for preparation of the preferred compounds of this invention. Compound 35 (e.g. S-lactic acid methylester) is contacted with excess pyrrolidine in methylene chloride for 24 hours at room temperature to give amide 36. Reaction of 36 and NaH with for example, benzyl halide in DMF gave 37. Selective reduction of amide 37 with a 3.4M solution of sodium bis(2-methoxyethoxy)aluminum hydride ("RED-Al") in toluene at –20° C. gave aldehyde 38. Reaction of aldehyde 38 with H₂NNHCHO in methanol gave 39 which was reacted with a Grignard reagent e.g. ethylmagnesium bromide in dry ether at a temperature of –10° C. to room temperature for 24 hours to give 40 wherein the ratio of the S,S isomer: S,R isomer was 94:6. When the Grignard reaction was done in the presence of 1.2 equivalents of bis(trimethylsilyl)acetamide the SS to SR ratio was 99:1. Compound 40 was reacted with compound 17F of Scheme V in toluene in the presence of DBU (1,8-diazabicyclo [5.4.0]undec-7-ene) for six hours at 80° C. Cyclization was effected by raising the temperature to 100°–110° C. and continuing to maintain this temperature overnight. After purification via TLC, 20F was obtained. Treatment of 20F with hydrogen and palladium black in methanol containing formic acid heated to 60° C. gave the crude product which was isolated and purified (via TLC) to give compound 20F i.e. the compound of formula III wherein

52



and X=F, Mt=701*

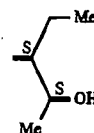
The reaction of the Grignard reagent on the propanimine 39 produces 40 wherein the absolute stereochemistry induced at the new chiral center in 40 is substantially the same (i.e., S) as that at the chiral carbon in 39. By the term "substantially the same" as used herein is meant the ratio of S:S to S:R (in e.g., 40) is greater than 9:1, preferably is greater than 15:1 and most preferably is at least 99:1.

The mass spectral data presented herein as M⁺ are parent ions which were determined by Fast Atom Bombardment (FAB) technique and represent the [M+H]⁺, i.e. {molecular ion+1} peaks.

We consider that Schemes I–VI set forth the preferred processes to prepare the alcohol compounds of this invention. Other processes for preparing the alcohol compounds of this invention are disclosed in commonly-assigned U.S. patent application Ser. No. 08/425,129, filed Apr. 19, 1995 and Ser. No. (Attorney's Docket No. CD0475) filed Apr. 19, 1995; and these two patent applications are hereby incorporated by reference.

Scheme VII provides a general method for preparation of the polyether esters of alcohols of the present invention. The alcoholate of alcohol ether 42 e.g. CH₃(OCH₂CH₂)₃OH i.e., 42 wherein R₁=H and t=3, was prepared by reaction, of 42 with excess strong base e.g. NaH in an anhydrous ether e.g. THF at ice bath temperatures. The so-formed reaction mixture was stirred for several hours i.e., 2 or more and the sodium salt of acid 43 e.g. sodium salt of chloroacetic acid (43 wherein LG=Cl, R₁=H and s=1) was added thereto. The so-formed reaction mixture was stirred at ice-bath temperatures and stirring was continued as temperature was allowed to warm to temperature. Water was carefully added to the reaction mixture and the polyether acid 44 was separated and purified by conventional techniques.

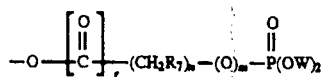
To a solution of 44 in CH₂Cl₂ was added 1.3–1.5 equivalents of the base 4-(N,N-dimethylamino)pyridine ("DMAP") and 20F wherein



The temperature of the so formed reaction mixture was lowered by use of an ice bath and 1.3–1.5 equivalents of dicyclohexylcarbodiimide ("DCCD") was added thereto. The so-formed reaction mixture was continuously stirred as the temperature was allowed to warm to room temperature. The dicyclohexyl urea precipitate was removed and the crude product isolated by conventional techniques. The so formed residue was purified by chromatography on silica gel to provide the pure compound [M+H]⁺=906 by FAB. By the appropriate substitution of different starting materials 42 and 43 the compounds 45 listed in Table for Scheme VII were prepared. The MS values for products listed under 45 in the Table for Scheme VII were measured by Fast Atom Bombardment ("FAB").

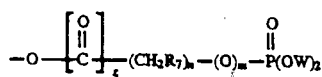
Schemes VIII A–C illustrate the generalised methods for preparing phosphate esters of the alcohols of this invention.

Scheme VIIIA provides a method for preparation of phosphate esters of formula IV wherein R_6 is

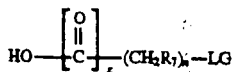


and $z=m=n=0$. Compound 20F of Scheme II in methylene chloride at room temperature was reacted with 1.5 equivalents of *N,N*-diisopropyl-dibenzylphosphoramidate, and 3 equivalents of a base such as tetrazole, followed by 1.5 equivalents of *tert*-butyl peroxide (3M in iso-octane) for several hours. The progress of the reaction was followed by TLC (5% methanol:EtOAc v:v) on silica gel. The crude product in EtOAc was washed with sodium thiosulfate and purified using standard techniques to provide the dibenzylphosphate ester 46. The dibenzyl ester groups of 46 were removed to give 47 by treatment of 46 dissolved in equal volumes of ethanol and glacial acetic acid in the presence of 10% Pd/C under a hydrogen atmosphere at room temperature in a stirred reactor overnight. The reaction was continued until no starting material was found by TLC (or NMR). The catalyst was removed by filtration and the crude phosphate ester 47 was purified by standard techniques. Treatment of 47 in methanol at room temperature with two equivalents of base e.g. NMG (or Et_3N) provided 47-2NMG. The compounds 46 and 47 prepared in accordance with Scheme VIIIA are listed in the Table for Scheme VIIA.

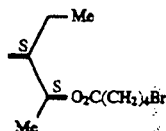
Scheme VIIIB illustrates preparation of phosphate esters of formula IV wherein R_6 =



$z=m=1$ and $n=0$. Compound 20F dissolved in methylene chloride was treated with 1.3 equivalents of DMAD 1.3 equivalents of DCCD and 1.3 equivalents of the acid 49 of the formula



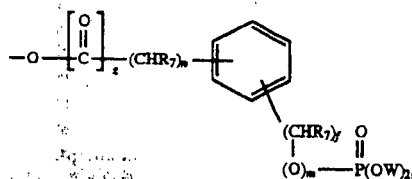
e.g., $\text{HO}_2\text{C}(\text{CH}_2)_4\text{Br}$, i.e., $z=1$, $n=4$, $R_7=\text{H}$ and the leaving group LG is Br. The reaction was stirred at room temperature until no starting material was found by TLC purification of the crude product gave bromide 50, a white solid wherein



The bromide 50 in benzene was heated at 80° C. overnight with 1.5 equivalents of silver dibenzylphosphate (available from Sigma Chemical Co., St. Louis). The reaction mixture was cooled and washed with aqueous base, e.g., K_2CO_3 . The crude product was separated and purified by silica gel column chromatography to give the dibenzyl phosphate ester 51. Treatment of 51 in ethanol/glacial acetic acid with 10% Pd/C under a hydrogen atmosphere overnight at room temperature gave phosphate ester 52. Treatment of 52 in methanol with two equivalents of base e.g. NMG (or Et_3N) gave 52-2NMG.

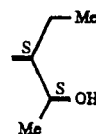
Scheme VIIIC provides an alternative procedure for preparation of phosphate esters of formula IV wherein R_6 is as defined above for Scheme VIIIB and $z=1$ and $n=1$. The benzyl ether of methyl acetate 53 in methanol-water and excess base e.g. K_2CO_3 were stirred overnight at room temperature to give the benzyl ether 54. Reaction of a solution of 20F and 54 in methylene chloride with a 1.3–1.5 equivalents of DCCD and DMAP at room temperature overnight gave ester 55. The benzyl ether group of 55 was removed by treatment with excess 10% Pd/C in ethanol-glacial acid under a hydrogen atmosphere at room temperature overnight. Purification of the crude product gave 56. Treatment of 56 with 1.5 equivalents of *N,N*-diisopropyl-dibenzylphosphoramidate and 3 equivalents of tetrazole and followed by 1.5 equivalents of *tert*-butyl peroxide in accordance with the procedure of Scheme VIIIB gave dibenzyl ester 57. Removal of the dibenzyl groups with 10% Pd/C in ethanol-glacial acetic acid under hydrogen atmosphere gave (as described hereinabove) phosphate ester 58. Treatment of 58 with two equivalents of base, e.g. NMG, gave 58-2NMG.

Additional phosphate esters of this invention of the formula



wherein $z=m=1$, $n=1$ or 2, $f=0$ or 1, and $W=\text{H}$, such as listed in the Table for Scheme VIIIC are prepared from compound 20F wherein R_1 is the same as that used in Scheme VIIIA by substituting equivalent amounts of starting materials 54a and 54b for compound 54 and thereafter following the procedures shown in Scheme VIIIC.

Scheme IX illustrates the preparation of heterocyclic esters of the present invention. Compound 20F, wherein

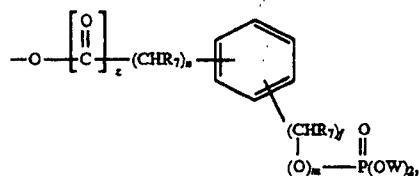


dissolved in methylene chloride is reacted with compound 62 in the ($\text{Hal}=\text{Br}$ or Cl , $w=1-5$, e.g., $\text{Cl—CH}_2\text{—COCl}$) in presence of a base such as pyridine at a temperature of 0°–5° C. for four hours. The reaction was placed in a refrigerator overnight. Additional compound 62 and base could be added, if necessary, and the reaction continued until no 20F is present by TLC. Purification of the crude product by column chromatography on silica gel gave pure 59 ($w=1$, $\text{Hal}=\text{Cl}$). Reaction of 59 with excess of the nitrogen heterocyclic compound 60 (e.g., $\text{Y}=\text{NH}$, $R_7=\text{H}$ and $q=4$) at a temperature of 50°–60° C. for 1 hour produced 61. Substitution of nitrogen heterocyclic compound 60 with a five and six membered compounds, e.g. morpholine, *N*-methylpiperidine provided the compounds listed in Table below Scheme IX.

Scheme X illustrates preparation of the amino acid ester derivatives of the compounds of this invention. Compound 20F is contacted with excess *N*-(*t*-butoxy carbonyl α -amino acid or α -amino alkanolate in the presence of DCCD and DMAP in an aprotic solvent such as CH_2Cl_2 at 0° to 25° C.

The reaction is followed by TLC and additional α -amino acid and DCCD are added, if necessary, to insure the starting material 20F is completely converted into amino acid ester derivative 64. Compound 64 is treated HCl in dioxane to provide the α -amino acid ester as the acid addition salt 65. Purification of the crude products is accomplished by standard techniques. When carbobenzoxy is used as the protecting group, hydrogen over palladium black is used to remove the protecting group in step b. Other protecting groups may be used such as those disclosed in "Protective Groups in Organic Synthesis" by T. W. Green and P. G. M. Wuts. John Wiley and Sons 1991 New York at pages 97-98 or 389-394.

Schemes XIA and XIB illustrate the preparation of additional phosphate esters of this invention of the formula



wherein $z=f=m=1$, $n=0$ formula or I, and $W=H$.

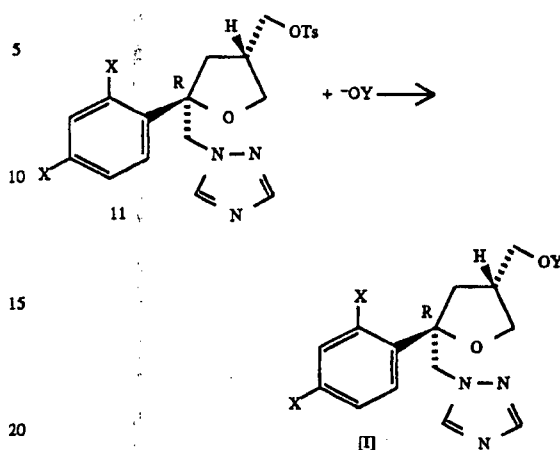
In Scheme XIA, the benzoyl bromide 70 is treated with 2,2,2-trichloroethanol to produce the corresponding trichloroethyl ester. Treatment of the trichloroethyl ester with excess silver dibenzyl phosphate under conditions similar to those used in Scheme VIIC converted the benzyl bromide into a dibenzyl phosphate ester. Removal of the trichloroethyl ester group was accomplished by use of zinc in acetic acid-THF to give the dibenzyl phosphate ester 72. Treatment of 72 with thionyl chloride gave the corresponding acid chloride which was contacted with a solution of 20F in methylene chloride under conditions of step one in Scheme VIIC to give ester 74. Removal of the dibenzyl ester groups of 74 with 10% Pd/C under a hydrogen atmosphere as described in Scheme VIIIA produced the corresponding phosphate ester which was treated with two equivalents of base e.g. NMG to provide 76. In Scheme XIB, the 2-methylphenylacetic acid 80 was esterified with 2,2,2-trichloroethanol and the so-formed ester was converted into corresponding the benzyl bromide by treatment with N-bromosuccinimide. The treatment of the benzyl bromide with excess silver dibenzyl phosphate under conditions of Scheme VIIC provided the corresponding dibenzyl phosphate ester. Removal of the trichloroethyl ester group was accomplished with zinc in acetic acid-THF to give dibenzyl phosphate 82. Treatment of a solution of 82 and 20F (wherein R_1 is the same as in Scheme VIIIA) with DCCD and DMAP provided the corresponding phosphate ester. Treatment of the phosphate ester with two equivalents of NMG gave compound 84.

The alkanoate and alkenoate esters of 20F are conveniently prepared by standard synthetic techniques, (for example, by reaction of the anhydride or acid halide of the alkanic acid or alkenic acid in the presence of base e.g., pyridine) produced the alkanoate or alkenoates of the compounds of formula I.

The sulfate esters may be prepared by reaction of the alcohol compounds of formulas I to IV with sulfur trioxide in the presence of excess pyridine at temperatures of 70°-90° C. for at least 2 hours in accordance with the procedure of R. M. Moriarty et al. *Tetrahedron Letters*, Vol. 35, No. 44, p 8103-8106 (1994).

The compounds of formula I may also be prepared by reaction of compound 11 with alcohols of formula HOY in

the presence of a strong base, e.g., NaH in an aprotic solvent, such as DMSO.



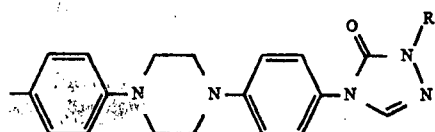
(R)-"Tosylate" Series

See Example 15

wherein

$X=F$ or Cl

$Y=$



and $R_1=a$ (C_3-C_8) alkyl group substituted by one or two hydroxy moieties.

Compounds represented by formula I exhibit broad spectrum antifungal activity, in conventional antifungal screening tests, against human and animal pathogens, such as the following: *Aspergillus*, *Blastomyces*, *Candida*, *Cryptococcus*, *Coccidioides*, *Epidermophyton*, *Fonsecaea*, *Fusarium*, *Mucor*, *Saccharomyces*, *Torulopsis*, *Trichophyton*, *Trichosporon*, *Sporothrix* and *Pneumocystis*.

The preferred compounds of formula IV exhibit topical, oral and parenteral antifungal activity in in vivo tests in animals and such activity is unexpectedly better than that of existing antifungal agents e.g. itraconazole and fluconazole as well as that of the azole compounds specifically disclosed by Saksena et al. in U.S. Pat. No. 5,039,676 and International Publication No. WO 93/09114.

The antifungal compounds of formula I and pharmaceutical compositions of this invention are expected to exhibit anti-allergic, anti-inflammatory and immunomodulating activities, broad spectrum anti-infective activity, e.g., antibacterial, anti-protozoal and anthelmintic activities.

The present invention also provides a composition for treating or preventing fungal infections comprising an antifungally effective amount of a compound represented by formula I or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier or diluent therefor.

The pharmaceutical compositions of the present invention may also contain a fungicidally effective amount of other antifungal compounds such as cell wall active compound. The term "cell wall active compound", as used herein, means any compound that interferes with the fungal cell

wall and includes, but is not limited to, compounds such as papulacandins, echinocandins, and aculeacins as well as fungal cell wall inhibitors such as nikkomycins, e.g., nikkomycin K and others which are described in U.S. Pat. No. 5,006,513 which is hereby incorporated by reference.

The pharmaceutically acceptable salts of the compounds of the present invention include pharmaceutically acceptable acid and base addition salts.

The preferred pharmaceutically acceptable acid addition salts are nontoxic acid addition salts formed by adding to the compounds of the present invention about a calculated amount of a mineral acid, such as HCl, HBr, H₂SO₄, HNO₃, or H₃PO₄, or of an organic acid, such as an alkyl or arylsulfonic acid such as methanesulfonic, isithionic, paratoluenesulfonic, naphthylsulfonic and the like.

The pharmaceutically acceptable bases found suitable for use in the present invention are those which form pharmaceutically acceptable salts of the acidic pharmaceutically acceptable esters of the antifungal compounds of formulas I, II, III or IV and include suitable organic and inorganic bases. Suitable organic bases include primary, secondary and tertiary alkyl amines, alkanolamines, aromatic amines, alkylaromatic amines and cyclic amines. Exemplary organic amines include the pharmaceutically acceptable bases selected from chloroprocaine, procaine, piperazine, glucamine, N-methylglucamine, N-N-dimethyl glucamine ethylenediamine, diethanolamine, diisopropylamine, diethylamine, N-benzylenediamine, diethanolamine, diisopropylamine, diethylamine, N-benzyl-2-phenylethylamine, N-n-dibenzylethylenediamine, choline, clemizole, triethylamine ("ET₃N"), tris(hydroxymethyl)aminomethane, or D-glucosamine. The preferred organic bases include N-methyl glucamine ("NMG"), diethanolamine, and tris(hydroxymethyl)aminomethane ("TRIS"). Use of two equivalents of NMG in this invention is more preferred. The suitable inorganic bases also include alkali metal hydroxides such as sodium hydroxide.

The pharmaceutical compositions of the present invention may be adapted for any mode of administration e.g., for oral, parenteral, e.g., SC, IM, IV and IP, topical or vaginal administration or by inhalation (orally or intranasally) Such compositions are formulated by combining the compound of formula I or an equivalent amount of a pharmaceutically acceptable salt of compound I with a suitable, inert, pharmaceutically acceptable carrier or diluent.

Examples of suitable compositions include solid or liquid compositions for oral administration such as tablets, capsules, pills, powders, granules, solutions, suppositories, troches, lozenges, suspensions or emulsions. A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders or tablet disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active compound. In the tablet, the active compound is mixed with carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

Topical dosage forms may be prepared according to procedures well known in the art, and may contain a variety of ingredients, excipients and additives. The formulations for topical use include ointments, creams, lotions, powders, aerosols, pessaries and sprays.

For preparing suppositories, a low melting wax such as a mixture of fatty acid glycerides or cocoa butter is first melted, and the active ingredients are dispersed homogeneously therein as by stirring. The molten homogeneous

mixture is then poured into convenient sized molds, allowed to cool and thereby solidify.

Liquid form preparations include solutions, suspensions and emulsions. As an example may be mentioned water or water-propylene glycol solutions for parenteral injection. Liquid preparations can also be formulated in solution with an appropriate amount of a hydroxypropyl α - β or γ -cyclodextrin having 2 to 11 hydroxypropyl groups per molecule of cyclodextrin, polyethylene glycol, e.g., PEG-200 or propylene glycol, which solutions may also contain water. Aqueous solutions suitable for oral use can be prepared by adding the active component in water and adding suitable colorants, flavors, stabilizing, sweetening, solubilizing and thickening agents as desired. Aqueous suspensions suitable for oral use can be made by dispersing the active component in finely divided form in water. A particularly preferred aqueous pharmaceutical composition may be prepared from the compounds of formulas I to IV together with hydroxypropyl- β -cyclodextrin in water. The use of derivatives of α -, β - and γ -cyclodextrins, for example, hydroxypropyl- β -cyclodextrin are disclosed by N. Bodor U.S. Pat. No. 4,983,586, Pitha U.S. Pat. No. 4,727,064 and Janssen Pharmaceutical International patent application Ser. No. PCT/EP 84/00417.

The pharmaceutical compositions of the present invention may be prepared by admixing the pharmaceutically acceptable carrier, e.g., a hydroxypropyl- β -cyclodextrin in water, and adding thereto an antifungally effective amount of a drug of the present invention. The solution so formed is filtered, and optionally, the water may be removed by well known methods, e.g., rotatory evaporation or lyophilization. The formation of the solution may take place at a temperature of about 15° to 35° C. The water is normally sterilized and may also contain pharmaceutically acceptable salts and buffers, e.g., phosphate or citrate as well as preservatives. The molar ratio of the antifungal compound of formula I hydroxypropyl- β -cyclodextrin is about 1:1 to 1:80, preferably 1:1 to 1:2. Normally the hydroxypropyl- β -cyclodextrin is present in molar excess.

Also included are solid form preparations which are intended to be converted, shortly before use, into liquid form preparations for either oral or parenteral administration. The solid form preparations intended to be converted to liquid form may contain, in addition, to the active materials, such as compounds of this invention, and optionally a cell wall active compound, especially a fungal cell wall inhibitor, e.g., a nikkomycin, flavorants, colorants, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents and the like. The solvent utilized for preparing the liquid form preparations may be water, isotonic water, ethanol, glycerin, polyethylene glycols, propylene glycol, and the like, as well as mixtures thereof.

Parenteral forms to be injected intravenously, intramuscularly, or subcutaneously are usually in the form of a sterile solution, and may contain salts or glucose to make the solution isotonic.

The topical dosage for humans for antifungal use in the form of a pharmaceutical formulation comprising a compound of formula I (usually in the concentration in the range from about 0.1% to about 20% preferably from about 0.5% to about 10% by weight) together with a non-toxic, pharmaceutically acceptable topical carrier, is applied daily to the affected skin until the condition has improved.

In general, the oral dosage for humans for antifungal use ranges from about 1 mg per kilogram of body weight to about 30 mg per kilogram of body weight per day, in single or divided doses, with about 1 mg per kilogram of body

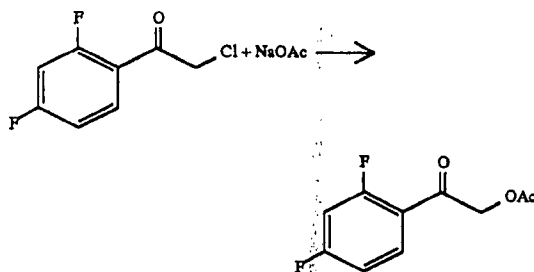
weight to about 20 mg per kilogram of body weight per day being preferred and the dose of about 1 mg per kilogram of body weight to about 10 mg per kilogram of body weight per day being most preferred.

In general, the parenteral dosage for humans for antifungal use ranges from about 0.25 mg per kilogram of body weight per day to about 20 mg per kilogram of body weight per day, in single or divided doses, with about 0.5 to about 10 mg per kilogram of body weight per day being preferred.

The exact amount, frequency and period of administration of the compounds of the present invention for antifungal use will vary, of course, depending upon the sex, age and medical condition of the patient as well as the severity of the infection as determined by the attending clinician.

GENERAL EXPERIMENTAL

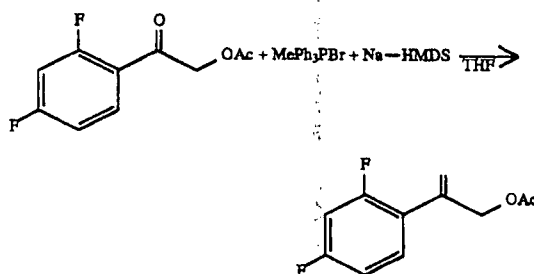
The compounds of this invention are prepared in accordance with Schemes I-IX hereinabove and the following Examples using commercially available starting materials.



EXAMPLE 1a

2-Acetoxy-1-(2,4-difluorophenyl)ethanone

Add 191 g of 2-chloro-2-(2,4-difluorophenyl)ethanone (Aldrich Chemical Co.) to a mixture of 246 g of sodium acetate, 3 g of NaI, and 3 L of DMF. Stir the mixture at 20° C. for 18 hr. then concentrate it to 1 L. Pour the residue into 6 L of cold dilute aqueous HCl and extract with EtOAc. Wash the extract with brine, dry it over anhydrous Na₂SO₄, filter the so-formed mixture, and evaporate the filtrate to leave a residue. Chromatograph the residue on silica gel, eluting with CH₂Cl₂-2-hexane to obtain 198 g of the title compound.

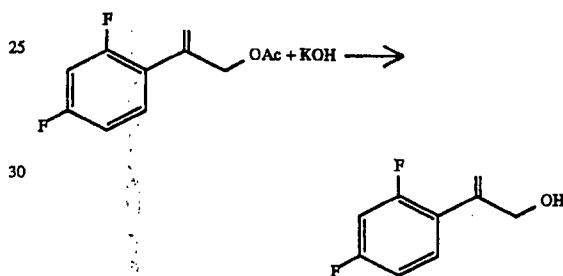


EXAMPLE 1b

1-[2-(2,4-Difluorophenyl)]-2-propenol acetate

Suspend 131 g of MePh₃PBr in 270 mL of mechanically-stirred, dry THF at 20° C. Add 393 mL of 1M NaN(Me₃Si)₂ in THF, slowly at first, then rapidly over 5 min. while

applying just enough ice cooling to maintain the temperature at <23° C. Stir the so-formed mixture for 1 hr at 20°-24° C., cool it to -70° C., and stir it another ½ hr. Then add thereto a solution of 65.5 g of the product of Example 1a in 140 mL of dry THF, at a rate slow enough to keep the temperature below -70° C. Continue to stir the so-formed reaction mixture in the cold bath overnight during which the temperature rises to 20° C. Add 50 mL of EtOAc to the so-formed suspension, and then add 3 L of hexane. Allow the so-formed mixture to stand for ~15 min., and suction-filter to remove Ph₃PO. While the filter cake is still damp, transfer it to a beaker. Triturate the cake thoroughly with ½ L of hexane and suction-filter again to remove the remainder of product. Wash the combined hexane filtrates with 2x1 L of a 1:1 (v/v) MeOH-water, and then with brine. Dry the organic layer over MgSO₄, filter and evaporate the filtrate to leave a red oil. Add 1.5 L of hexane and suction-filter through a Celite pad to leave a clear yellow solution. Chromatograph the yellow oil on silica gel, eluting with ½ L of hexane, then 1 L of 15:1 (v/v) hexane-EtOAc. Combine the homogeneous fractions to yield 38.6 g of the title compound as an oil.



EXAMPLE 1c

2-(2,4-Difluorophenyl)-2-propenol

Dissolve 40 g of the product of Example 1b in 400 mL of dioxane. Add a solution of 18 g of 85% KOH in 315 mL of water. Stir the so-formed mixture vigorously for 1 hr, and then pour the mixture into 1 L of Et₂O. Separate the aqueous layer and extract it with 250 mL of Et₂O. Combine the organic extracts, and wash them with water and then brine. Dry the organic extract over anhydrous K₂CO₃, and add 10 g of charcoal thereto. Filter, and evaporate the filtrate to leave 31.3 g of the title compound as a straw-colored oil.

EXAMPLE 1d

(S)-(-)-[2-[2-(2,4-Difluorophenyl)]oxiranyl] methanol

Add 33 g of activated 3 Å molecular sieve powder to a solution of 13 g of L-(+)-diethyl tartarate in 2.3 L of CH₂Cl₂, and cool the so-formed mixture to -5° C. Add a solution of 15.4 mL of titanium tetra-isopropoxide in 100 mL of CH₂Cl₂ over 2-3 minutes and then cool the so-formed mixture to -22° C. Add 109.5 mL of a 5.5M solution of tert-butylhydroperoxide in 2,2,4-trimethylpentane over 4-6 minutes, and cool the so-formed mixture to -25° C. Stir the mixture at -25° C. for 25 minutes and then add a solution of 40g of 2-(2,4-difluorophenyl)-3-propenol of Example 1c in 100 mL of CH₂Cl₂ over 3-4 minutes. Stir the so-formed mixture at -27° C. for 4½ hour. Add 102 mL of 30% aqueous sodium hydroxide saturated with NaCl and stir the so-formed mixture while warming to +10° C. over a ½ hour

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period. Add thereto 100 g of anhydrous MgSO_4 and 33 g of Celite, and stir $\frac{1}{2}$ hour at $+10^\circ\text{C}$. Suction-filter the mixture, wash the so-formed filter cake with 1.2 L of diethyl ether (Et_2O) and then 1.5 L of toluene, and dry the combined organic layers over anhydrous MgSO_4 . Filter the organic layer, and evaporate the filtrate in vacuo to form a residue. Dissolve the residue in 1 L of Et_2O and suction-filter the mixture to remove insolubles. Suction-filter the filtrate through 100 g of silica gel, and wash the pad with 200 mL of fresh Et_2O . Evaporate the filtrate in vacuo to give 41 g (94%) of the crude title compound as a yellowish oil, $[\alpha]_D^{25} -36.7^\circ$ ($c=1$, MeOH); PMR (CDCl_3) δ 7.40(m, 1H), 6.85(m, 2H), 3.95(m, 2H), 3.31(d, 1H), 2.84(d, 1H), 1.91(m, 1H, deuterium exchangeable).

EXAMPLE 2

(R)-(+)-[2-[2-(2,4-Difluorophenyl)]oxiranyl]
methanol

Follow the procedure of Example 1d, except substitute an equivalent amount of D-(-) diethyl tartarate in place of L-(+) diethyl tartarate to give the crude title compound, $[\alpha]_D^{25} +33.9^\circ$ ($c=1$, MeOH).

Purify a portion of the crude compound by silica gel chromatography to obtain a sample homogeneous by TLC, $[\alpha]_D^{25} +40.0^\circ$ ($c=1$, MeOH).

EXAMPLE 3

(R)-(-)-2-(2,4-Difluorophenyl)-3-(1,2,4-triazol-1-yl)-1,2-propanediol

Dissolve 8.91 g of 1H-1,2,4-triazole in 150 mL of anhydrous DMF and cool so-formed mixture to $0^\circ\text{--}5^\circ\text{C}$. Add 2.81 g of sodium hydride (60% oil dispersion) and stir the so-formed mixture 30 minutes at room temperature. Add thereto 10.9 g of the product of Example 1d. Stir the so-formed reaction mixture for 2 hours at $60^\circ\text{--}70^\circ\text{C}$. Cool the mixture to room temperature, add thereto 10 mL of H_2O and evaporate it in vacuo to give a residue. Dissolve the residue in 100 mL of H_2O and 900 mL of ethyl acetate (EtOAc). Extract the H_2O layer with another 250 mL of EtOAc. Wash the combined EtOAc extracts with 100 mL of brine. Dry the EtOAc extracts over anhydrous MgSO_4 and evaporate. Triturate the so-formed oily residue with 10 mL of CH_2Cl_2 and add 100 mL of Et_2O . Stir the CH_2Cl_2 - Et_2O mixture for 1 hour at room temperature. Filter to give 11.2 g (75%) of the title compound, $[\alpha]_D^{25} -70.7^\circ$ ($c=1.0$, MeOH), mass spectrum (FAB): 256 $[\text{M}+\text{H}]^+$. Recrystallize 1.0 g of the filtered product from 5 mL of CH_3CN to give 0.83 g of the title compound, m.p. $99^\circ\text{--}100^\circ\text{C}$; $[\alpha]_D^{25} -71.5^\circ$ ($c=1.0$, MeOH); elemental analysis: Calculated for $\text{C}_{11}\text{H}_{11}\text{F}_2\text{N}_3\text{O}_2 \cdot \frac{1}{2}\text{CH}_3\text{CN}$: 52.27C, 4.57H, 17.78N, 13.78F; Found: 52.26C, 4.58H, 17.54N, 13.78F; PMR(DMSO) δ 8.25(s, 1), 7.66(s, 1), 7.33(m, 1), 7.09(t, 1), 6.90(t, 1), 5.72(s, 1), 5.05(t, 1), 4.53(s, 2), 3.61(m, 2).

EXAMPLE 4

(S)-(+)-2-(2,4-Difluorophenyl)-3-(1,2,4-triazol-1-yl)-1,2-propanediol

Follow the procedure of Example 3, except substitute an equivalent quantity of the product of Example 2 in place of the product of Example 1 to give the title compound; MP: $95^\circ\text{--}101^\circ\text{C}$; $[\alpha]_D^{25} +70.0^\circ$ ($c=1.0$, MeOH). The PMR and Mass spectra were consistent with the structure of the title compound.

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EXAMPLE 5

(R)-2-(2,4-Difluorophenyl)-3-(1,2,4-triazol-1-yl)-1,2-propanediol-1-methanesulfonate

Suspend 10.9 g of the powdered product of Example 3 in 150 mL of CH_2Cl_2 . Add thereto 8.95 mL of triethylamine and cool to the so-formed mixture $0^\circ\text{--}5^\circ\text{C}$. Add 3.64 mL of methanesulfonyl chloride in 20 mL of CH_2Cl_2 over 10 min. Stir the so-formed mixture for 1 hour at room temperature. Cool it to $0^\circ\text{--}5^\circ\text{C}$, extract with 100 mL of cold ($0^\circ\text{--}5^\circ\text{C}$) 5% KH_2PO_4 , followed by 100 mL of cold ($0^\circ\text{--}5^\circ\text{C}$) H_2O , followed by 50 mL of brine. Dry the separated organic layer over anhydrous MgSO_4 and evaporate to obtain 13.7 g (96%) of the title $[\text{M}+\text{H}]^+$; PMR (CDCl_3) δ 7.95(s, 1), 7.82(s, 1), 7.53(m, 1), 6.81(m, 2), 4.84(d, 1), 4.65(d, 1), 4.46(m, 2), 3.05(s, 3).

EXAMPLE 6

(S)-2-(2,4-Difluorophenyl)-3-(1,2,4-triazol-1-yl)-1,2-propanediol-1-methanesulfonate

Follow the procedure of Example 5, except substitute an equivalent quantity of the product of Example 4 in place of the product of Example 3 to give the title compound. The PMR is consistent with the structure of the title compound.

EXAMPLE 7

(R)-1-[2-[2-(2,4-Difluorophenyl)]oxiranylmethyl]-1,2,4-triazole

Dissolve 13.7 g of the product of Example 5 in 200 mL of anhydrous DMF and cool the so-formed solution to $10^\circ\text{--}15^\circ\text{C}$. Add thereto 1.71 g of sodium hydride (60% oil dispersion) and stir the so-formed reaction mixture at room temperature for 90 minutes. Concentrate in vacuo to 50 mL. Add thereto 200 mL of cold H_2O ($0^\circ\text{--}5^\circ\text{C}$) and extract with 3×200 mL portions of EtOAc. Wash the combined EtOAc extracts with 100 mL of brine. Dry the EtOAc extracts over anhydrous MgSO_4 and evaporate it to give 10.8 g of a residue. Apply the residue in CH_2Cl_2 to a column of 400 g of MPLC grade silican gel previously prepared by slurry packing with CH_2Cl_2 containing 1 mL of Et_3N per liter. Elute with 1 liter, each of 25, 50 and 75% EtOAc; CH_2Cl_2 (v/v) followed by 2 liters of EtOAc. Combine the fractions to give 6.92 g (68%) of the title compound. Mass spectrum (FAB): 238 $[\text{M}+\text{H}]^+$; PMR (CDCl_3) δ 7.97(s, 1), 7.77(s, 1), 7.07(m, 1), 6.73(m, 2), 4.73(d, 1), 4.41(d, 1), 2.84(d, 1), 2.78(d, 1).

EXAMPLE 8

(S)-1-[2-[2-(2,4-difluorophenyl)]oxiranylmethyl]-1,2,4-triazole

Follow the procedure of Example 7, except substitute an equivalent amount of the product of Example 6 in place of the product of Example 5 to give the title compound. [PMR is consistent with the structure of the title compound].

EXAMPLE 9

Ethyl(5R-cis)- and (5R-trans)-5-(2,4-Difluorophenyl)-2-oxo-5-[(1H-1,2,4-triazol-1-yl)methyl]tetrahydro-3-furancarboxylate

Dissolve 9.35 mL of diethyl malonate in 70 mL of anhydrous DMSO. Add 2.24 g of sodium hydride (60% oil

dispersion) in 2 portions and stir the so-formed reaction mixture at room temperature for 1 hour. Add 6.65 g of the product of Example 7 and stir 18 hours at 50°–55° C. Cool to room temperature and pour the reaction mixture into a well-stirred mixture of 500 mL of KH_2PO_4 , 500 mL of brine, and 1 liter of EtOAc. Separate and extract the H_2O layer with another 300 mL of EtOAc. Wash the combined EtOAc extracts with 500 mL of brine. Dry the EtOAc extracts over anhydrous MgSO_4 and evaporate to give an oil. Apply the oil with CH_2Cl_2 to a column of 400 g MPLC grade silica gel prepared with hexane. Elute with 500 mL of hexane, followed by 2 liters of 50% EtOAc: hexane (v/v), followed by 2 liters of EtOAc. Combine fractions to give 8.66 g (80%) of the title compound. Mass spectrum (FAB): $352[\text{M}+\text{H}]^+$, PMR (CDCl_3) δ 8.08(s,2), 7.91(s,1), 7.71(s,1), 7.42(m,1), 7.13(m,1), 7.85(m,2), 4.60(m,4), 4.10(m,4), 3.49(t,1), 3.14(t,1), 3.89(m,4), 1.18(m,6).

EXAMPLE 10

Ethyl(5S-cis), and (5S-trans)-5-(2,4-Difluorophenyl)-2-oxo-5-(1H-1,2,4-triazol-1-yl)methyl tetrahydro-3-furancarboxylate

Follow the procedure of Example 9, except substitute an equivalent amount of the product of Example 8 in place of the product of Example 7 to give the title compound. [PMR and mass spectra are consistent with the structure of the title compound].

EXAMPLE 11

(R)-(-)-4-(2,4-Difluorophenyl)-2-hydroxymethyl-5-[1H-(1,2,4-triazol-1-yl)]-1,4-pentanediol

Dissolve 8.5 g of the product of Example 9 in 125 mL of EtOH and add 2.15 g of LiCl. Cool the stirred mixture to 0° C. and add 1.92 g of NaBH_4 in portions. Stir the mixture for 18 hr without further cooling. Add 125 mL of MeOH and 10 mL of H_2O to the mixture and stir for 4 hr. Evaporate the mixture to dryness and extract the precipitate with warm EtOH. Evaporate the extract to dryness, add 200 mL of THF to the residue, and sonicate the stirred mixture for 15 min. Filter the mixture and evaporate the filtrate. Chromatograph the residue on silica gel, eluting with CH_2Cl_2 -MeOH- NH_4OH (95:5:1) v/v/v to obtain 3.9 g of the title compound. Mass spectrum (FAB): 314 ($\text{M}+\text{H}^+$); PMR (DMSO) δ 8.25(s,1), 7.69(s,1), 7.35(m,1), 7.13(m,1), 6.94(m,1), 6.27(s,1), 5.16(m,1), 4.44(m,4), 3.39(m,1), 3.20(m,1), 3.05(t,2), 2.11(m,1), 1.52(m,1).

EXAMPLE 12

(S)-(+)-4-(2,4-Difluorophenyl)-2-hydroxymethyl-5-[1H-(1,2,4-triazolyl)]-1,4-pentanediol

Follow the procedure of Example 11, except substitute an equivalent amount of the product of Example 10 in place of the product of Example 9 to give the title compound. Chromatograph a portion of the crude product on silica gel eluting with CH_2Cl_2 -MeOH- NH_4OH to give a product homogeneous by TLC. Dissolve the material in H_2O and filter, and lyophilize the filtrate to give the title compound. $[\alpha]_D^{25}+54.50$ ($c=1.0$, MeOH)

EXAMPLE 13

(R)-(-)-4-(2,4-Difluorophenyl)-2-[[4-(4-methylphenyl)-sulfonyloxy]methyl]-5-[1H-(1,2,4-triazolyl)]-1,4-pentanediol-1-(4-methylbenzene)sulfonate

Dissolve 4.4 g of the product of Example 11 in 50 mL of CH_2Cl_2 -THF (1:1, v/v). Add 4.7 mL of Et_3N and 180 mg of

N,N -dimethylaminopyridine, and cool the solution to 0° C. Add thereto 5.9 g of p -toluenesulfonyl chloride in portions and stir the so-formed reaction mixture at 0° C. for ½ hour, and then stir it at room temperature for 5 hours. Add 100 mL of EtOAc and suction-filter the mixture. Concentrate the filtrate; add thereto 150 mL of EtOAc, and wash with 5% aqueous KH_2PO_4 . Wash the organic layer with cold aqueous 5% NaHCO_3 , then with saturated brine, and then dry it over anhydrous MgSO_4 . Filter the mixture, and evaporate the filtrate. Chromatograph the residue on silica gel, eluting with EtOAc-hexane to give 6.4 g (73%) of the title compound, PMR (CDCl_3) δ 7.95(s,1), 7.67(m,5), 7.30(m,6), 6.70(t,2), 4.74(d,1), 4.53(d,1), 4.13(m,1), 3.97(m,1), 3.8(m,2), 2.43(s,6), 1.95(m,2), 1.77(m,1). Mass spectrum (FAB): $622[\text{M}+\text{H}]^+$.

EXAMPLE 14

(S)-(+)-4-(2,4-Difluorophenyl)-2-[[4-(4-methylphenyl)-sulfonyloxy]methyl]-5-[1H-(1,2,4-triazolyl)]-1,4-pentanediol-1-(4-methylbenzene)sulfonate

Follow the procedure of Example 13 except substitute an equivalent amount of the product of Example 12 in place of the product of Example 11 to obtain the title compound, $[\alpha]_D^{25}+14.2^\circ$ ($c=1$, MeOH).

EXAMPLE 15

(-)-(5R-cis)-5-(2,4-Difluorophenyl)-5-[1H-(1,2,4-triazol-1-yl)methyl]-tetrahydro-3-furanmethanol,4-toluenesulphonate

Dissolve 6.3 g of the product of Example 13 in 150 mL of toluene and heat the so-formed solution to 100° C. Add 2.4 g of 60% NaH dispersion in oil portionwise, and then heat the so-formed reaction mixture at reflux until cyclization is complete (approx. 3–4 hours). Cool the mixture and decant the solution from excess NaH. Wash the solution with cold 5% aqueous KH_2PO_4 . Evaporate the organic layer to form a residue and chromatograph the residue on silica gel, eluting with acetone-hexane to obtain 1.6 g (35%) of the title compound as the less polar of the two products, $[\alpha]_D^{25}-39.4^\circ$ ($c=1$, CHCl_3); PMR (CDCl_3) δ 8.09 (s,1), 7.88 (m,3), 7.31 (m,3), 6.81(m,2), 4.52(ABq,2), 3.99(m,1), 3.85(m,1), 3.70(m,1), 3.59(m,1), 2.49(m,2), 2.47(s,3), 1.90(m,1). Mass spectrum (FAD): 450 ($\text{M}+\text{H}^+$).

EXAMPLE 16

(+)-(5S-cis)-5-(2,4-Difluorophenyl)-5-[1H-(1,2,4-triazol-1-yl)methyl]-tetrahydro-3-furanmethanol,4-toluenesulphonate

Follow the procedure of Example 15, except substitute an equivalent amount of the product of Example 14 in place of the product of Example 13 to give the title compound, $[\alpha]_D^{25}+40.3^\circ$ ($c=0.3$, CHCl_3), mp 96°–98° C.

EXAMPLE 17

(-)-[(2R)-cis]-4-[4-[4-[5-(2,4-difluorophenyl)-tetrahydro-5-(1H-1,2,4-triazol-1-yl)methyl]furan-3-yl]methoxy]phenyl-1-piperazinyl]phenyl-2,4-dihydro-3H-1,2,4-triazol-3-one

The title compound is prepared starting with the tosylate of Example 15 and 4-[4-(4-nitrophenyl)-1-piperazinyl]phenol (Example 3a of U.S. Pat. No. 4,791,111) and using the synthetic scheme outlined in Scheme V and J. Heeres, et al., *J. Med. Chem* 1984, Vol 27, p894–900 at 898 and 900.

EXAMPLE 18

(-)-[(2R)-cis]-4-[4-[4-[[5-(2,4-Difluorophenyl)-Tetrahydro-5-(1H-1,2,4-Triazol-1-ylmethyl)-3-Furanyl]Methoxy]Phenyl]-1-Piperazinyl]Phenyl]-2,4-Dihydro-2-[1(S)-Methyl-2(R)-Hydroxypropyl]-3H-1,2,4-Triazol-3-One

a. 2-O-SEM Ether of (2R,3R)-2,3-Butanediol

To a stirred solution of 4.95 g of (2R, 3R)-2,3-butanediol (55 mmoles) and 9.3 g of SEM-Cl (55.7 mmoles) in 55 ml of anhydrous DMF at 0° C. were added in four portions 2.34 g of 60% oil-dispersed NaH (58.5 mmoles) over 10 min. The resulting mixture was stirred at 0° C. for 4 hours and at ambient temperature overnight. The turbid reaction mixture was poured onto 0.5 L of 5% KH_2PO_4 solution and extracted with 2×300 ml of ether; the combined ethereal solution was washed once with distilled water, saturated brine, dried over MgSO_4 and evaporated to give a colorless liquid. Flash chromatography over 350 g silica gel with 1 L of 7% ETOAC/Hexane, 2 L of 10% ETOAC/Hexane and 1 L of 15% ETOAC/Hexane gave 1.74 g of the title compound (yield 14.4%) MS:(M+H)⁺=221.

Brosylation

A mixture of 0.7 g of the 2-O-SEM ether of Example 18(a), (3.18 mmoles) and 0.97 g of 4-bromobenzenesulfonyl chloride (3.82 mmoles) in 5 ml of anhydrous pyridine was stirred under N_2 atmosphere at ambient temperature for 6 hours. The reddish slurry reaction mixture was diluted with 50 ml of ice-cold water, extracted with 2×25 ml of ether. The combined ethereal solution was washed with 2×25 ml of 1% CuSO_4 solution, distilled water, saturated brine, dried over MgSO_4 and evaporated to give a reddish oily residue. Flash chromatography over 50 g silica gel with 1 L of 10% ETOAC/Hexane gave 1.02 g of the brosylate as a colorless liquid (yield 72.9%) $[\alpha]_D^{23} = -3.69^\circ$ (CHCl_3 ; c=1)

c. Alkylation Reaction

A mixture of 0.98 g of the brosylate of Example 18(b) (2.23 mmoles), 0.69 g of the 3H-1,2,4-triazol-3-one of Example 17 (1.12 mmoles) and 0.37 g of cesium carbonate (1.12 mmoles) in 20 ml of anhydrous DMF was stirred at 80° C. under N_2 overnight (~20 hours). The reaction mixture was diluted with 100 ml of ice-cold water, extracted with 2×50 ml of ethyl acetate. The combined organic solution was washed once with distilled water, saturated brine, dried over MgSO_4 and evaporated to give a brown solid residue. Flash chromatography of the residue over 125 g silica gel with 1.2 L of 80% ETOAC/Hexane gave 0.327 g of the product as a tan solid (yield 35.7%) MS=(M+H)⁺=81.7.

d. Acidic Hydrolysis of 18(c) to the title product

A mixture of 0.32 g of the SEM-ether of Example 18(c) and 6 ml of 6N HCl solution in 6 ml of methanol was stirred at ambient temperature for 4 hours and was evaporated under reduced pressure. The residue was diluted with 5 ml of ice water, carefully basified with 10% Na_2CO_3 solution until pH=8-9 was obtained. Extraction of the so-formed reaction mixture with 2×25 ml of CH_2Cl_2 followed by washing with saturated brine, drying over MgSO_4 and evaporation gave a tan solid. Filtration of the tan solid through a 50 g silica gel column and elution with 0.75 L of 4% MeOH/ CH_2Cl_2 gave 0.26 g of title product as a tan solid, yield 96.6%. MS=(M+H)⁺=687; $[\alpha]_D^{23} = -23.65^\circ$ (CHCl_3 ; c=1)

EXAMPLE 19

(-)-[(2R)-cis]-4-[4-[4-[[5-(2,4-Difluorophenyl)-Tetrahydro-5-(1H-1,2,4-Triazol-1-ylmethyl)-3-Furanyl]Methoxy]Phenyl]-1-Piperazinyl]Phenyl]-2,4-Dihydro-2-[1(R)-Methyl-2(R)-Hydroxypropyl]-3H-1,2,4-Triazol-3-one

a. Mitsunobu Reaction

To a stirred solution of 0.72 g of the 2-O-SEM ether of Example 18(a) (3.27 mmoles), 2.1 g of triphenyl phosphine (8.06 g) and 1.2 g of p-nitrobenzoic acid (7.17 mmoles) in 30 ml of dry benzene at 0° C. were added, dropwise, 1.25 ml (8.06 mmoles) of diethyl azodicarboxylate ("DEAD"). The so-formed clear yellow solution became turbid and the mixture was stirred at ambient temperature for 2 hours, and mixture loaded on a 100 g silica gel column. Elution of the column with 15% ETOAC/Hexane gave 1.5 g of the 3-β-nitrobenzoate having the S absolute configuration (95% yield) MS: 219 (M⁺-150), 252 (M⁺-117).

b. Basic Hydrolysis of the D-Nitrobenzoate

A solution of 1.12 g of the p-nitrobenzoate of Example 19(a) (3 mmoles) and 3.5 ml of 1N NaOH solution in 20 ml of methanol was stirred at ambient temperature for 3 hours. Solvents were evaporated and the residue was diluted with 10 ml of distilled water, and extracted with 2×20 ml of ether. The combined ethereal solution was washed once with saturated brine, dried over MgSO_4 and evaporated to give 0.67 g of the corresponding alcohol as a colorless liquid (~100%), which was used directly for the next reaction without further purification.

c. Brosylation, Alkylation and Acidic Hydrolysis

Following the procedures of Example 18(c) and (d), the title compound was prepared in 32% overall yield in 3 steps from the products of Example 19(b) and of Example 17. MS: [M+H]⁺=687; $[\alpha]_D^{23} = -23.65^\circ$ (CHCl_3 ; c=1)

EXAMPLE 20

(-)-[(2R)-cis]-4-[4-[4-[[5-(2,4-Difluorophenyl)-Tetrahydro-5-(1H-1,2,4-Triazol-1-ylmethyl)-3-Furanyl]Methoxy]Phenyl]-1-Piperazinyl]Phenyl]-2,4-Dihydro-2-[1(S)-Methyl-3-Hydroxypropyl]-3H-1,2,4-Triazol-3-one

a. Formation of TBDPS Ether

To a solution of 0.9 g or (R)-(-)-1,3-butanediol (10 mmoles), 1.5 g of imidazole (22 mmoles) in 10 ml of anhydrous DMF at 0° C. were added 3 ml of t-butylchlorodiphenylsilane ("TBDPS") (11 mmoles) over 3 minutes. The reaction mixture was stirred at 0° C. for 4 hours, diluted with 50 ml of ice-cold water and extracted with 2×30 ml of ether. The aqueous phase was back extracted with 50 ml of ether and the combined ethereal solution was washed once with saturated brine, dried over MgSO_4 and evaporated to give a colorless residue. Flash chromatography over 150 g silica gel with 1.5 L of 5% EtOAC/Hexane and 1 L of 10% EtOAC/Hexane gave 2.87 g of the TBDPS ether (87.5%) MS: [M+H]⁺: 329; $[\alpha]_D^{23} = +0.64^\circ$ (CHCl_3 ; c=1)

b. Brosylation

To a solution of 0.984 g of TBDPS ether of Example 20(a) (3 mmoles) in 7 ml of anhydrous pyridine were added 0.845 g of 4-bromobenzenesulfonyl chloride (3.3 mmoles). The reaction was run and worked-up and purified in accordance with the procedure of Example 18(b) and 1.02 g of the brosylate was obtained in 61.1% yield; MS: [M+23]⁺=569/571; $[\alpha]_D^{23} = +2.45^\circ$ (CHCl_3 ; c=1)

c. Alkylation

The brosylate of Example 20(b), 0.959 (1.74 mmoles) was reacted with the compound of Example 17 according to the procedure of Example 18(c) to provide 0.499 g of corresponding alkylated product, yield 60.3% MS: (M+H)⁺925 [α]_D²³ = -32.27° (CHCl₃; c=1)

d. Acidic Hydrolysis

The compound of Example 20(c), 0.329 g, (0.35 mmoles) was hydrolyzed by 6N HCl solution in accordance with the procedure of Example 18(d) to give 0.229 g of the title compound (yield 92.4%); MS: M⁺=686; [M+Na]⁺=709; [α]_D²³ = -38.52° (CHCl₃; c=1).

Alternatively a solution of 0.19 g of the compound of Example 20(c) and 60 mg of tetrabutylammonium fluoride (0.23 mmoles) in 5 ml of THF was stirred at ambient temperature for 24 hours. The brown solution was concentrated to a syrup. Flash chromatography of the syrup over 50 g silica gel with 0.5 L each of 2% and 4% MeOH/CH₂Cl₂, gave 0.11 g of the title compound (yield 88.7%).

EXAMPLE 21

(-)-[(2R)-cis]-4-[4-[4-[5-(2,4-Difluorophenyl)-Tetrahydro-5-(1H-1,2,4-Triazol-1-ylmethyl)-3-Furanyl]Methoxy]Phenyl]-1-Piperazinyl]Phenyl]-2,4-Dihydro-2-[(R)-1-Methyl-3-Hydroxypropyl]-3H-1,2,4-Triazol-3-one

The procedures of Example 20 were followed except an equivalent amount of S-(+)-1,3-butanediol was substituted for the corresponding R enantiomer. An overall 31.8% yield of the title compound was obtained in four steps; MS=[M+H]⁺=687.

EXAMPLE 22

(-)-[(2R)-cis]-4-[4-[4-[5-(2,4-Difluorophenyl)-Tetrahydro-5-(1H-1,2,4-Triazol-1-ylmethyl)-3-Furanyl]Methoxy]Phenyl]-1-Piperazinyl]Phenyl]-2,4-Dihydro-2-[1(S)-Methyl-2(S)-Hydroxypropyl]-3H-1,2,4-Triazol-3-one

a. Benzlation

To a solution of 10 g of (2R, 3R)-(-)-2,3-butanediol (111 mmoles) in 40 ml of anhydrous CH₂Cl₂ and 80 ml of cyclohexane at 0° C. were added 1 ml of trifluoromethanesulfonic acid (TfOH), followed by dropwise addition of 21 ml of benzyl trichloroacetimidate (113 mmoles). The resulting slurry was stirred at ambient temperature overnight, diluted with 125 ml of hexane and filtered. The combined filtrate was concentrated to a yellow syrup. Flash chromatography of the yellow syrup over 250 g silica gel with 1.5 L of 7% ETOAC/Hexane, 2 L of 15% ETOAC/Hexane and 2 L of 25% ETOAC/Hexane, 1.5 L of 10% MeOH/CH₂Cl₂ gave 11.88 g of the 2-monobenzyl ether of the starting material (74.5% yield) and 2.03 g of unreacted starting material MS: [M+H]⁺: 181.

b. Mitsunobu Reaction

The 2-monobenzyl ether of Example 22(a), 5.4 g, was converted into 6.6 g of the 3-benzoate ester (yield 66.9%) by Mitsunobu reaction in accordance with the procedure of Example 19(a); MS: [M+H]⁺=330.

c. Alkaline Hydrolysis

The 5.3 g of the product of Example 22(b) was subjected to alkaline hydrolysis according to the procedure of Example 19(b) to give 2.33 g of the 2-monobenzyl ether of (2R,3S)-2,3-butanediol (yield 80.3%) (M+H)⁺=181; [α]_D²³ = -23.75° (CHCl₃, c=1)

d. Formation of the SEM Ether

To a stirred solution of 3.14 g of the product of Example 22(c) (17.44 mmoles) and 3.8 ml of di-isopropylethylamine (2.82 g, 21.8 mmoles) in 30 ml of anhydrous CH₂Cl₂ at ambient temperature were added 3.8 ml of SEM-Cl (3.64 g, 21.8 mmoles) in one portion. Fuming formed and the resulting yellow solution was stirred for 20 hours. The orange-colored reaction mixture was evaporated under reduced pressure and the solid residues were partitioned between ether and water. The ethereal solution was washed once with distilled water, saturated brine, dried over mg 504 and concentrated to give the crude product. Flash chromatography of the crude product over 200 g silica gel with 2 L of 3% ETOAC/Hexane gave 5.3 g of the 3-O-SEM ether of the product of Example 22(c) (98% yield) as a colorless liquid; MS: [M+H]⁺=311.

e. Hydrogenolysis

A mixture of 5.25 g of the product of Example 22(d) (16.94 mmoles) and 0.5 g of 10% Pd/C in 150 ml of methanol was hydrogenated under atmospheric pressure for 6 hours. Catalysts were filtered and washed with additional methanol. The combined filtrate was concentrated to give a colorless liquid. Flash chromatography of the liquid over 100 g silica gel with 2 L of 10% ETOAC/hexane 3.53 g of the free alcohol (yield 95%) as a colorless liquid; MS: 174, 103.

f. Brosylation

The product of Example 22(e) 1 g was converted into 1.52 g of the corresponding brosylate in 76.2% yield in accordance with the procedure of 18(b); [α]_D²³ = -1.53° (CHCl₃; c=1)

g. Alkylation Reaction

The brosylate of Example 22(f), 1.48 g of was reacted with the product of Example 17 to give 0.75 g of the 2-alkylated triazol-3-one (yield 54.3%); [α]_D²³ = -32.69° (CHCl₃; c=1)

h. Acidic Hydrolysis

Hydrolysis of 0.7 g of the product of Example 22(g) in accordance with the procedure of Example 18(d) gave 0.51 g of the title compound as a cream-colored solid (yield 86.7%); [α]_D²³ = -32.69° (CHCl₃; c=1)

EXAMPLE 23

(-)-[(2R)-cis]-4-[4-[4-[5-(2,4-Difluorophenyl)-Tetrahydro-5-(1H-1,2,4-Triazol-1-ylmethyl)-3-Furanyl]Methoxy]Phenyl]-1-Piperazinyl]Phenyl]-2,4-Dihydro-2-[1(R)-Methyl-2(S)-Hydroxypropyl]-3H-1,2,4-Triazol-3-one

a. Mitsunobu Reaction

The product of step e of Example 22 (1.99 g, 9.05 mmoles) was reacted with p-nitrobenzoic acid in accordance with the procedure Example 19(a) to give 3.3 g of product (yield 98.8%); MS=[M+H]⁺=221.

b. Alkaline Hydrolysis

The product of step (a) of this Example (2.36 g, 6.4 mmoles) was hydrolyzed by 7 ml of 1N NaOAc to give 1.18 g of the 3-O-SEM ether of (2S,3S)-2,3-butanediol (yield 83.7%). MS: [M+H]⁺=221 [α]_D²³ = +55.15° (CHCl₃; C=1)

c. Brosylate Formation

The product of step (b) of this Example (1.15 g were converted into the brosylate in accordance with the procedure of Example 18(b) to give 3.47 g of the brosylate (yield 97.7%).

d. Alkylation and Acidic Hydrolysis

The procedures of Example 18(c) and (d) were followed except the product of Example 23(c) was substituted for that of 18(b) to give the title compound.

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EXAMPLE 24

(2R-cis)-4-[4-[4-[[5-(2,4-difluorophenyl)-
tetrahydro-5-(1H-1,2,4-triazol-1-ylmethyl)furan-3-
yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-
dihydro-2-[(S)-1-ethyl-2(S)-hydroxypropyl]-3H-1,2,
4-Triazol-3-One

a. The methyl ester of (S)-lactic acid was converted into the corresponding benzyloxymethyl ether in accordance with the procedure of W. C. Still, et al. *Tetrahedron Letters*, 21, 1035-1038 (1980).

b. Reduction to the Aldehyde

DIBAL-H, 37.7 ml of a 1M solution, was added dropwise to a stirred solution of 7.67 g of the ester of step (a) of this Example in toluene at -78° C. (dry ice/acetone bath) under an atmosphere of nitrogen. After 6 min. methanol (10 ml) followed by an aqueous solution of Rochelles salt were added. After warming to room temperature the moisture was partitioned between ETOAc and water. The organic phase was separated, washed with water, dried (MgSO₄) and concentrated to produce the crude aldehyde which was used in the next step without purification.

b. Grignard Step

The THF solution of 80 ml of 1 molar solution of the ethyl magnesium bromide Grignard reagent was added dropwise to a stirred THF solution of the crude aldehyde obtained from step (b) of this Example at -78° C. (dry ice/acetone bath) under an atmosphere of nitrogen. After the addition was complete, the resulting mixture was allowed to warm slowly to room temperature overnight and stirred for a further period of 48 h. An aqueous solution of Rochelles salt was added and then the resulting mixture was partitioned between acetone and water. The organic phase was separated, washed with water, dried (MgSO₄) and concentrated. The residue was purified by column chromatography on silica gel using ETOAc/Hexane (1:10) as eluant to give (i) non-polar alcohol (2S,3S) 2.31 g; 31%, as a colorless oil.

(ii) a mixture of both alcohols, 1.23 g; 41% and

(iii) polar alcohol (2S,3R) 1.23 g; 16%, as a colorless oil.

c. Brosylation of polar alcohol

4-Bromobenzenesulphonyl chloride (1.035 g, 4.1 mmoles) was added to a stirred solution of (0.605 g, 2.7 mmoles) the polar (2S, 3R) alcohol of step (b) of this Example and 2.20 g (5.9 mmoles) of DMAP in CH₂Cl₂ at room temperature under an atmosphere of nitrogen. The resulting mixture was stirred for 12 h. and then partitioned between ETOAc and water. The organic phase was separated, washed with water, dried and concentrated. The residue was purified by column chromatography on silica gel using ETOAc/Hexane (1:10) as eluant to give the desired brosylate (85%) as a colorless oil.

d. Alkylation and acidic hydrolysis

The procedures of Example 18(c) and (d) were followed except the (2S, 3R) brosylate of step (c) of this Example was substituted for that used in Example 18(c). The acidic hydrolysis produced the title compound as a white solid, mp 170°-172° C.

EXAMPLE 25

(2R-cis)-4-[4-[4-[4-[[5-(2,4-difluorophenyl)-
tetrahydro-5-(1H-1,2,4-triazol-1-ylmethyl)furan-3-
yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-
dihydro-2-[(R)-1-ethyl-2(S)-hydroxypropyl]-3H-1,2,
4-triazol-3-one

The procedures of Example 24 were followed except the non-polar (2S,3S) alcohol from step (b) of Example 24 was

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converted into the (2S,3S)-3-brosylate. Alkylation of the brosylate followed by acidic hydrolysis of the SEM protecting group in accordance with the procedures of Example 24(d) provided the title compound.

EXAMPLE 26

(2R-cis)-4-[4-[4-[4-[[5-(2,4-difluorophenyl)-
tetrahydro-5-(1H-1,2,4-triazol-1-ylmethyl)furan-3-
yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-
dihydro-2-[(R)-1-ethyl-2(R)-hydroxypropyl]-3H-1,2,
4-triazol-3-One

The procedures of Example 24 were followed except the methyl ester of (R) lactic ester was substituted for the methyl ester of (S)-lactic acid in step (a) of Example 24. The (2R, 3S) alcohol was used in steps (c) and (d) to provide the title compound.

EXAMPLE 27

(2R-cis)-4-[4-[4-[4-[[5-(2,4-difluorophenyl)-
tetrahydro-5-(1H-1,2,4-triazol-1-ylmethyl)furan-3-
yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-
dihydro-2-[(S)-1-ethyl-2(R)-hydroxypropyl]-3H-1,
2,4-triazol-3-One

The procedures of Example 26 were followed except the (2R, 3R) alcohol was used in steps (c) and (d) to provide the title compound.

EXAMPLE 28

(2R-cis)-4-[4-[4-[4-[[5-(2,4-difluorophenyl)-
tetrahydro-5-(1H-1,2,4-triazol-1-ylmethyl)furan-3-
yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-
dihydro-2-[(R)-1-ethyl-3-hydroxypropyl]-3H-1,2,4-
triazol-3-One

a. Reduction

To methyl (3R)-hydroxyvalerate (5.289, 40.0 mmoles) dissolved in 100 ml of anhydrous THF at 0°-5° C. was added dropwise 60 ml of a 1M THF solution of LiAlH₄ (60 mmoles). The solution was allowed to warm to ambient temperature and to the so-formed mixture was added sequentially: 2.5 mL of water, dropwise, 2.5mL of 15% NaOH and 7.5mL of water. The so-formed reaction mixture was stirred at ambient temperature for 4 h. The inorganic solids were removed by filtration and the filtrate was evaporated to give 4.31 g of (3R)-1,3-pentanediol.

b. 1-O-SEM ether formation

The procedure of Example 18(a) was followed except an equivalent quantity of the product of step (a) of this Example was substituted for the (2R, 3R)-2,3-butanediol to provide the title compound.

c. Mitsunobu Reaction

The procedure of Example 19(a) was followed except that an equivalent quantity of the product of step (b) of this Example was substituted for the 2-SEM ether of (2R,3R)-2,3-butanediol to give 3.34 g of the corresponding p-nitrobenzoate.

d. Basic Hydrolysis

The procedure of Example 19(b) was followed except that an equivalent quantity of the p-nitrobenzoate ester of step (c) of this Example was used to provide 1.88 g of the 1-O-SEM ether of (3S)-1,3-pentanediol.

e. Brosylation, Alkylation and Acid Hydrolysis

The procedures of Example 18 (b), (c), and (d) were followed except that an equivalent quantity of the product of

step (d) of this Example was substituted for the corresponding 1-O-SEM ether of (2R, 3R) 2,3-butanediol used in Example 19(b) to produce 1.04 g of the title compound of this Example $[\alpha]_D^{25} = -8.42^\circ$ (CHCl_3 ; $c=1$)

EXAMPLE 29

(2R-cis)-4-[4-[4-[[5-(2,4-difluorophenyl)-tetrahydro-5-(1H-1,2,4-triazol-1-ylmethyl)furan-3-yl]methoxy]phenyl]1-piperazinyl]phenyl]-2,4-dihydro-2-[(S)-1-ethyl-3-hydroxypropyl]-3H-1,2,4-triazol-3-One

The procedures (a) and (b) of Example 28 were followed to produce the 1-O-SEM-(3R)-1,3-pentanediol which was converted directly into the 3R brosylate by following the procedures of Example 18(b). The 3R brosylate was used to alkylate the product of Example 17 in accordance with the procedures of Example 18(c). The so-formed product was subjected to acidic hydrolysis in accordance with the procedures of Example 18(d) to provide 368mg (90% yield) of the title compound; $[\alpha]_D^{25} = -47.11^\circ$ (CHCl_3 ; $c=1$)

EXAMPLE 30

(2R-cis)-4-[4-[4-[[5-(2,4-difluorophenyl)-tetrahydro-5-(1H-1,2,4-triazol-1-ylmethyl)-3-furanyl]methoxy]phenyl]1-piperazinyl]phenyl]-2,4-dihydro-2-[1-hydroxy-(2R)-butyl]-3H-1,2,4-triazol-3-One

a. Preparation of (2S)-1,2,-butanediol

A solution of (2S)-3-butene-1,2-diol which was purchased from Eastman Kodak, (3 g, 0.034 mmoles) in 40 mL of ethanol was hydrogenated in the presence of 300 mg of 10% Pd/C overnight. The so-formed reaction mixture was filtered through celite. The so-formed filter cake was washed with ethanol and the combined filtrates were evaporated to provide 2.08 g (68% yield) of the title compound.

b. 1-O-SEM ether formation, brosylaton, alkylation and acidic hydrolysis

The procedures of Example 18(a)-(d) were followed except that an equivalent amount of the product of step (a) of this Example was substituted for the (2R, 3R) 2,3-butanediol of Example 18 to provide the title compound $[\alpha]_D^{25} = -24.3^\circ$ (CHCl_3 ; $c=1$)

EXAMPLE 31

(2R-cis)-4-[4-[4-[[5-(2,4-difluorophenyl)-tetrahydro-5-(1H-1,2,4-triazol-1-ylmethyl)-3-furanyl]methoxy]phenyl]1-piperazinyl]phenyl]-2,4-dihydro-2-[1-hydroxy(2S)-butyl]-3H-1,2,4-triazol-3-one

The procedures of Example 30 were followed except that an equivalent quantity of (2R)-3-butene-1,2-diol (available from Eastman Kodak) was substituted for (2S)-3-butene-1,2-diol in step (a) of Example 30. The procedures of Example 30(b) were there after followed to produce the title compound $[\alpha]_D^{25} = -29.4^\circ$ (CHCl_3 ; $c=1$)

EXAMPLE 32

(-)(2R-cis)-4-[4-[4-[[5-(2,4-difluorophenyl)-tetrahydro-5-(1H-1,2,4-triazol-1-ylmethyl)furan-3-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-[(S)-1-ethyl-2(S)-hydroxypropyl]-3H-1,2,4-triazol-3-one

a. (S)-2-(benzyloxy) propionaldehyde by selective reduction of (S)-(O-benzyl)lactic acid pyrrolidine amide: To a

solution of the S-(O-benzyl)lactic acid pyrrolidine amide prepared in accordance with the procedure of *Tetrahedron*, 1989, vol. 45, pages 57-67 (5 g, 0.0214 mol.) dissolved in 20 ml of toluene cooled to in a ice methanol bath was added slowly with stirring 4.25 ml of RED-AL (3.4M solution of sodium bis(2-methoxyethoxy) aluminum hydride) in toluene available from Aldrich Chemical Catalogue #19, 619-3). The solution was stirred for 5 hrs., quenched with 2.5 ml of acetone and thereafter with 35 ml of 2NHCl. The so-formed mixture was extracted with EtOAc. The organic extracts were washed with water, NaHCO_3 and brine, dried over Na_2SO_4 and evaporated to give the titled product.

b. (S)-2-(Benzyloxy)-N-(Formylamino) propanimine. The propionaldehyde of step (a) (1 g, 16.09 mmol) was added dropwise to a solution of formyl hydrazine (0.73 g, 12.18 mmol) dissolved in 5 ml of methanol. The so-formed reaction mixture was stirred overnight. The solvent was removed by evaporation and the so-formed residue was stirred with ethyl ether. The undissolved excess formyl hydrazine was removed by filtration and the ether was removed to provide a residue which was chromatographed on silica gel(f) using 20% EtOAc: hexane (v:v) to give 805 mg of the title product as a light yellow waxy solid having strong UV activity; $\text{ms}[\text{M}+\text{H}]^+ = 207$.

c. 2-[3-(2S, 3S)-2-(Benzyloxy)pentyl]formic acid hydrazide Ethylmagnesium bromide (1.3 ml, 3.9 mmol, 3.0 molar in ethyl ether) was added to a stirred solution of 200 mg, 0.97 mmol of the propanimine of step (b) in 10 ml of ethyl ether at 0°C . The so-formed reaction mixture was stirred overnight at room temperature and quenched with water. The organic layer was separated and the solvent removed to provide a residue which was chromatographed on silica gel using 30 to 50% of EtOAc:hexane (v:v) to provide 113 mg; (50 % yield) of the title compound as an oil. The ratio of S,S isomer: S,R isomer in the product was 94:6. When the reaction was repeated in the presence of 1.2 equivalent of bis(trimethylsilyl) acetamide the S,S:S,R ratio improved to 99:1 MS: $[\text{M}+\text{H}]^+ = 237$

d. Cyclization Reaction

A solution of 156.3 mg, 0.66 mmol of the product of step (c) and 400 mg 0.60 mmol of 17F of Scheme V and 1 mole of DBU (1,8-diaza bicyclo [5.4.0]undec-7-ene) in volume was stirred at 80°C for six hours; the temperature was raised to 100° to 110°C and stirring was continued at this temperature overnight. The reaction mixture was allowed to cool to room temperature and the stirring was continued over the weekend. The solvent was removed by evaporation and the crude product was purified on preparative TLC (80% EtOAc) hexane, v:v) to provide 200 mg of the benzyl ether of the title product of this example as a foamy solid; MS: $[\text{M}+\text{H}]^+ = 792$ This cyclization reaction is the invention of Mergelsberg, Gala et. al. which is disclosed in commonly-owned U.S. patent application Ser. No. (attorney's Docket #CD0475), filed Apr. 19, 1995.

e. Hydrogenolysis

To the solution of the benzyl ether (190 mgs, 0.24 mmol) of step d dissolved in 10 ml of methanol was added 40 mg of Pd black on carbon and 4 ml of formic acid. The reaction flask was sealed with a balloon and heated at 60°C for four hours. The catalyst was removed by filtration through a celite cake and the filtrate was poured into cold water. The pH of the so-formed solution was adjusted to a value of 4 to 5 with ammonia. The so-formed mixture was extracted with EtOAc. The organic layer was separated and dried over Na_2SO_4 . The solvent was removed to provide a crude product which was purified on preparative TLC (5% methanol: CH_2Cl_2 , v:v) to give 95 mg of the title compound of

73

this example. (57% yield) as a tan solid. MS: $[M+H]^+=701$. $[\alpha]_D^{25}=-28.4$ (c, =1.0, $CHCl_3$)

EXAMPLE 33.

(-)-[(2R)-cis]-4-[4-[4-[4-[5-(2,4-Difluorophenyl)-Tetrahydro-5-(1H-1,2,4-Triazol-1-ylmethyl)-3-Furanyl]Methoxy]Phenyl]-1-Piperazinyl]Phenyl]-2,4-Dihydro-2-[1(S)-Ethyl-2(S)-Hydroxypropyl]-3H-1,2,4-Triazol-3-one, Ester with Glycine (as Hydrochloride);

A. To a solution of N-Cbz-glycine (315 mg), N,N-dimethylaminopyridine (DMAP, 200 mg), and compound of Example 24 (900 mg) in CH_2Cl_2 (50 mL) at 0° C., add dicyclohexylcarbodiimide (DCCD, 290 mg). Stir the solution at 0° C. for 30 min., then at room temperature for 1 hr. Add additional N-Cbz-glycine (700 mg) and then increments of DCCD at 20 min. intervals until the reaction is

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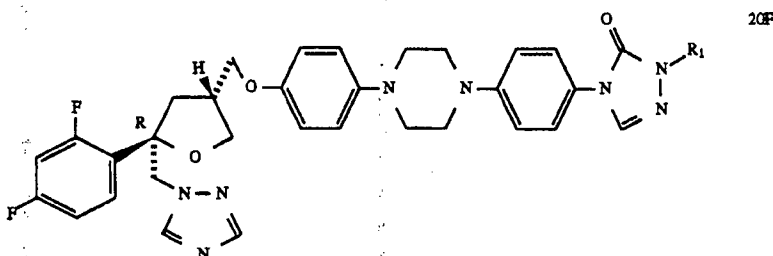
EXAMPLE 36

(-)-(2R)-cis-4-[4-[4-[4-[5-(2,4-Difluorophenyl)-Tetrahydro-5-(1H-1,2,4-Triazol-1-ylmethyl)-3-Furanyl]Methoxy]Phenyl]-1-Piperazinyl]Phenyl]-2,4-Dihydro-2-[1(S)-Ethyl-2(S)-Hydroxypropyl]-3H-1,2,4-Triazol-3-one, Ester with L-Alanins (as Hydrochloride salt)

¹⁰ Follow the procedure of Example 33 except substitute an equivalent quantity of N-carbobenzoxy-L-alanine for N-Cbz-glycine to obtain the title compound.

EXAMPLE 37

The preferred compounds of formula 20F prepared in accordance with the procedures of Examples 1-32 and Schemes I-VI are listed hereinbelow:



complete by TLC. Pour the reaction mixture into 5% aqueous KH_2PO_4 and extract with EtOAc. Wash the EtOAc extracts three times with 5% aqueous KH_2PO_4 , then with brine, and dry the extracts over anhydrous MgSO_4 . Filter, evaporate the filtrate, and chromatograph the residue to obtain the N-Cbz- γ -glycyl ester (1.3 g). [Mass spec. found: (FAB) 892 ($\text{M}+\text{H}^+$).]

B. Stir a solution of the N-Cbz-glycyl ester of step A above in 100 mL MeOH-96% HCOOH (10:1) in sealed flask with a safety valve. Add 30 mg increments of palladium black at 30 min intervals until the reaction is complete by TLC (6-14 hr). Suction-filter the mixture, add 12N HCl (0.5 mL) to the filtrate and evaporate the so-formed mixture to dryness. Add water (100 mL) and activated carbon (0.8 g) to the residue, suction-filter on a 0.45 μ nylon membrane. Lyophilize the filtrate to provide 356 mg of the title compound. [Mass spec. found: (FAB) 795 (M+H⁺).]

EXAMPLE 34

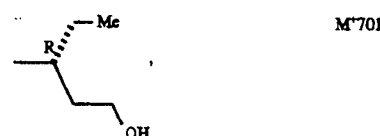
Follow the procedure of Example 33 except substitute an equivalent amount of any other N-carbenzoxycarbonyl or N-tert-butoxycarbonyl protected natural amino acid to obtain the corresponding natural α -amino acid ester hydrochloride of the compound of Example 32.

EXAMPLE 35

(-)-[(2R)-cis]-4-[4-[4-[5-(2,4-Difluorophenyl)-Tetrahydro-5-(1H-1,2,4-Triazol-1-ylmethyl)-3-Furanyl]Methoxy]Phenyl]-1-Piperazinyl]Phenyl]-2,4-Dihydro-2-[1 (S)-Ethyl-2(S)-Hydroxypropyl]-3H-1,2,4-Triazol-3-one, Ester with 2,4-Diaminobutanoic acid (as Di-Hydrochloride salt)

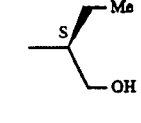
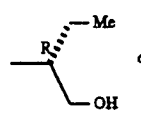
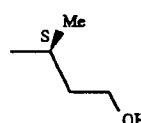
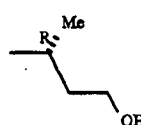
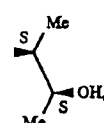
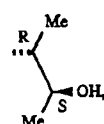
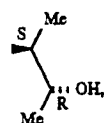
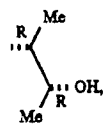
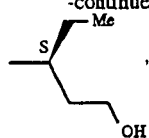
Follow the procedure of Example 33 except substitute an equivalent quantity of N,N'-dicarbobenzoxy 2,4-
diaminobutanoic acid for N-Cbz-glycine to obtain the title
compound.

wherein R₁ is:

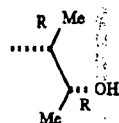


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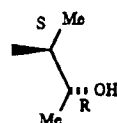


the above-listed compound 20F wherein $R_1 =$



was prepared by substitution of an equivalent amount of (2R,3R)-2,3-butanediol for the 2-monobenzyl ether of (2R, 3S) butanediol used in step d of Example 22. The product so formed was treated in accordance with the procedures of steps f, g, and h of Example 22. The above-listed compound 20F wherein $R_1 =$

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M*701

5

M*687

10

was prepared by substitution of an equivalent amount of the 2-O-SEM ether of 2R,3R butanediol for the starting material used in step d of Example 22. The product so formed was thereafter treated in accordance with the procedures of steps c, f, g, and h of Example 22.

What is claimed is:

1. A compound represented by the formula

M*687

15

M*687

20

M*687

25

M*687

30

M*687

35

M*687

40

M*687

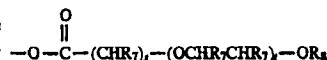
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or an ester group thereof convertible in vivo into OH; or a pharmaceutically acceptable salt thereof.

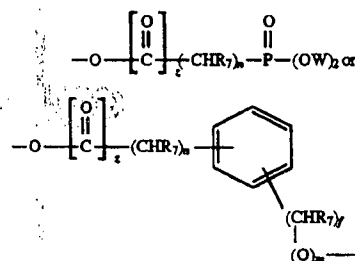
2. A compound of claim 1 wherein the ester group convertible in vivo into OH is a polyether ester, a phosphate ester, a sulfate ester, a heterocyclic ester, an alkanate ester, an amino acid ester or an acid ester.

3. A compound of claim 1 wherein the ester group convertible in vivo into OH is a polyether ester represented by the formula



wherein R_7 is a (C_1-C_6) straight or branched chain alkyl group or H, s is an integer from 1 to 6, t is an integer from 1 to 6, and R_8 is R_7 or $-CHR_7-CO_2R_7$.

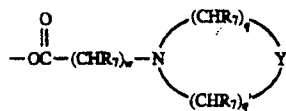
4. A compound of claim 1 wherein the ester group convertible in vivo into OH is a phosphate ester represented by the formula



wherein z is 0 or 1, R_7 is a (C_1-C_6) straight or branched chain alkyl group or H, f and n are independently an integer from 0 to 6, m is zero or 1 and W is H, CH_2Ar or and Ar is phenyl, phenyl substituted by halo, nitro, cyano or trihalomethyl.

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5. A compound of claim 1 wherein the ester group convertible in vivo into OH is a heterocyclic ester represented by the formula



wherein R_7 is a (C_1-C_6) straight or branched chain alkyl group or H; w is an integer of from 1 to 5; q and q' are independently 1 to 4 and Y is CHR_7 , $-O-$, NH , NR_7 , S , SO or SO_2 .

6. A compound of claim 1 wherein the ester group convertible in vivo into OH is an α -amine acid ester.

7. A compound of claim 1 wherein the esters of the compound represented by the formula of claim 1 have a solubility in a pharmaceutically acceptable aqueous medium of at least about 1 to about 50 mg/mL.

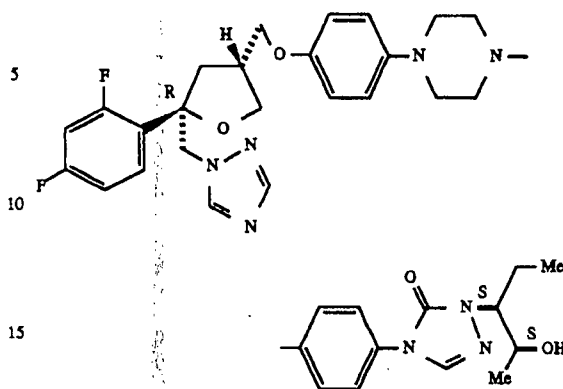
8. A pharmaceutical composition for treating or preventing fungal infection comprising an antifungally effective amount of a compound of claim 1 together with a pharmaceutically acceptable carrier therefor.

9. A method of treating and/or preventing fungal infections in a mammal afflicted with same which comprises administering an antifungally effective amount of a compound of claim 1 sufficient for such treating or preventing.

10. The pharmaceutical composition of claim 8 wherein the mode of administration is oral or parenteral.

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11. A compound represented by the formula



or a pharmaceutically acceptable salt thereof.

12. A pharmaceutical composition for treating or preventing a fungal infection comprising an antifungally effective amount of the compound of claim 11 together with a pharmaceutically acceptable carrier therefor.

13. A method of treating and/or preventing a fungal infection in a mammal afflicted with same which comprises administering an antifungally effective amount of the compound of claim 11 sufficient for such treating or preventing.

* * * * *

Comparative bioavailability of two medroxyprogesterone acetate suspensions

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Key words: Medroxyprogesterone acetate; Comparative bioavailability; Bioequivalence; Bioinequivalence; Pharmacokinetics; Oral administration; Intramuscular administration; Suspension formulation; Particle size; Ultrastructure

Summary

The drug particle-size characteristics of two medroxyprogesterone acetate (MPA) sterile suspension products (Depo-Provera sterile aqueous suspension, DP, Upjohn; Farlutal Depot sterile aqueous suspension, FD, Farmitalia) were compared, followed by bioequivalence studies of these preparations in humans after both oral and intramuscular administration. By means of scanning electron microscopy, DP was shown to contain smaller drug particles than FD. After oral administration of the suspensions in a single-dose, randomized cross-over study involving 16 healthy males, DP-treated subjects exhibited a significantly higher mean individual peak serum MPA concentration (C_{max}) and a significantly larger mean area under the serum MPA concentration vs time curve compared with FD-treated subjects. In a separate single-dose, randomized, non-cross-over study involving 38 healthy males, log-transformed data showed the mean C_{max} to be significantly higher in DP-treated subjects than in FD-treated subjects after intramuscular administration of the drug. We conclude that the bioequivalence of MPA formulations may be attributed largely to variations in the overall size of the component drug particles.

Introduction

Different brands of drug products containing a chemically equivalent active compound may exhibit inequivalent physicochemical behavior. This can sometimes be a result of interproduct differences in the ultrastructure of the active compound. In particular, interproduct particle size differences can lead to profound disparities in the

rates of solution and, ultimately, the bioavailability of pharmaceutical compounds (Hoener and Becket, 1979; Higuchi et al., 1985). Such bioinequivalencies become important clinically when they result in an unexpected treatment response.

High-dose medroxyprogesterone acetate (MPA) therapy has been shown to produce improvements in the rate of induced remission of several types of carcinoma. The normal route of administration in such uses is intramuscular injection of a sterile aqueous suspension; however, this route is associated with a 14% incidence of abscess formation and a 15% incidence of infiltration at the injection

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site (Lober, 1983). As an alternative, oral MPA administration has been shown to induce an anti-blastic activity in advanced breast cancer similar to that seen in objective remissions obtained after intramuscular injections (Pannuti et al., 1980).

Several companies have introduced MPA sterile aqueous suspension products into the market. It is unlikely that all of these products contain drug with identical particle characteristics, bringing to question the bioequivalency, or lack thereof, of these products.

The purposes of this study were to compare the drug particle-size characteristics of two internationally marketed brands of MPA sterile suspension (Depo-ProveraTM sterile aqueous suspension, Upjohn; Farlutal DepotTM sterile aqueous suspension, Farmitalia) and to see if any observed particle size differences would result in differing absorption characteristics after either oral or intramuscular administration.

Materials and Methods

Analyses of drug samples

Depo-Provera source. Both lots of Depo-Provera sterile aqueous suspension (Upjohn), containing 150 mg MPA per ml, were obtained in-house from the manufacturer. Lot B914N was used in the oral administration study, and lot B774N was used in the intramuscular administration study. All Depo-Provera lots contain bulk drug that has been micronized.

Farlutal Depot source. The Farlutal Depot sterile aqueous suspension (Farmitalia) sample, containing 200 mg MPA per ml, was obtained on the open market in Italy. This lot (2012) was used in both the oral and intramuscular administration studies.

Scanning electron microscopy and elemental analysis. A 1 ml aliquot of each drug suspension was diluted with 2.0 ml of double-distilled water. The resulting dilution was mixed well, and an aliquot of less than 0.05 ml was placed on a carbon stub. The stub was sputter-coated with gold, and the drug samples were photographed with a Cambridge Stereoscan 150 Scanning Electron Microscope. The MPA content of these sam-

ples was confirmed via an analysis for elemental content using a Kevex Unispec 7000 System 77 Energy Dispersive Spectrometer.

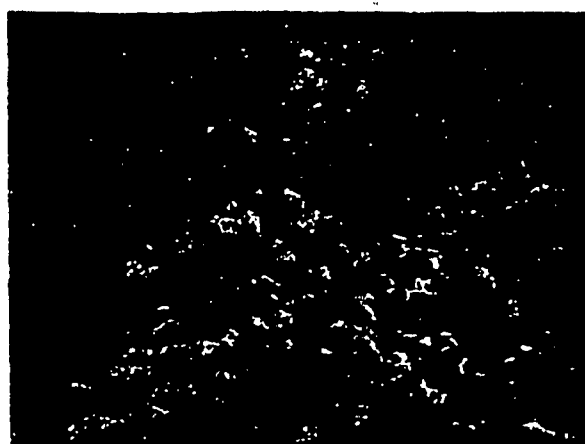
Potency assay. The potency of each lot used in this study was confirmed by means of a high-performance liquid chromatographic assay specific for MPA.

Particle size analysis. Scanning electron micrographs of the samples were visually inspected, and the diameter of individual particles was measured with a graduated linear scale. While a statistical data design for measuring particle size and/or including a specific sample was not attempted with this general morphological inspection, the MPA samples and fields chosen for evaluation were consistent with what was observed overall.

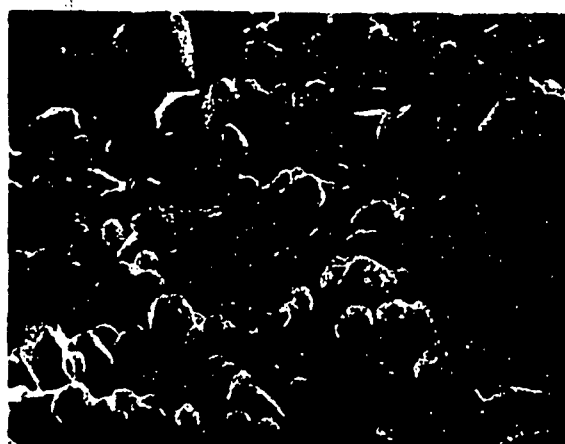
Comparative bioavailability studies

After oral administration. Sixteen healthy males (mean age: 26.8 ± 8.1 years; mean weight: 74.3 ± 6.1 kg) took single 500-mg oral doses of MPA in a randomized cross-over study. Subjects took either Depo-Provera or Farlutal Depot (mixed in 50 ml of orange juice and followed by 6 oz of water) and, after a minimum of 28 days, crossed over and took the other brand. Blood samples were drawn just before drug administration (time 0) and at the following hours after administration: 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120 and 144. The time 0 blood sample was 10 ml in volume; the remaining samples were each 7 ml. Serum was harvested from all samples as soon as possible after allowing 40 min for clot formation. Serum samples were frozen until they were radioimmunoassayed for MPA-like material, using the method of Cornette et al. (1971). This method is non-specific for both MPA and its metabolites.

After intramuscular administration. Thirty-eight healthy males (mean age: 33.0 ± 7.5 years; mean weight: 77.4 ± 12.9 kg) were given single 500-mg intramuscular doses of MPA in a randomized non-cross-over study. Subjects received either Depo-Provera ($n = 20$) or Farlutal Depot ($n = 18$); injections were made deeply into the gluteal muscle in the upper outer quadrant of the buttock. Blood samples were drawn just before drug administration (time 0) and at the following hours after administration: 1, 2, 4, 6, 8, 10, 12, 16,



20 μm



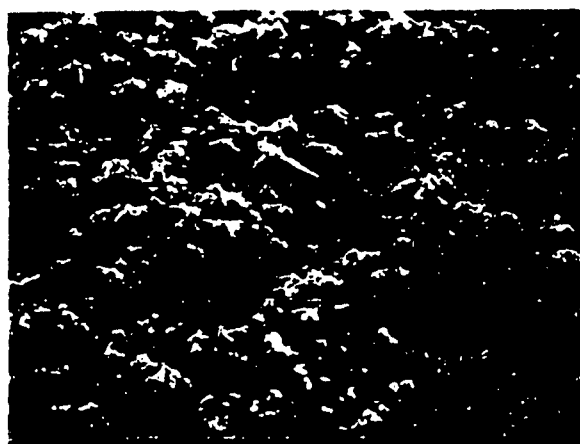
4 μm



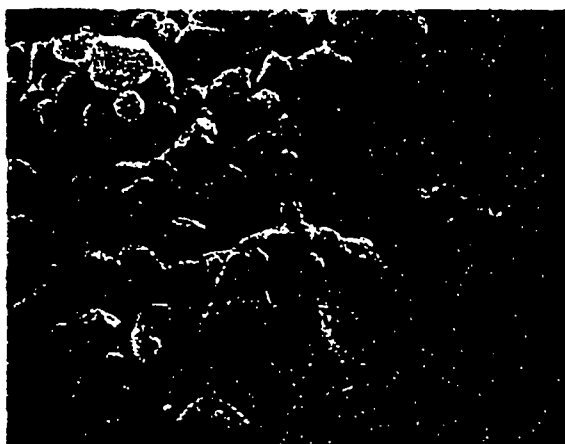
20 μm



4 μm



20 μm



4 μm

Fig. 1. Scanning electron micrographs of MPA drug particles. Depo-Provera lots B914N (top row) and B774N (bottom row), Farlutal Depot lot 2012 (middle row).

24, 28, 36 and 48; in addition, samples were collected each morning on the following days after drug administration: 3, 4, 5, 7, 9, 11, 14, 17, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 105, 112 and 119. All blood samples were 7 ml in volume. Serum was harvested from all samples within 45 min after sampling and then stored and assayed as in the oral administration study.

Pharmacokinetic calculations and statistical methods

Serum levels of MPA and the resultant bioavailability parameters were statistically compared between treatments by means of analysis of variance for cross-over design (oral administration study) or unpaired *t*-test analysis (intramuscular administration study). AUCs were determined using the trapezoidal rule. All doses were normalized to 500 mg, based upon assayed potency. Individual maximum serum MPA concentration data from the intramuscular administration study were subjected to log transformation for statistical analysis because the original values were found to be non-normally distributed and heteroscedastic by Bartlett's test analysis. Unless otherwise indicated, "statistically significant" and similar phrases imply $P < 0.05$.

Results

MPA particle characteristics

The ultrastructures of representative MPA particles (taken from the lots used in the bioavailability studies) can be seen in the scanning electron

TABLE I

Bioavailability parameters following oral administration of 500 mg medroxyprogesterone acetate ($n = 16$, cross-over)

Bioavailability parameter	Depo-Provera	Farlutal Depot	P-Value (ANOVA)
Individual peak serum MPA concentration, C_{max} (ng/ml)	106 (44.9) *	75.4 (30.1)	0.002
Time of peak serum MPA concentration, t_{max} (h)	5.1 (6.2)	10.3 (23.5)	NS **
AUC serum MPA concentration vs time, 0-144 h, AUC, (ng·h/ml)	5733 (1996)	4675 (1602)	0.02

* Standard deviations are given in parentheses.

** Not significant ($P > 0.05$).

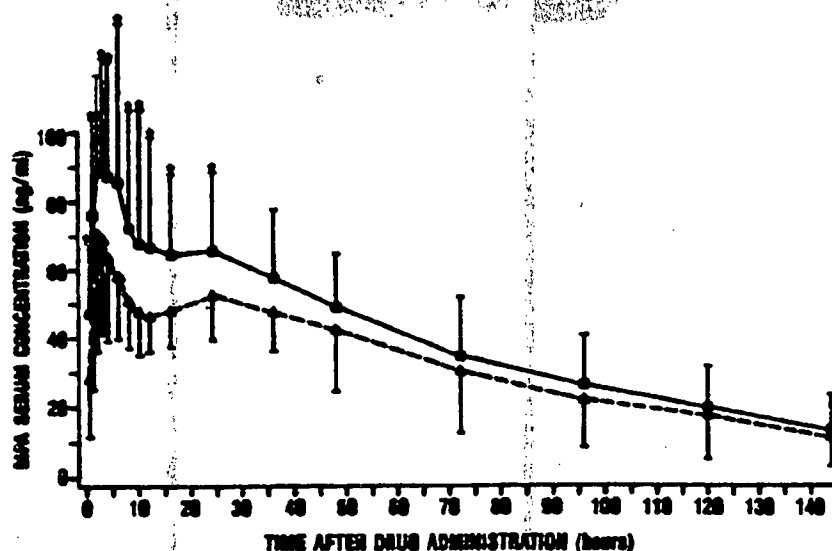


Fig. 2. Serum MPA concentrations following oral administration of two MPA suspensions ■, Depo-Provera; ▲, Farlutal Depot; ($n = 16$, crossover). *, Significantly different ($P < 0.05$). Doses have been normalized to 500 mg.

micrographs shown in Fig. 1. It is readily apparent that the two Depo-Provera drug samples had overall MPA particle-sizes similar to one another and smaller than those of the Farlutal Depot sample. Indeed, the MPA particles in Depo-Provera had diameters between 0.5 and 4.0 μm , while those in Farlutal Depot had diameters between 1.0 and 47 μm . In addition, the MPA particles in Depo-Provera were of a uniform, spherical shape in contrast to the irregularly shaped particles in Farlutal Depot.

Bioavailability

Table 1 contains statistical information concerning the pharmacokinetic data gathered after oral administration of the two preparations, and Fig. 2 graphically presents mean serum MPA concentrations throughout the study after oral administration. Both the individual peak serum MPA concentration (C_{max}) and area under the serum MPA concentration vs time curve (AUC) were significantly higher for Depo-Provera than for Farlutal Depot. The time of peak serum MPA concentration (t_{max}) occurred earlier with Depo-Provera than with Farlutal Depot but not significantly so. Statistically significant between-group differences in serum MPA concentrations were

TABLE 2

Bioavailability parameters following intramuscular administration of 500 mg MPA

Bioavailability parameter	Depo-Provera (n = 20)	Farlutal Depot (n = 18)	P-Value (t-Test)
Individual peak serum MPA concentration, C_{max} (ng/ml)	26.0 (25.0) *	15.7 (8.3)	0.03
Time of peak serum MPA concentration, t_{max} (days)	14.0 (22.1)	26.8 (29.8)	NS *
AUC Serum MPA Concentration vs Time, 0-14 days, AUC ($\mu\text{g} \cdot \text{h}/\text{ml}$)	5.1 (1.9)	3.7 (2.4)	0.05
AUC Serum MPA Concentration vs Time, 0-119 days, AUC ($\mu\text{g} \cdot \text{h}/\text{ml}$)	26.77 (8.6)	25.54 (15.4)	NS

Values are means $\pm$ S.D.

* Not significant ($P > 0.05$).

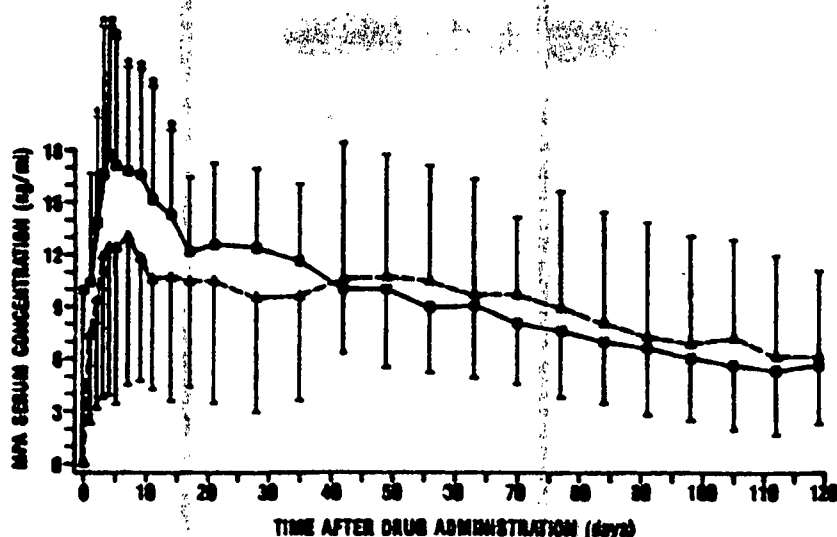


Fig. 3. Serum MPA concentrations following intramuscular administration of two MPA suspensions. $\blacksquare$, Depo-ProveraTM (n = 20); $\blacktriangle$, Farlutal DepotTM (n = 18). *, Marginally significantly different ($P < 0.1$). Doses have been normalized to 500 mg.

observed at the 0.5, 1, 3, 4, 6, 8, 10, 12, 16, and 24-h post-dosing sampling times, with Depo-Provera producing the higher concentrations. The terminal phase half-life of both products was approximately 40 h.

Table 2 contains statistical information concerning the pharmacokinetic data gathered after intramuscular administration of the two MPA preparations; mean serum MPA concentrations after intramuscular administration are presented graphically in Fig. 3. Log-transformed data yielded mean C_{max} significantly higher for Depo-Provera than for Farlutal Depot. Upon visual inspection of Fig. 3, it is apparent that both products produced large intersubject variability in serum MPA concentration. Marginally significant ($P < 0.1$) between-group differences were observed at the 48 h and 3, 4, 5, 7, 9, 11, and 14-day post-dosing sampling times, with Depo-Provera producing the higher concentrations. AUCs from 0 to 336 h (day 14) were significantly different, although those from 0 to 119 days were not. t_{max} occurred earlier with Depo-Provera than with Farlutal Depot, but the difference was not statistically significant. The terminal phase half-life of both products was approximately 50 days.

Discussion

Depo-Provera and Farlutal Depot were shown to have absorption characteristics clearly different from one another after both oral and intramuscular administration. While other formulation dissimilarities may have influenced the results to some extent, we attribute these differences to the smaller overall size of the drug particles in Depo-Provera.

We found that after oral administration, both rate and extent of absorption appeared to be affected by particle size difference, based upon the resultant significant differences in serum MPA concentrations at early (through 24 h) sampling times, as well as C_{max} and $AUC_{0-144 h}$.

After intramuscular administration, differences in serum MPA concentrations appeared to be limited to relatively early sampling timepoints.

Marginally significant or significant differences were found in serum MPA concentrations and AUC values calculated up to 14 days; C_{max} also differed significantly between the two treatments. As time progressed, serum MPA concentration differences decreased, resulting in similar concentration profiles. This could be the result of physicochemical or host-originated factors, such as encapsulation of the injection site, limited availability of dissolution media, or aggregation of drug particles, that would negate the effect of the original MPA surface-area differences over time.

The MPA used in manufacturing Depo-Provera is subjected to a controlled micronization process. It is well-known that reducing the particle size of a drug increases its total surface area and often its dissolution rate. A faster dissolution rate, in turn, can lead to a more rapid and, occasionally, greater extent of absorption. As early as the mid-1960s, it was shown that MPA particle size had an inverse relationship to MPA bioavailability after oral tablet administration (Helmreich and Huseby, 1965; Smith et al., 1966). Since drug in an aqueous suspension is analogous to the dispersion of tablet particles that exists after disintegration, it was not surprising to find similar results in our study.

High doses of MPA have been associated with much variability in pharmacokinetic parameters. Blosser et al. (1982) reported that oral (tablet) MPA doses of 1.5 mg/day produced a broad range of plasma MPA concentrations in patients with advanced breast cancer. In a study conducted in postmenopausal patients with metastatic breast cancer, the interpatient variability of plasma MPA levels in the plateau state increased significantly with oral dose (Blosser et al., 1984). Such interpatient differences can only be magnified by interproduct formulation differences of the kind found in this investigation. As a result, the therapeutic activity of the drug may, in theory, be greatly exaggerated or depressed. Since our data indicate that clinicians most likely see varying responses to MPA among their patients regardless of the drug brand, indiscriminately prescribing or dispensing bioequivalent MPA products would possibly introduce additional variability and unpredictability of patient response. Because drug-band selection is a controllable variable, it seems prudent for

clinicians to limit their prescribing and dispensing practices to one MPA brand when possible.

We conclude that there is apparently a difference in the overall rate and extent of absorption of Depo-Provera compared with that of Fardolal Depot after oral administration and a difference in the rate (but not extent) of absorption after intramuscular administration. It is likely that the dissimilarities of the two suspensions can largely be attributed to the smaller overall size of the MPA particles in Depo-Provera and, further, appear to be dependent upon the route of administration and time after dosing. The clinical consequences of these observations are unknown.

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Research Article

Development of a Glyburide-Metformin Fixed Combination Tablet With Optimized Glyburide Particle Size

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT The bioavailability-guided development of a fixed combination tablet formulation of glyburide and metformin hydrochloride, resulting in the identification of specific particle properties for the glyburide component that confer desirable in vivo performance, is described. Four separate pharmacokinetic studies were undertaken in healthy volunteers using fixed combination tablet formulations containing glyburide with differing particle properties. These studies directed first to the appropriate particle size in laboratory scale studies and subsequently confirmed the required, narrow particle size range in product made at commercial scale. Glyburide with particle size properties of 25% volume undersize of $\leq 6 \mu\text{m}$, 50% volume undersize in the range 7–10 μm , and 75% volume undersize of $\leq 21 \mu\text{m}$ was identified for use in a fixed combination with metformin hydrochloride. The optimized combination provided for bioequivalence to single entity metformin and acceptable bioavailability, though not bioequivalence, to single entity glyburide whilst having unique and potentially clinically valuable pharmacokinetic properties. Drug Dev. Res. 66:25–35, 2006. © 2006 Wiley-Liss, Inc.

Key words: formulation; particle size; metformin; glyburide; bioavailability

INTRODUCTION

Type 2 diabetes involves defective insulin secretion and insulin resistance [DeFronzo, 1999]. Hence, effective treatment would include targeting both of these disease aspects. Sulfonylureas such as glyburide (known as glibenclamide outside of North America) act to stimulate insulin secretion [Groop, 1992; Groop et al., 1991] and the biguanide metformin acts on insulin resistance, behaving as an insulin sensitizer, decreasing hepatic gluconeogenesis [Wollen and Bailey, 1988; Argaud et al., 1993] and increasing glucose uptake in peripheral tissues [DeFronzo et al., 1991]. Treating patients with a combination therapy of metformin and a sulfonylurea, such as glyburide, would

therefore be appropriate in dealing with the two primary defects of type 2 diabetes. Indeed, use of

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metformin together with a sulfonylurea has been a commonly used approach where monotherapy has failed to provide for adequate glycaemic control [Bailey, 2000; Hermann, 1990; Hermann et al., 1994a,b]. The availability in the United States of a fixed combination of metformin and glyburide, optimally formulated for effective oral delivery of the two agents together (GlucovanceTM, Bristol-Myers Squibb) has enabled compliant, safe, efficacious, and cost-effective treatment of patients failing on monotherapy [Marre et al., 2002; Blonde et al., 2002; Melikian et al., 2002; Bell, 2004] and provides a superior treatment compared with monotherapy as an initial pharmacological intervention [Garber et al., 2002].

The unique benefits of the fixed dose combination tablets may be associated with the pharmacokinetic profile of the two therapeutic agents described in previously published studies of this combination product formulation [Marathe et al., 2000]. In healthy volunteers dosed under fasting conditions, metformin from the combination formulation showed bioequivalence to a reference formulation co-administered with glyburide, whilst glyburide from the combination formulation showed comparable bioavailability to a reference glyburide tablet co-administered with metformin [Marathe et al., 2000]. Despite having comparable bioavailability to commercially-available glyburide tablets, the area under the glyburide concentration-time curve up to 3 h post-dosing was twofold greater from the combination tablet when dosed to type 2 diabetics along with a liquid meal as compared with that from co-administered glyburide and metformin tablets [Donahue et al., 2002]. This difference was suggested as the reason for the improvement in control of post-prandial glucose excursions with the combination tablet relative to co-administering commercial formulations of the individual drugs [Donahue et al., 2002].

The active medications in the combination tablet have very different physicochemical and biopharmaceutical properties, belonging to two different classes defined in the Biopharmaceutical Classification System as applied by the US Food and Drug Administration [Amidon et al., 1995]. Metformin shows high water solubility (>300 mg/ml) and poor intestinal permeability, indicated by the fact that when administered orally, less than 60% of the dose is absorbed [Sirtoli et al., 1978; Pentikäinen et al., 1979; Noel, 1980]. Glyburide exhibits very low water solubility (≤ 0.01 mg/ml) but, based on the relatively complete oral bioavailability of the drug after oral administration [Pearson, 1985], has high intestinal permeability. Drugs with absorption and solubility properties like those of glyburide show dissolution-controlled bioavailability.

Hence, including glyburide of appropriate controlled particle size distribution properties in the fixed-combination formulation is essential to obtaining the reproducible, desired pharmacokinetic and pharmacodynamic characteristics in the product.

We have previously [Balan et al., 2000] described a relationship between drug release rate and bioavailability for glyburide in a glyburide-metformin fixed combination tablets, where the different drug release rates were achieved by varying the particle size distribution of the glyburide. The studies described in this work complement and continue that earlier publication in detailing the bioavailability-guided development of the controlled physical properties for glyburide incorporated into the optimized, commercial fixed combination with metformin (GlucovanceTM, Bristol-Myers Squibb), and the potential clinical importance of these properties is discussed.

MATERIALS AND METHODS

Characterization of Glyburide Physical Properties

Glyburide drug substance

Glyburide drug substance was obtained from commercial sources. Collaboration with the manufacturers produced materials with differing particle properties through carefully controlled air jet milling processes.

Laser light scattering particle size analysis

Glyburide drug substance particle size distribution was evaluated using a laser light scattering instrument (Mastersizer X, Malvern Instruments, UK). A suspension of the drug in a suitable vehicle, appropriately dispersed to minimize the presence of significant agglomerates as validated by microscopy, was introduced into the instrument and data collected. Particle size distributions are mean data from analysis of three individual samples of each batch of drug substance, reported as the 25% volume undersize (D25), 50% volume undersize (D50), and 75% volume undersize (D75). The percentage volume undersize is a cumulative term where the percentage quoted defines the total volume of particles in the distribution falling below the specified particle size diameter.

Glyburide specific surface area analysis

The specific surface area of glyburide drug substance lots was determined by nitrogen absorption using a Gemini 2360 instrument (Micromeritics) with samples being preconditioned for analysis by degassing at 120°C and purging with nitrogen. Results given are the mean of three individual sample analyses.

Dosage Forms

Metformin 500-mg tablets were commercial formulation (GlucophageTM, Bristol-Myers Squibb). Different batches of glyburide-metformin fixed dose combination tablets were manufactured employing comparable processes and equipment, incorporating glyburide drug substance with differing particle size distribution characteristics. The two active ingredients were wet granulated with binder and disintegrant. The resultant granules were dried, milled, and blended with diluent/compression aid and lubricant before being compressed into tablets. Glyburide tablets 5 mg were an approved commercial formulation (MicronaseTM, Pfizer).

Drug In Vitro Release Studies

In vitro release of glyburide from the combination tablet product was undertaken using the method described in the FDA Draft Guidance for Bioequivalence Studies on glyburide tablets [Nerurkar, 1993]. This employs the USP rotating paddle method with agitation at 75 r.p.m. and 500 ml of pH 9.5 borate buffer as drug release test medium.

Bioavailability Studies

Studies were designed to assess the effect of glyburide particle size on its bioavailability and to optimize the particle size characteristics for material made at commercial scale. Four separate studies were conducted. The first two studies were direction finding and based on small-scale preparation of drug substance and formulated drug product (manufacturing scale for drug product 1 kg), and the latter two were used for optimization of material properties at a commercial operating scale (manufacturing scale for drug product 405 kg). Each study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. All subjects gave informed, written consent prior to participation.

Healthy volunteers (male and female, 18 to 40 years of age), with a body weight within $\pm 15\%$ of the desirable weight for their height, were eligible for the studies. In addition, women of childbearing potential were required to have a negative serum or urine pregnancy test and to be using an effective method of contraception. Subjects were excluded from the study if they had donated blood within the past 60 days, had a history or current evidence of cardiovascular, hepatic, haematopoietic, renal, respiratory, or gastrointestinal disease, had a history of drug abuse, or had ingested alcohol within 48 h prior to the dose of study medication.

Drug products used in these studies are given in Table 1. In all studies, treatment was administered at

8:00 AM after an overnight fast, along with 240 mL of a 20% glucose solution. Subjects were also given 60 mL of a 20% glucose solution every 15 min for 4 h post-dose to prevent hypoglycemic episodes.

The two initial bioavailability studies were open-label, single-site, randomized, 4-treatment, 4-period, single-dose, complete crossover studies. Study 1 was conducted in 12 healthy volunteers and study 2 was conducted in 24 healthy volunteers. Each subject was randomly assigned to 1 of 4 treatments and 1 of 4 treatment sequences. Each subject was expected to complete all 4 treatments, with a minimum of a 1-week washout period between each treatment. During each treatment period for studies 1 and 2, subjects received a single dose of metformin (1,000 mg) and glyburide (5 mg), either as one of the formulations of a glyburide-metformin 2.5 mg/500 mg combination tablet (Formulation 1, 2, 3, or 4) or as co-administered metformin tablets and a glyburide tablet. In addition, 2 different lots of the marketed glyburide tablets were used in Study 1 to assess lot-to-lot variability in glyburide pharmacokinetics. Study 2 included a treatment leg used to evaluate a metformin formulation (Formulation 5) that was outside the scope of the work reported here. Consequently, this formulation will not be discussed further in this report.

Following identification of an approximate target glyburide particle size, Studies 3 and 4 were conducted to establish an appropriate particle size specification for the commercial fixed-dose combination product. Study 3 was an open-label, single-dose, two-period crossover study conducted in 28 healthy volunteers. During each treatment period, subjects received a single dose of metformin (1,000 mg) and glyburide (5 mg), either as the scaled-up formulation of glyburide-metformin 2.5 mg/500 mg combination tablet (Formulation 6) or as co-administered metformin tablets and a glyburide tablet.

Study 4 was an open-label, randomised single dose study, three-treatment, three-period crossover study in 36 subjects. There was a minimum of one week washout period between treatments. Three different lots of glyburide-metformin tablets 2.5 mg/500 mg, which differed slightly in glyburide particle size range, were used (Formulation 6, 7, and 8). Two tablets from a lot were administered to each subject in each leg of the study.

Sample Collection and Analysis

Serial venous blood samples (7 mL) were collected prior to administration of study medication and at 0.5, 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, and 24 h post-dose. In addition, samples were also collected at 30, 36, and 48 h post-dose in Studies 2, 3, and 4.

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TABLE 1. Drug Products Used in the Four Bioavailability Studies

Study	Treatment/drug product	Glyburide batch number ^a
1	2 × glyburide/metformin 2.5 mg/500 mg	SN/96/K7
	Formulation 1	
	2 × glyburide/metformin 2.5 mg/500 mg	SN/96/K6
	Formulation 2	
	2 × metformin 500 mg tablet and 1 × glyburide 5 mg lot A	—
	2 × metformin 500 mg tablet and 1 × glyburide 5 mg lot B	—
2	2 × glyburide/metformin 2.5 mg/500 mg	SN/96/L2
	Formulation 3	
	2 × glyburide/metformin 2.5 mg/500 mg	SN/97/D10
	Formulation 4	
	Formulation 5 ^b	—
3	2 × metformin 500 mg tablet and 1 × glyburide 5 mg tablet lot A	—
	2 × glyburide/metformin 2.5 mg/500 mg	268585
	Formulation 6	
	2 × metformin 500 mg tablet and 1 × glyburide 5 mg lot C	—
4	2 × glyburide/metformin 2.5 mg/500 mg	268585
	Formulation 6	
	2 × glyburide/metformin 2.5 mg/500 mg	SN/97/G1
	Formulation 7	
	2 × glyburide/metformin 2.5 mg/500 mg	268484
	Formulation 8	

^aUsed in preparation of test dosage forms.^bSee text.

Plasma samples were assayed for metformin concentrations using a validated gas chromatography (GC) method (Study 1) or a gas chromatography/mass spectrometry (GC/MS) method (Studies 2, 3, and 4). The lower limit of quantitation (LLQ) was established at 10 ng/mL for metformin (standard curve range 10 to 2,500 ng/mL). The standard curves for metformin in plasma had R-squared values of ≥ 0.994 , and the overall between- and within-run variability of the analytical quality control (QC) samples were less than 16% relative standard deviation (RSD). The mean observed concentrations of the analytical QC samples in plasma deviated less than 5% from the nominal values.

Plasma glyburide concentrations were measured using a validated liquid chromatography/mass spectroscopy (LC/MS) method. The LLQ was established at 1 ng/mL (standard curve range 1 to 500 ng/mL). The standard curves for glyburide in plasma had R-squared values of ≥ 0.997 . The overall between- and within-run variability of the analytical QC samples in plasma were $< 12\%$ RSD. The mean observed concentrations of the analytical QC samples in plasma deviated $< 7\%$ from the nominal values.

Pharmacokinetic Evaluation

Pharmacokinetic parameters were calculated using noncompartmental methods [Gibaldi and Perrier, 1982]. C_{max} and time to reach C_{max} (T_{max}) were calculated for metformin and glyburide from the plasma concentration versus time profile. In addition, area under the plasma concentration versus time curve to the last quantifiable time point (AUC_{0-T}) was calculated for glyburide only, and area under plasma concentration versus time curve from time zero to infinity (AUC_{INF}) was calculated for metformin only. Determination of AUC_{INF} for glyburide was not possible due to lack of a discernible terminal elimination phase.

Statistical Methods

For each pharmacokinetic variable, an analysis of variance (ANOVA) was performed with treatment sequence, subjects within sequence, period, direct effect of treatment, and first-order carryover effect of treatment as factors. All statistical tests were carried out at the 5% level of significance and all treatment comparisons were 2-sided. Interval estimates for

treatment differences and ratios were 2-sided and calculated using 90% confidence coefficients for C_{max} and AUC only, and no adjustments were made for multiple comparisons.

RESULTS

Glyburide Particle Size and Surface Area

The particle size distributions and specific surface areas of the glyburide batches employed in making the fixed combination tablets used in the pharmacokinetic studies are given in Table 2, along with data for commercially available micronized material, commercially available non-micronized material, and a further lot of material prepared to meet the specification for inclusion in an optimized combination formulation. It was not possible to determine the particle size distribution of glyburide in the commercially available single entity reference formulation but it is known that it does not employ micronized material.

Drug release data for glyburide from the experimental fixed combination formulations is shown in Fig. 1. The data in Table 2 show clearly that the material used in the fixed-dose combination tablet has been specifically engineered to produce a distinct distribution, as it is distinct in particle size and surface area properties from typical commercially available glyburide material, both of micronized and non-micronized grades.

Pharmacokinetics Results

Bioavailability Study 1

Metformin and glyburide pharmacokinetic parameters are presented in Tables 3 and 4, respectively. The 90% confidence intervals (CI) of the ratio of the means for the metformin component from Formulation 1 compared with the metformin reference tablet were within the acceptable range of 0.80 to 1.25 for all

pharmacokinetic parameters tested. However, for metformin from Formulation 2 compared with metformin reference tablets co-administered with reference glyburide Lot A tablets, the 90% CI was slightly outside the acceptable range for C_{max} . All other 90% CI for Formulation 2 compared with metformin co-administered with either lot of the reference glyburide tablet were within acceptable limits. For glyburide, the 90% CIs were outside the acceptable limits for demonstration of bioequivalence for all parameters tested for both Formulation 1 and Formulation 2 when compared with both lots of glyburide reference tablets. It is also apparent from Tables 3 and 4 that C_{max} and AUC_{0-1} values for glyburide differed substantially from

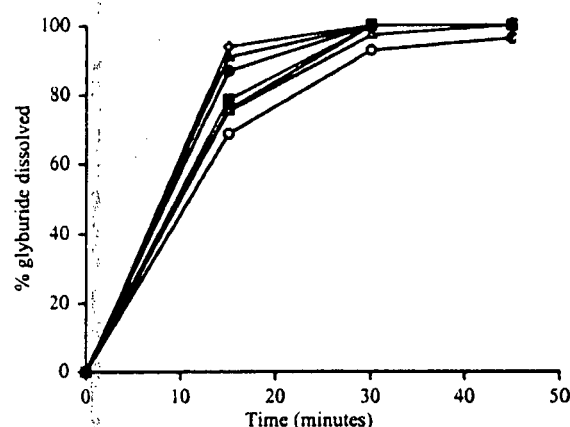


Fig. 1. *In vitro* release profiles for glyburide from the different glyburide-metformin tablets studied in the bioavailability studies. □, glyburide-metformin 2.5 mg/500 mg Formulation 1; ○, glyburide-metformin 2.5 mg/500 mg Formulation 2; △, glyburide-metformin 2.5 mg/500 mg Formulation 3; ■, glyburide-metformin 2.5 mg/500 mg Formulation 4; ●, glyburide-metformin 2.5 mg/500 mg Formulation 6; ▲, glyburide-metformin 2.5 mg/500 mg Formulation 7; ◇, glyburide-metformin 2.5 mg/500 mg Formulation 8.

TABLE 2. Particle Size Distribution and Surface Area Data for Glyburide Drug Substance Used in the Preparation of Combination Glyburide-Metformin Hydrochloride Dosage Forms^a

Glyburide reference	Particle size (μm)			Surface area m ² /g	Comment
	D25	D50	D75		
SN/96/K7	15	33	62	0.5	Used in Formulation 1
SN/96/K6	28	58	88	ND	Used in Formulation 2
SN/96/L2	10	25	52	ND	Used in Formulation 3
SN/97/D10	6	11	19	ND	Used in Formulation 4
268585	5	9	18	ND	Used in Formulation 6: optimized specification
SN 97/G1	7	14	26	ND	Used in Formulation 7
268484	4	8	18	1.7	Used in Formulation 8: optimized specification
280304	13	29	56	0.5	Example of commercial non-micronized glyburide
288384	4	8	17	1.8	Optimized specification material
288990	3	5	7	3.7	Example of commercial micronized material

^aND: not determined.

TABLE 3. Pharmacokinetic Parameters of Metformin From Formulation 1, Formulation 2, Metformin Coadministered with Glyburide Lot A, and Metformin Coadministered With Glyburide Lot B

Treatment	Parameter	Mean (SD)	Ratio of means (90% CI) ^a	Ratio of means (90% CI) ^b
Formulation 1	C _{max} (ng/mL)	1,560 (417)	1.00 (0.91,1.11)	1.12 (1.01,1.23)
	AUC(INF) (ng.h/mL)	9,684 (1765)	0.97 (0.91,1.05)	1.05 (0.98,1.12)
	t _{max} (h)	2.5 (1,4)	—	—
Formulation 2	C _{max} (ng/mL)	1,581 (278)	1.03 (0.93,1.14)	1.14 (1.04,1.26)
	AUC(INF) (ng.h/mL)	9,854 (1,793)	0.99 (0.92,1.07)	1.07 (0.99,1.14)
	t _{max} (h)	2.5 (2,3.5)	—	—
Metformin+glyburide A	C _{max} (ng/mL)	1,541 (367)	—	—
	AUC(INF) (ng.h/mL)	9814 (1,816)	—	—
	t _{max} (h)	2.5 (2,3.5)	—	—
Metformin+glyburide B	C _{max} (ng/mL)	1,388 (285)	—	—
	AUC(INF) (ng.h/mL)	9,244 (1,635)	—	—
	t _{max} (h)	2.8 (2,4)	—	—

^aRelative to metformin + glyburide A.^bRelative to metformin + glyburide B.**TABLE 4. Pharmacokinetic Parameters of Glyburide From Formulation 1, Formulation 2, Metformin Coadministered with Glyburide Lot A, and Metformin Coadministered With Glyburide Lot B**

Treatment	Parameter	Mean (SD)	Ratio of means (90% CI) ^a	Ratio of means (90% CI) ^b
Formulation 1	C _{max} (ng/mL)	76.3 (27.3)	0.83 (0.62,1.11)	0.92 (0.69,1.22)
	AUC(0-T) (ng.h/mL)	493.5 (222.1)	0.72 (0.56,0.92)	0.60 (0.47,0.76)
	t _{max} (h)	4.7 (2,16)	—	—
Formulation 2	C _{max} (ng/mL)	54.2 (17.7)	0.59 (0.44,0.79)	0.65 (0.49,0.87)
	AUC(0-T) (ng.h/mL)	338.7 (74.3)	0.52 (0.41,0.67)	0.52 (0.41,0.67)
	t _{max} (h)	4.6 (2,12)	—	—
Metformin+glyburide A	C _{max} (ng/mL)	98.9 (52.7)	—	—
	AUC(0-T) (ng.h/mL)	720.0 (385.7)	—	—
	t _{max} (h)	9.1 (3,24)	—	—
Metformin+glyburide B	C _{max} (ng/mL)	82.2 (29.0)	—	—
	AUC(0-T) (ng.h/mL)	792.8 (233.7)	—	—
	t _{max} (h)	9.3 (2.5,24)	—	—

^aRelative to metformin + glyburide A.^bRelative to metformin + glyburide B.

reference glyburide formulation Lot A to Lot B, which led to differences in the statistical comparison of combination Formulation 1 and 2 depending on whether the comparison was being made with reference glyburide Lot A or reference glyburide Lot B.

Bioavailability Study 2

Metformin and glyburide pharmacokinetic parameters are presented in Tables 5 and 6, respectively. When compared with metformin co-administered with glyburide, bioequivalence was concluded for the metformin component of all formulations based on

the ratio of means and 90% CI for both C_{max} and AUC_{INF}. The mean plasma concentration profiles for metformin from all glyburide-metformin tablets were superimposable with that of metformin co-administered with glyburide.

The glyburide component of the glyburide-metformin combination tablets showed two distinct peaks in the plasma concentration-time profile for all subjects and in the mean plasma concentration-time profile for the co-administered treatment leg there was also a very small early peak, although this was very much less prominent and was not seen in all

TABLE 5. Pharmacokinetic Parameters of Metformin From Formulation 3 and 4 Compared to Metformin Coadministered With Glyburide (Study 2)

Treatment	Parameter	Mean (SD)	Ratio of means ^a	
			Point estimate	90% CI
Formulation 3	C _{max} (ng/mL)	1,328 (465)	1.08	(0.99, 1.18)
	AUC(INF) (ng/h/mL)	8,879 (2,051)	1.06	(0.99, 1.13)
	t _{max} (hours)	2.0 (1, 4.5)	—	—
Formulation 4	C _{max} (ng/mL)	1,258 (356)	1.05	(1.00, 1.14)
	AUC(INF) (ng/h/mL)	8,471 (2,262)	1.01	(0.95, 1.08)
	t _{max} (h)	2.0 (1, 3.5)	—	—
glyburide+metformin	C _{max} (ng/mL)	1,201 (359)	—	—
	AUC(INF) (ng/h/mL)	8,238 (2,101)	—	—
	t _{max} (h)	2.5 (1, 4.5)	—	—

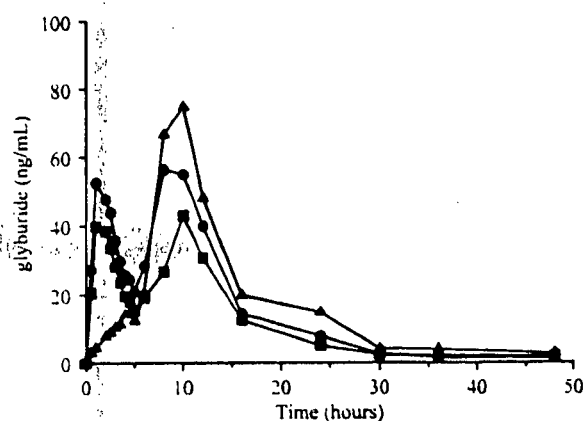
^aRelative to glyburide + metformin treatment based on adjusted means derived from ANOVA.**TABLE 6.** Pharmacokinetic Parameters of Glyburide From Formulation 3 and 4 Compared to Metformin Coadministered With Glyburide (Study 2)

Treatment	Parameter	Mean (SD)	Ratio of means ^a	
			Point estimate	90% CI
Formulation 3	C _{max} (ng/mL)	66.6 (25.1)	0.69	(0.57, 0.84)
	AUC(0-T) (ng/h/mL)	531.4 (121.7)	0.70	(0.60, 0.81)
	t _{max} (h)	8.0 (1, 12)	—	—
Formulation 4	C _{max} (ng/mL)	92.7 (30.5)	0.95	(0.79, 1.15)
	AUC(0-T) (ng/h/mL)	715.7 (251.8)	0.87	(0.75, 1.01)
	t _{max} (h)	8.0 (0.5, 12)	—	—
Glyburide + metformin	C _{max} (ng/mL)	103.0 (42.4)	—	—
	AUC(0-T) (ng/h/mL)	773.1 (263.2)	—	—
	t _{max} (h)	8.0 (4.5, 24)	—	—

^aRelative to glyburide + metformin treatment based on adjusted means derived from ANOVA.

volunteers. The two peaks with the glyburide-metformin tablets are seen approximately 1 to 3 h and 6 to 12 h post-dose (Fig. 2).

Glyburide-metformin Formulation 3 demonstrated bioavailability of 70% (based on AUC_{0-T}), respectively, for glyburide in comparison with glyburide co-administered with metformin, whereas glyburide-metformin Formulation 4 demonstrated more acceptable and comparable bioavailability to that of glyburide co-administered with metformin based on C_{max} and AUC_{0-T} ratios of 0.952 and 0.871, respectively (Table 6). However, for all the tests to reference comparisons of glyburide, bioequivalence could not be concluded for either C_{max} or AUC_{0-T} because the lower confidence limit was <0.80. Nevertheless, among glyburide-metformin tablet treatments evaluated, Formulation 4 was found to be an optimal prototype

**Fig. 2.** Plasma concentration time curves for glyburide from different glyburide/metformin tablet formulations (study 2). ■, Formulation 3; ●, Formulation 4; ▲, glyburide co-administered with metformin.

formulation for glyburide compared with the reference treatment (glyburide co-administered with metformin) in terms of providing appropriate exposure and suitability for treatment of patients. This is appropriate where one component of therapy (glyburide) is available as multiple branded products, which may or may not be equivalent and where therapy is in any case titrated to control.

Bioavailability Study 3

Pharmacokinetic results for metformin and glyburide are given along with statistical comparisons of parameters in Table 7. C_{max} and AUC for metformin for the scaled-up combination tablet (Formulation 6) met bioequivalence criteria, with 90% confidence

intervals being contained within (0.80, 1.25). As in the case of the laboratory scale formulation 4 evaluated in study 2, although bioequivalence was not demonstrated for the glyburide component from the combination product to glyburide from MicronaseTM co-administered with metformin, bioavailability based on AUC and C_{max} was comparable, with the C_{max} ratio 1.10 and the AUC_{0-T} 1.18. Such glyburide exposure would be appropriate for fixed-dose combination therapy even in the absence of bioequivalence.

Bioavailability Study 4

Pharmacokinetic parameters for metformin and glyburide are presented in Table 8. In this study, some long terminal elimination half-life values for metformin

TABLE 7. Pharmacokinetic Parameters of Metformin and Glyburide From Formulation 6 Compared to Metformin Coadministered With Glyburide (Study 3)

Treatment	Parameter	Mean (SD)	Ratio of means ^a	
			Point estimate	90% CI
Metformin	Formulation 6			
	C_{max} (ng/mL)	1,507 (392)	1.02	(0.98, 1.06)
	AUC(INF) (ng.h/mL)	10,425 (2,032)	0.99	(0.95, 1.03)
	t_{max} (h)	2.5 (1.4.5)	—	—
	Glyburide + metformin			
	C_{max} (ng/mL)	1,458 (315)	—	—
Glyburide	Formulation 6			
	AUC(0-T) (ng.h/mL)	10,539 (1,801)	—	—
	t_{max} (h)	2.5 (1.3.5)	—	—
	Glyburide + metformin			
	C_{max} (ng/mL)	119 (36.6)	1.10	(0.98, 1.25)
	AUC(0-T) (ng.h/mL)	971 (368)	1.18	(1.08, 1.29)
Glyburide	Formulation 6			
	t_{max} (h)	8 (1.12)	—	—
	Glyburide + metformin			
	C_{max} (ng/mL)	115 (54.4)	—	—
	AUC(0-T) (ng.h/mL)	854 (385)	—	—
	t_{max} (h)	8 (2.5, 12)	—	—

^aRelative to glyburide + metformin treatment based on adjusted means derived from ANOVA.

TABLE 8. Pharmacokinetic Parameters of Metformin and Glyburide From Formulation 7 and Tablet 8 Compared to Formulation 6 (Study 4)

Treatment	Parameter	Mean (SD)	Ratio of means ^a	
			Point estimate	90% CI
Metformin	Formulation 7			
	C_{max} (ng/mL)	1,461 (444)	0.96	(0.90, 1.03)
	AUC(INF) (ng.h/mL)	9,515 (2,349)	0.97	(0.92, 1.02)
	Formulation 8			
	C_{max} (ng/mL)	1,403 (336)	0.93	(0.87, 1.00)
	AUC(INF) (ng.h/mL)	9,462 (2,024)	0.97	(0.92, 1.02)
Glyburide	Formulation 6			
	C_{max} (ng/mL)	1,503 (377)	—	—
	AUC(INF) (ng.h/mL)	9,764 (2,020)	—	—
	Formulation 7			
	C_{max} (ng/mL)	90.2 (34.7)	0.74	(0.65, 0.84)
	AUC(0-T) (ng.h/mL)	633 (277)	0.84	(0.78, 0.90)
Glyburide	Formulation 8			
	C_{max} (ng/mL)	125 (69.9)	0.99	(0.87, 1.12)
	AUC(0-T) (ng.h/mL)	689 (247)	0.91	(0.86, 0.97)
	Formulation 6			
	C_{max} (ng/mL)	121 (47.4)	—	—
	AUC(0-T) (ng.h/mL)	757 (308)	—	—

^aRelative to tablet 6 based on adjusted means derived from ANOVA.

were observed in a small number of subjects. However, as AUC(0-T) in these instances accounted for at least 88% of the overall AUC, the resultant calculated AUC(INF) are considered reliable. These calculations excluded those already noted subjects where outlier terminal half-lives were observed. Bioequivalence was demonstrated for metformin from the two test formulations relative to the test formulation.

Statistical comparison of glyburide pharmacokinetic parameters is also provided in Table 8. Formulation 8 was found to be bioequivalent to combination Formulation 6, but bioequivalence for the glyburide component of combination Formulation 7, which employed a very slightly coarser particle size grade of glyburide, could not be established.

DISCUSSION

After preliminary pharmacokinetic studies indicated that the formulation of commercially available micronized glyburide in a fixed combination tablet with metformin resulted in a very high early single peak C_{max} with a resultant risk of hypoglycemia (Merck Kga, unpublished observations), development efforts were directed away from employing micronized drugs.

Bioequivalence for metformin was demonstrated for all combination products investigated relative to metformin tablets administered with glyburide tablets, except for Formulation 2, which showed an upper confidence interval for C_{max} of 1.26, slightly outside the accepted criteria for bioequivalence. The variable absorption of metformin and the small size of the group of volunteers employed may have contributed to this effect. Bioequivalence was not unexpected as rapid *in vitro* metformin release is seen for all formulations with more than 85% of the contained drug being liberated within 15 min. Such drug release properties for a drug falling into Biopharmaceutical Classification System class III would result, in the absence of any specific promoting or retarding influence of excipients, in its *in vivo* performance being similar to that of a solution of a drug [Yu et al., 2001] and, hence, bioequivalence of such drug products.

Results for Formulations 1, 2, 3, and 4 affirm the expected relationship between glyburide particle size/surface area and *in vivo* performance. Reducing the mean particle size and so increasing the powder surface area for glyburide was previously shown to improve the rate and extent of absorption [Armqvist et al., 1983; Karttunen et al., 1985; Neugebauer et al., 1985; Cui et al., 1993]. Balan and colleagues [2000] had demonstrated an *in vitro-in vivo* correlation between drug release rate and bioavailability for glyburide administered as glyburide-metformin fixed combination tablets where the different drug release rates were

achieved by undisclosed variation in the glyburide particle size distribution. It is notable that the controlled particle size of glyburide used in the scaled-up, optimized fixed-dose combination tablet (Formulation 6 and 8) is distinct from both typical commercially available non-micronized and micronized glyburide (Table 2). Typical micronized materials have particle sizes less than 10 μm [Midoux et al., 1999] and for glyburide, bioavailability has been previously claimed as optimized when micronized material has a particle size of around 2 μm and a surface area of not less than 3 m^2/g [Rothe et al., 1977]. Glyburide sized to be suitable for the fixed combination product has a D75 particle size $\leq$ approximately 20 μm and a surface area of 2 m^2/g or less, but the drug substance is not as fine as micronized material (Table 2). Data from tablets made at a large scale (Formulation 6, 7, and 8) show that it is possible to create the product in a commercial manufacturing environment and that a very narrow particle size range is required (note C_{max} properties of Formulation 7). Evaluation of data from these batches led to the definition of particle size range of glyburide for the optimized product for future clinical and commercial use as 25% volume undersize $\leq 6 \mu\text{m}$, 50% volume undersize within the range 7–10 μm , and 75% volume undersize of $\leq 21 \mu\text{m}$.

In these studies, and in clinical studies in the literature, administration of commercial glyburide tablets results in a single plasma peak. In the case of established micronized glyburide formulations, the single plasma peak occurs at around 1.3 to 2.3 h [Armqvist et al., 1983; Karttunen et al., 1985; Neugebauer et al., 1985; Cui et al., 1993], whereas with established non-micronized glyburide formulations, the single plasma peak occurs at around 2.0 to 7.0 h [Marathe et al., 2000; Armqvist et al., 1983; Karttunen et al., 1985; Neugebauer et al., 1985; Cui et al., 1993; Sartor et al., 1980; Coppack et al., 1990]. The novel particle size distribution employed in the combination product is distinct from both micronized material and typical non-micronized material (Table 2). The bulk of the combination tablet is highly soluble metformin hydrochloride, which will be expected to rapidly and completely dissolve in stomach contents, so *in vivo* the fixed dose tablet is effectively delivering a solution of metformin and a suspension of the controlled particle size glyburide. The particle size distribution of glyburide was identified through these healthy fasted (with glucose supplementation) volunteer pharmacokinetic studies to provide similar exposure to a conventional non-micronized formulation single component formulation, but from a highly water soluble matrix. The optimized combination tablet formulation provides for a pharmacokinetic profile in healthy fasted subjects

when administered with 20% glucose solution (Fig. 2) and in Type 2 diabetics when dosed with a liquid meal [Donahue et al., 2002] not seen with the single entity glyburide formulations studied in this work or reported in the literature. Since the significant double peak phenomenon is only observed in the case of the combination product, it may be presumed that the absorption profile of the product is associated with the formulation and the glyburide particle size distribution. The double peak phenomenon is not a consequence of inter-subject variation in the absorption of glyburide, a phenomenon previously described where it has been shown that there may be subpopulations of patients, one group that absorbs the drug rapidly and a second that show delayed absorption [Ikegami et al., 1986]. In that study, only a single maximum glyburide plasma concentration peak was observed in individual subjects.

The faster onset glyburide absorption from this fixed dose combination product suggested by the first plasma peak (note that T_{max} data in the tables only indicates the second, larger peak) relative to conventional glyburide formulations, may indeed be a highly desirable attribute. It translates to a twofold increase in area under the concentration-time curve to 3 h compared with co-administered glyburide and metformin, which may contribute to the better postprandial glycaemic control compared with the co-administered drugs [Donahue et al., 2002]. Administered twice daily, it could enhance insulin secretion and utilization better timed to deal with postprandial glucose surges [Howlett et al., 2003]. The double peak profile observed in the current studies, translated to a twice daily dosing of the glyburide-metformin combination product containing the specific particle size distribution glyburide, is in fact similar to the estimated optimal glyburide plasma concentration profile for treatment of type 2 diabetes derived from a system approach to pharmacodynamics [Gillespie et al., 1988] with peak drug level closely aligned with mealtimes, affirming the value of the combination formulation.

As commercially available, single-entity non-micronized glyburide formulations do not apparently exhibit these absorption characteristics, co-administration of commercial single component glyburide tablets with metformin tablets may not offer the same benefit of early exposure and impact on postprandial glucose. The optimum particle size distribution material utilized in the combination product is patented [Cave and Nicholson, 2001]. Hence, the same *in vivo* properties may not necessarily be exhibited by other fixed combination formulations of glyburide-metformin. This may be the case even though bioequivalence has been demonstrated, as conventional demonstration of

bioequivalence only requires maximum attained plasma concentration and area under the plasma concentration-time curve to be equivalent and may not necessarily reflect true equivalence of clinical efficacy [Walson, 2003].

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Appendix C
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POSACONAZOLE NDA AMENDMENT: REVISED PARTICLE SIZE SPECIFICATION

Summary

Posaconazole Oral Suspension is an immediate-release oral suspension formulation, with the proposed first indication for the treatment of refractory invasive fungal infections (r-IFI). During drug product manufacturing, the particle size of the poorly soluble drug substance is reduced using a microfluidization process. A relatively narrow range of particle size batches was used during the clinical program. A clinical bioequivalence study was conducted to assess the feasibility of changing the drug product particle size specification, in the context of possible future manufacturing scale and equipment changes. The results of the bioequivalence study show that posaconazole drug product batches with median particle sizes 1.7 and 2.3 μm are not bioequivalent. (SAS transport files of the individual subject pharmacokinetic data in xpt format are provided.) Based on these results and an assessment of the capability of the current manufacturing process, the drug product release/shelf-life median particle size specification has been tightened and minor revisions to the manufacturing process and an in-process control have been implemented.

The relevant changes are:

- the finished product median particle size specification has been tightened from a range of 1.6 - 2.2 μm to a range of 1.5 - 2.0 μm . This tightened specification continues to bracket the median particle size range of 1.6 - 1.8 μm used in the pivotal clinical efficacy trial.
- the in-process median particle size specification has been tightened from 1.3 - 1.6 μm to 1.3 - 1.5 μm
- the batch temperature during the holding period for the bulk compounded product is now controlled at $24 \pm 3^\circ\text{C}$ under low agitation conditions.

These changes are justified by:

- calculation of theoretical dissolution and subsequent absorption based on Fick's law, which shows that, even if the assumptions inherent in this worst case analysis were true, suspension with a median particle size of 2.0 μm would have an average of 85% of the absorption of a 1.7 μm suspension.



This is within the range of the variability seen between different clinical studies using drug product batches of similar particle size.

- review of previous studies (both single-dose and steady-state AUCs vs. particle size) which, although not definitive, shows that there is significant variability within each group and that there is no strong correlation between absorption and particle size. Furthermore, there is considerable overlap in the ranges of AUCs observed from capsule formulations (with drug substance particle sizes of 3.0 – 3.2 μm) and suspension formulations (drug substance particle sizes 1.5 – 2.3 μm).
- the capability of the manufacturing process which, following introduction of two minor modifications to the process and a reduction in the in-process particle size specification at the end of microfluidization, will be able to consistently meet the revised specifications. This assessment indicates that the particle size results for the majority of finished product batches will be consistent with the range used in the pivotal clinical efficacy study.

1 INTRODUCTION

Posaconazole Oral Suspension is an immediate-release oral suspension formulation, with the proposed first indication for the treatment of r-IFI. During drug product manufacturing, the particle size of the poorly soluble drug substance is reduced using a microfluidization process. The current particle size specification for the finished product (median 1.6 - 2.2 μm ; not less than 65% under 3 μm ; not less than 99% under 10 μm) was chosen based on our manufacturing experience at the commercial site, as noted in the justification of specifications (NDA Section 3.2.P.5.6). The median particle sizes of the clinical batches used in the pivotal clinical program (Study P00041) were 1.6 - 1.8 μm (NDA Module 2.7.1). The broader specification was considered appropriate since development work in early clinical studies using different particle size drug substance in different dosage forms had not indicated a strong correlation between particle size and bioavailability (NDA Section 3.2.P.2.2.1, Formulation Development, and Section 3.2.P.2.7.1, Summary of Biopharmaceutic Studies and Associated Analytical Methods).

A time-consuming part of the drug product manufacturing process is the particle size reduction (i.e. microfluidization) step, in which the particle size of the



input drug substance is reduced to a range specified by an in-process control (NDA Section 3.2.P.3.3). Manufacturing process and scale changes are being evaluated, to improve the throughput of the process. To determine if there could be an allowance for relaxing the microfluidization in-process specification, and the resulting drug product particle size specification, Schering-Plough undertook a bioequivalence study to demonstrate that an oral suspension batch deliberately manufactured with a median particle size of 2.3 μm was bioequivalent to a typical batch with a median particle size of 1.7 μm . We recognized the inherent challenges in attempting to show bioequivalence for a drug with highly variable pharmacokinetic (PK) properties, but proceeded with the study since the available clinical PK data indicated that the two products should behave similarly. In vitro dissolution testing showed similar results by f_2 analysis for these two batches; results for both particle size products were consistent with the proposed specifications.

The bioequivalence study is now complete and the results show that the two batches of different particle sizes are not bioequivalent. In view of this result, we are revising the median particle size specification for the finished drug product from a range of 1.6 - 2.2 μm to a range of 1.5 - 2.0 μm . This range brackets the clinical experience of median 1.6 - 1.8 μm . By a combination of reducing the upper limit of the microfluidization in-process particle size specification and implementing minor modifications to the hold time conditions post compounding, the process will be capable of consistently meeting these revised drug product specifications. However, further reduction in the upper limit is not practical because of the unacceptably long processing times required to achieve an even lower in-process control limit. In addition, the inherent variability of the process will not support a narrowing of the upper and lower specification limits beyond the proposed 1.5 - 2.0 μm range.

The purpose of this amendment is to provide the results of the recently completed BE study and to justify the revised median particle size specification for the product.



2 RESULTS OF BIOEQUIVALENCE STUDY

As outlined above, Study P03409 (Bioavailability of the Posaconazole Suspension When Administered as Two Different Median Particle Sizes: A Replicated Two-Way Crossover Study) was conducted to determine the bioequivalence of two posaconazole suspensions which differed in median particle size, following single dose administration.

2.1 Methods

This was a randomized, open label, single-dose, replicate, crossover (two sequences, four periods) bioavailability study performed at a single site. Forty healthy volunteer subjects were to be dosed with single doses of 400 mg posaconazole oral suspension (40 mg/mL) with a high fat meal in each of the four periods of the study. The subjects were to receive two doses of each of the following treatments:

- Treatment B (test product: also known as L) posaconazole suspension with a median particle size of 2.3 μm
- Treatment A (reference product: also known as S) posaconazole suspension with a median particle size of 1.7 μm .

Each subject was randomized to receive Treatment L and Treatment S in one of two sequences: LSLS or SLSL. Each treatment period was to be separated by at least a 14-day washout period between the single-dose administrations.

The log transformed AUC and Cmax values were statistically analyzed using a linear mixed effect model. Following the FDA guidance for assessing average bioequivalence using replicated designs, the model included sequence, period, and treatment as fixed effects. Subjects (nested in sequence) were considered random with grouped treatment forming a factor analytic (heterogeneous) covariance structure of degree zero. Bioequivalence was assessed by the 90% confidence intervals (90%CI) for the difference between two treatment least squares means



from the above linear mixed effect model. The error variances and associated Satterthwaite degrees of freedom from the linear mixed effect model were used in the calculation of the confidence interval and power.

2.2 Study Results

Forty-two subjects were enrolled and 39 completed all 4 treatment periods. The demographics of those enrolled are presented in **Appendix 1, Table 1**. There were no protocol deviations that would have had a significant impact on the study results.

The relative bioavailability (BA) of the large (2.3µm) particle size batch compared to the small (1.7µm) particle size batch was 76% with 90% confidence interval (CI) limits of 65-88 % (**Table 1**).

Table 1 The Relative BA and 90% Confidence Limit for Log-transformed AUC and Cmax (L- 2.3 µm and S- 1.7 µm)			
Study No. P03409			
Parameter	Number of Subjects	Relative BA (L/S; %)	90% CI
Cmax	39	78.9	69-90
AUC(tf)	39	75.8	66-88
AUC(l)	38 ^a	75.7	65-88
a: AUC(l) could not be determined in one subject			

The intrasubject and intersubject variability appeared to be higher for the large particle size test product compared to that for the small particle size reference product (**Table 2**). For the small particle size reference product, the intrasubject variability was lower than the intersubject variability, as would typically be expected. It is interesting to note that the intrasubject variability for the large particle size test product was greater than the intersubject variability. It is also worth noting that 4 subjects had very low ratio estimates (0.17 to 0.39) for the mean AUC value of the test product to that of the reference product. No obvious explanation for these observations was identified.



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Table 2 Intersubject and Intrasubject Variability in Study No. P03409 (Variance Estimate Based on Log-Transformed Data; L - 2.3 µm and S - 1.7 µm)				
Parameter	Inter-subject variability		Intra-subject variability	
	L	S	L	S
Cmax	0.17	0.09	0.20	0.07
AUC(tf)	0.19	0.13	0.23	0.07
AUC(l)	0.19	0.13	0.22	0.07

Although the analysis did not reveal any statistically significant sequence or period effect, the geometric mean AUC values for the group administered the small particle size batch in periods 1 and 3 (treatment sequence SL₁SL₃) were higher than those for the group who received the small particle size batch in periods 2 and 4 (treatment sequence L₂SL₄; Figure 1). In contrast, the geometric mean AUC values for the large particle size product were relatively consistent no matter which sequence the subject was enrolled into (i.e., the geometric mean AUC values were similar across all periods). As pointed out earlier, 4 subjects in the large particle size group had consistently low AUC values, but did not meet the statistical criteria to be considered outliers and cannot be excluded from the analysis. Therefore, high AUC values for subjects administered the small particle size batch in periods 1 and 3 (sequence SL₁SL₃) and consistently low AUC values for 4 subjects in the large particle size group may have in part contributed to the observed difference in bioavailability; however, this does not completely explain the difference in AUC values between these two batches.



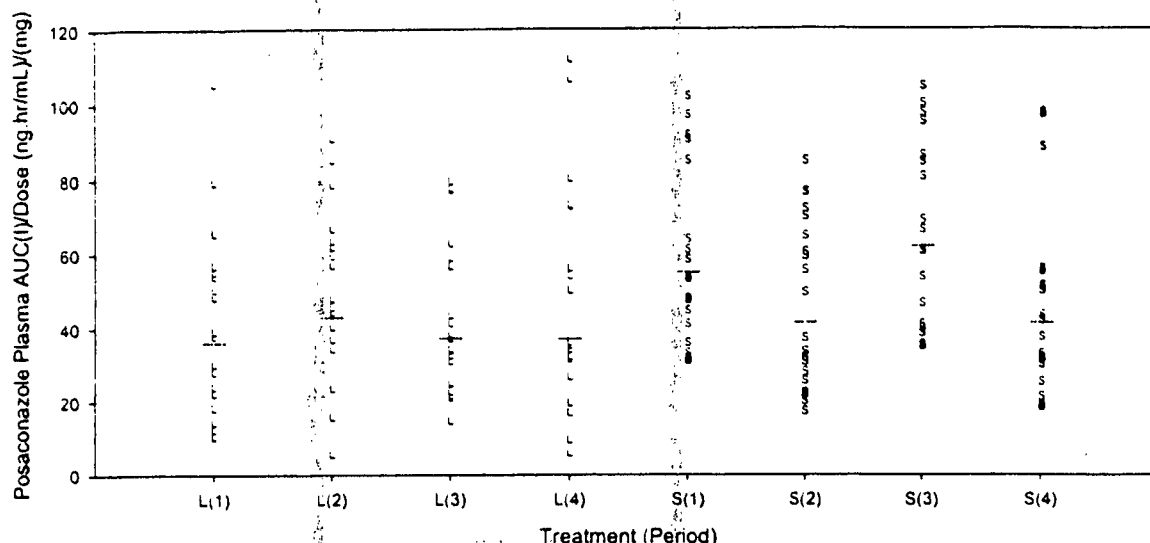


Figure 1 The Dose normalized (to 1 mg) Posaconazole Plasma AUC values in Healthy Volunteers for Large 2.3 µm (L) and Small 1.7 µm (S) particle size suspension for Periods 1, 2, 3, and 4.

There was a 3% difference in potency between the large particle size batch (99% label strength) and the small particle size batch (102% label strength). This difference arose from the fact that the small particle size batch was made using a 2% manufacturing overage whereas the large particle size batch did not include any overage. When this potency difference is taken into account, the difference in relative bioavailability of the large and small particle size batches is likely to be reduced by 3%.

2.3 Discussion

For a long half-life drug such as posaconazole, the key biopharmaceutic parameters from the standpoint of clinical efficacy are the AUC and the steady-state plasma levels achieved through repeated dosing. The 24% difference in AUC observed in P03409 for large vs. small particle size drug products should be viewed

in the context of the inherently variable pharmacokinetics of posaconazole, as illustrated by the following:

- Posaconazole pharmacokinetics exhibit moderate to high intersubject variability (~35%-50% CV; refer to Section 3.1.5 in Module 2.7.2 of the NDA). This can be seen in this BE study, as the range of AUC values for both formulations is large (**Appendix 1, Table 2 and Table 3**). In addition, some individual subjects showed a large difference in AUC after replicate administration of the same formulation (differences of 314 to 27668 ng·hr/mL and 189 to 22166 ng·hr/mL for the 2.3 µm and 1.7 µm suspensions, respectively). This highlights the inherent variability in the pharmacokinetics of the drug under single-dose study conditions.
- Food and fat content of a meal have a greater magnitude of effect on the absorption of posaconazole than the particle size effect observed in this study. The AUC of posaconazole was 2.6 times higher when administered with a nonfat meal or nutritional supplement and 4 times higher when administered with a high-fat meal compared to administration in the fasted state (refer to **Section 3.1.1** in Module 2.7.2 of the NDA).
- Results from the evaluation of pharmacokinetics against clinical outcome in the pivotal clinical efficacy study P00041 suggest there is an association between plasma concentration and response, as responders tended to have greater median plasma concentrations compared to nonresponders. However, it should be noted that the minimum and maximum concentrations were similar among responders and nonresponders. In particular, some subjects who had a positive response had undetectable plasma concentrations at the assessed time point, while other subjects considered nonresponders had relatively high mean concentrations (up to 3490 ng/mL), outlining the inherent variability in posaconazole pharmacokinetics (refer to **Section 3.5.1** of Module 2.7.2).

2.4 Conclusions

Posaconazole suspension with a median particle size of 2.3 µm is not bioequivalent to posaconazole suspension with a median particle size of 1.7 µm. Due to the high variability observed in this study, there is considerable overlap of individual exposures regardless of particle size.



3 REVISED MEDIAN PARTICLE SIZE SPECIFICATION

In view of the BE study result, we evaluated the possibility of revising the median particle size specification for the drug product to bring it more in line with the particle size range of the drug product batches used in the pivotal clinical efficacy study (P00041). This evaluation has established that we can revise the median specification from a range of 1.6 - 2.2 μm to a range of 1.5 - 2.0 μm . This range more evenly brackets the clinical experience of median 1.6 - 1.8 μm . Minor modifications were made to the microfluidization in-process control and the conditions of the post-final compounding holding period to ensure that the process can produce finished product consistently meeting these specifications. The following sections provide a justification for the acceptability of this revised specification.

3.1 Fick's Law

As mentioned in the introduction, this bioequivalence result was unexpected based on our review of earlier clinical study results, which had not indicated a strong correlation between particle size and bioavailability across a particle size range of 1.5 - 3.2 μm . Nevertheless, a thorough examination of the data from this bioequivalence study (race, gender, order of dose administration, etc.) provided no evidence that would impugn the result. In an attempt to rationalize this result using physicochemical principles, we conducted a comparison of predicted bioavailability based on particle size using Fick's First Law^a. This application of Fick's law assumes that dissolution alone is rate-determining for bioavailability - that is, it assumes that there are no other factors governing rate and extent of absorption of the drug other than diffusion-controlled dissolution of the particles. While this is often an oversimplification, this assumption predicts the "worst case" effect of

^a Hussain, A. Prediction of dissolution rates of slightly water-soluble powders from simple mathematical relationships (1972) *J. Pharm. Sci.*, 61 (5), 811-813.



particle size, i.e. it predicts the maximum anticipated theoretical effect for particles of different sizes, since the model assumes that there are no other factors affecting bioavailability that might dilute out or mask the effect of particle size differences.

The rate of dissolution of a poorly water-soluble drug, and the effect of particle size on dissolution, can be calculated from Fick's First Law as follows:

$$Q = \frac{A \times S \times D \times t}{h}$$

where Q = amount of drug dissolved in time t

A = surface area of the drug

S = solubility of the drug in the medium

D = diffusion coefficient of the drug in the medium

h = boundary layer thickness

When comparing suspensions of the same drug substance under equivalent conditions, the term (S.D)/h may be considered a constant; i.e. the quantity of drug Q dissolved in time t is proportional to surface area A. Assuming spherical particles and a mono-dispersed particle size distribution, the total surface area A presented by weight W of powder is given by:

$$A = \frac{W \times 3}{r}$$

where r = particle radius

Thus for a series of suspensions of the same drug substance, differing only in particle size, these equations reduce to:

Q is proportional to $1/r$

From this relationship, it is possible to predict the relative dissolution rates of drug suspensions of a range of particle sizes. Specifically, for posaconazole, we can predict the relative dissolution rates of suspensions of particle sizes ranging from 1.7 µm (the midpoint of the particle size range of the batches used in the pivotal clinical study P00041) to 2.3 µm (Table 3).

Table 3 Predicted relative dissolution rates of posaconazole suspensions of a range of particle sizes	
Particle Size (µm)	Predicted Relative Dissolution Rate ^a
1.7	1.00
2.0	0.85
2.3	0.74
3.0	0.57
a: Predicted from the relationship Q is proportional to 1/r, according to Hussain	

Assuming that dissolution is the only factor governing bioavailability, the predicted dissolution rates in **Table 3** would correspond to the relative bioavailability of the different products with these particle sizes. The results derived from Fick's law are in agreement with the 76% bioavailability value obtained from the BE study. Fick's law predicted a slightly lower bioavailability than was actually observed, again consistent with the model predicting the worst-case absorption level. The model also predicts an 85% bioavailability for a particle size of 2.0 µm. We have observed differences of 35 – 50% in pharmacokinetic parameters among subjects and across clinical studies using drug product of the same particle size, and consider a 15% relative difference between 1.7 and 2.0 µm particle sizes to be insignificant, since there appear to be a broad range of exposures that correlate with positive clinical responses to posaconazole.

3.2 Pharmacokinetics of Posaconazole Over a Broader Range of Drug Product Particle Sizes

The PK study results presented in **Section 2.4** (P03409) showed a difference in bioavailability between suspension particle sizes of 1.7 µm and 2.3 µm. However, a review of single-dose and multiple-dose PK data from studies using a broader range of particle sizes (1.5 – 3.2 µm) will put this difference, and the difference expected after tightening the specifications, into perspective.



3.2.1 Single-dose PK

Single-dose PK data after oral administration of suspension formulation in fed subjects are available from three previous studies. Corresponding data are also available on two different capsule formulations of posaconazole in fed subjects from two previous studies. Study and formulation information are provided in **Table 4** below. The compositions of these three formulations (the oral suspension, and two capsule formulations) are presented in **Table 5**. The dose-normalized (to 1mg) AUC(I) values for these studies are shown in **Figure 2**.

Table 4 Formulations used in single-dose PK studies			
Study	Number of Subjects	Formulation	Drug Substance Particle Size (µm)
I96099	18	Oral suspension	1.5
I97195	4	Oral suspension	1.5
P01940	6	Oral suspension	1.6 – 1.7
P03409	38	Oral suspension	1.7
P03409	38	Oral suspension	2.3
I95099	23	Capsule formulation 3151	3.0
I96172 ^a	24	Capsule formulation 3151	3.0
I96172 ^a	24	Capsule formulation 3227	3.2
a: Capsule formulations 3151 and 3227 were shown to be bioequivalent in Study I96172			



Table 5 Formulations of Posaconazole Oral Suspension and capsule formulations evaluated in single-dose PK studies				
Ingredient		Quantity (mg)		Function
Oral Suspension (normalized to 100mg dose)				
Posaconazole, Micronized		100		Active ingredient
Polysorbate 80		25		Surfactant
Simethicone		7.5		Anti-foaming Agent
Sodium Benzoate		5		Preservative
Sodium Citrate Dihydrate		1.5		Buffer
Citric Acid Monohydrate		3.75		Buffer
Glycerin (Glycerol)		250		Cap Anti-locking Agent
Xanthan Gum		7.5		Suspending agent
Liquid Glucose (Corn Syrup)		875		Sweetener
Titanium Dioxide		10		Opacifier
Artificial Cherry Flavor #13174		12.5		Flavoring agent
Purified Water		q.s. to 2.5 mL		Diluent
Capsule		Formulation # 3151	Formulation # 3227	
Posaconazole, Micronized		100	100	Active ingredient
Microcrystalline cellulose		300	245.25	Diluent
Poloxamer 188		50	50	Surfactant
Croscarmellose sodium		50	30	Disintegrant
Povidone		25	22.5	Binder
Magnesium stearate		2.5	2.25	Lubricant
Capsule shell, blue opaque		No. 0 Elongated	No. 0	
Total capsule fill weight		527.5	450	



Although the suspension and capsule drug products are fundamentally different formulations, pharmacokinetic comparisons between the two are still relevant. The first requisites for drug absorption from a capsule dosage form are disintegration of the capsule shell followed by wetting and dispersion of the powder fill to yield a suspension. We have determined that the drug substance lots used in these capsule formulations, when dispersed in an aqueous vehicle and tested by the particle size method used for the oral suspension, yield particle sizes of 3.0 and 3.2 μm (Table 4). Thus it can be assumed that after ingestion these capsule formulations would have yielded suspensions of median particle sizes of 3.0 to 3.2 μm in the gastro-intestinal contents. Thus comparison of capsule and oral suspension pharmacokinetic data is considered relevant.

Both formulations contain surfactants – polysorbate 80 in the oral suspension and poloxamer 188 in the capsules – at the levels shown in Table 5. There is no reason to believe that poloxamer 188 is substantially more effective than polysorbate 80 in enhancing the absorption of posaconazole, such that it might significantly affect the relative bioavailability of these formulations.

A comparison of dose-normalized single-dose AUC against particle size in the range of 1.5 to 3.2 μm from various Phase I studies did not reveal a clear trend of an effect of the particle size on the AUC after a single-dose oral administration (Figure 2). Rather, the dose-normalized AUC across this range appeared to be variable and independent of particle size. While there is considerable variability in the observed AUCs – consistent with posaconazole being a moderate to highly variable drug – there is also considerable overlap in the ranges of AUCs observed from the capsule formulations (with drug substance particle sizes of 3.0 – 3.2 μm) and suspension formulation (drug substance particle sizes 1.5 – 2.3 μm).

3.2.2 Steady-State PK

Steady-state PK data after oral administration of the suspension formulation in fed subjects are available from five previous studies; the particle sizes of the drug



product batches used in those studies ranged from 1.6 to 1.8 μm . Projected steady state AUC values were calculated for BE study P03409 by multiplying AUC(0-12 hr) from Day 1 data from P03409 (mean of the two doses of the same particle size for each subject) with known mean accumulation from Phase I studies (P02489, P02810, P02812, P02811, and P02862; refer to **Section 3.6** of Module 2.7.2 for discussion of accumulation). The dose-normalized (to 1mg) steady-state AUC values for these studies are shown in **Figure 3**.



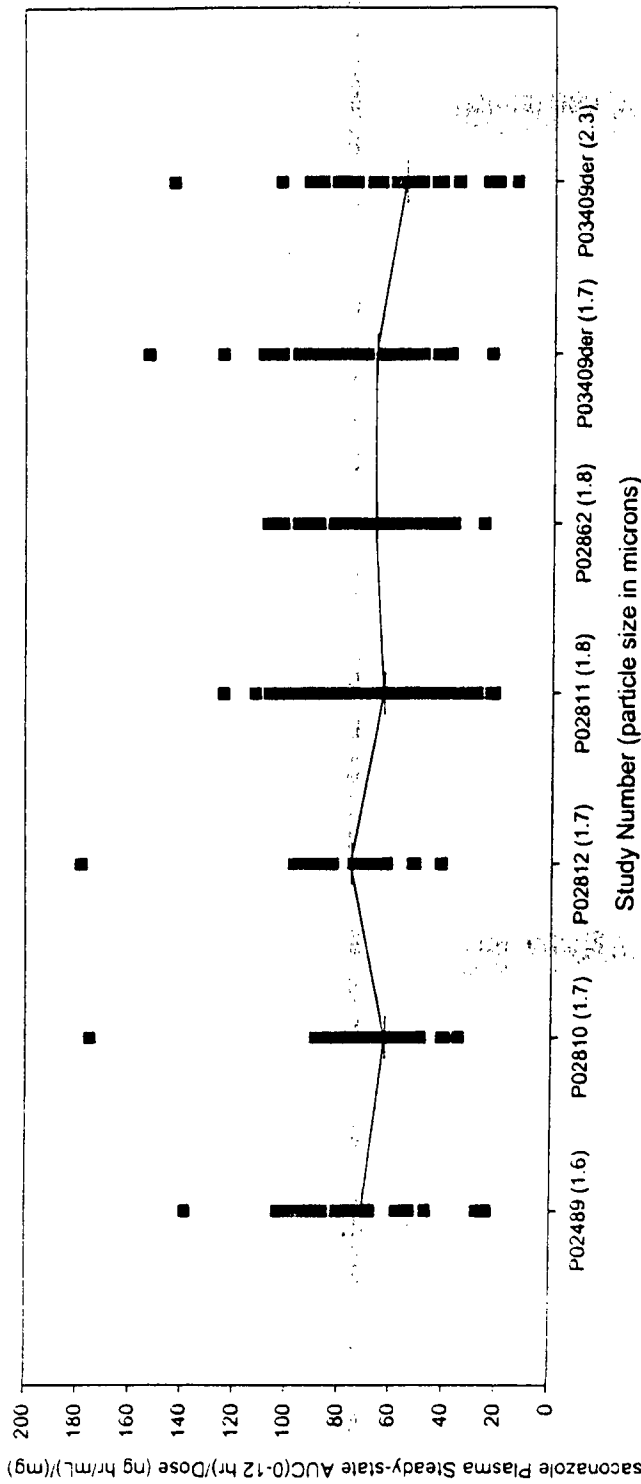


Figure 3 Dose-normalized (to 1 mg) steady state AUC (0-12 hr) values after oral administration of posaconazole suspension to fed subjects in Phase I studies with varying particle sizes. [Observed steady state AUC(0-12) from P02489 (n=12; includes two values from each of the 12 subjects, one on day 8 and one on day 10), P02810 (n=24), P02812 (n=23), P02811 (n=56), and P02862 (n=35); P03409der (n=39)- projected steady state AUC value for P03409. Horizontal dotted lines on plot denote geometric means and the solid line connects the geometric means.]



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Consistent with the single dose data, a comparison of dose-normalized steady state AUC against particle size over the range of 1.5 to 2.3 μm from various Phase I studies did not reveal a clear trend of steady state AUC with particle size (Figure 3). The steady state AUC appeared to be independent of particle size over this range, with considerable overlap in the ranges of AUCs observed from all particle sizes, up to and including 2.3 μm .

3.2.3 Conclusion

Single-dose and multiple-dose PK data from studies using a broader range of particle sizes (1.5 – 3.2 μm) were reviewed. This review suggests that the AUC for drug product at 2.0 μm (the upper limit of the revised particle size specification), which would presumably lie between the 1.7 and 2.3 μm data sets, would not be significantly different to drug product used in the pivotal clinical study (1.6 μm – 1.8 μm) in the context of the broad PK variability we see with posaconazole.

3.3 Optimization of the current manufacturing process

We intend to reduce the particle size specification of the finished drug product from 1.6 - 2.2 μm to 1.5 – 2.0 μm . The justification of specifications (NDA Section 3.2.P.5.6) provided a rationale for selection of the current median particle size specification, based in part on our manufacturing capability at the commercial site. We have subsequently identified a source of variability in one specific part of the drug product process, namely the bulk compounded product holding/filling period. During a review of the batch analysis data for the development site (Kenilworth) and the commercial site (Patheon), we noted that there was a trend towards slightly higher particle size medians at the commercial site, as summarized in Table 6. The shift to a higher median was traced to a greater increase in particle



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size growth in the Patheon batches after the initial compounding/microfluidization step.

Table 6 Summary of Historical Median Particle Size Results for Posaconazole Oral Suspension

	Drug Product Manufacturing Site		
	Schering Kenilworth, New Jersey	Patheon Whitby, Ontario, Canada	Overall Database
Number of Batches ^a	35	30	65
Average Drug Product Median Particle Size (µm)	1.7	1.9	1.8
Standard Deviation	0.11	0.10	0.16
Range (µm)	1.6 to 2.0	1.7 to 2.1 ^b	1.6 to 2.1 ^b
a: Includes all representative full-scale (200 L) batches manufactured at target processing conditions, including those manufactured subsequent to the NDA filing.			
b: One Patheon batch showed an unexpectedly high value of 2.4 µm. No definitive root cause for this one result could be identified, but it is attributed to process variability arising from a lack of temperature control during the final compounded drug product hold period.			

The median particle size of the finished drug product is slightly higher than that of the posaconazole concentrate at the end of microfluidization. The difference in median particle size values for the concentrate at the end of microfluidization and at any point in the process thereafter is referred to as “Delta”, and is denoted by the symbol “Δ”. As discussed in the Pharmaceutical Development section of the NDA (Section 3.2.P.2.2.1.3, Optimization of Drug Substance Particle Size in Drug Product), direct comparisons of the absolute particle size results for the microfluidized concentrate versus the finished product are difficult because these two determinations use slightly different analytical methods, and are performed on samples of different compositions. Nevertheless, the Δ values enable some comparisons to be made with regards to the total extent of particle size growth after microfluidization has been completed (i.e., throughout the holding period for the concentrate, during final compounding of the bulk suspension, throughout the



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holding period for the bulk compounded product, and throughout primary packaging/filling).

As mentioned above, we noted that batches manufactured at Patheon tended to exhibit more growth (i.e., higher Δ values) than batches manufactured at Schering's research facility in Kenilworth, New Jersey, as shown graphically in **Figure 4** and **Figure 5**. This observation led us to focus on potential differences between the Kenilworth and Patheon batches.

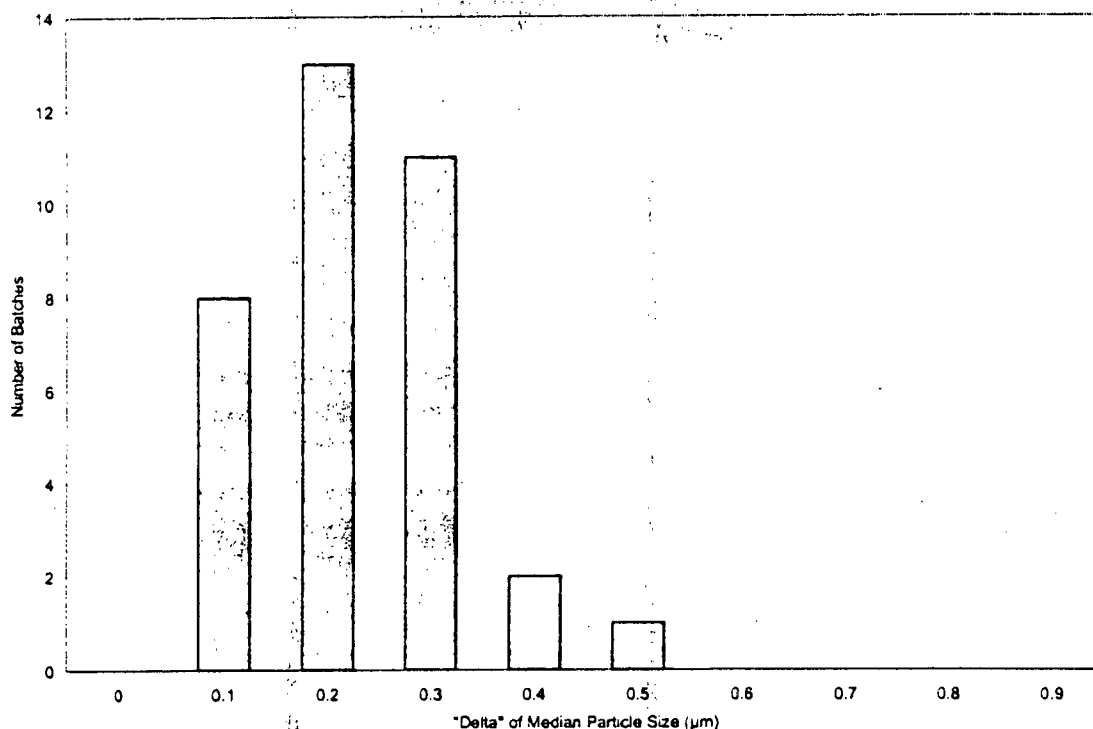


Figure 4 "Delta" of median particle size – Kenilworth batches

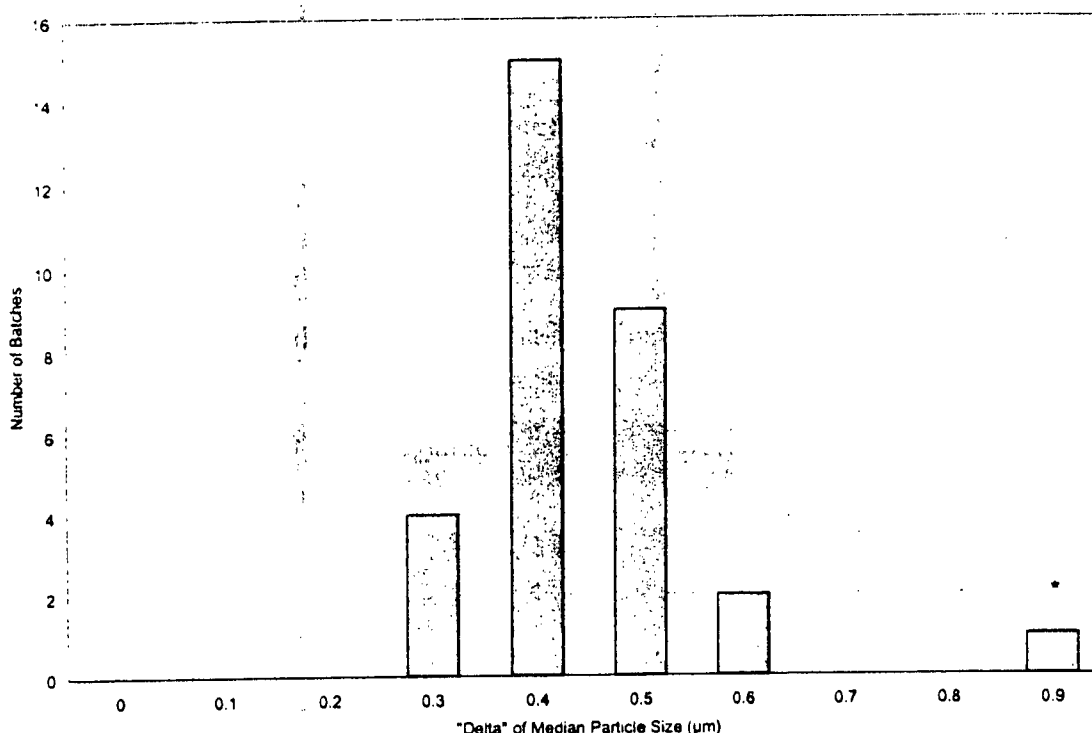


Figure 5 "Delta" of Median Particle Sizes – Patheon batches. (* An unexpectedly high delta value of 0.9 µm was observed for one Patheon batch, for which no definitive root cause could be identified.)

One of the factors that was noted to be different was that the agitation speed during the Final Compounding step and during the bulk compounded product holding period was generally lower in Kenilworth. A fixed agitation speed was not established for batches manufactured during development at the Kenilworth site. However, the agitation speed was typically in the range of about 100 to 275 RPM. At Patheon, the speed during the latter steps of Final Compounding as well as during the bulk compounded product hold was fixed at a target of 300 ± 10 RPM to ensure thorough mixing of the bulk suspension. An additional control that was introduced as part of technology transfer was that all batches made at Patheon incorporated temperature control of the batch at $24 \pm 3^\circ\text{C}$ during final compounding (NDA Section 3.2.P.2.3.6.3). However, temperature control was not implemented

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during the bulk compounded product holding period either at the Kenilworth or Patheon site.

We conducted an investigative study into the effects of agitation rate and the consequent batch temperature rise on particle growth. Following some initial experiments, a definitive laboratory experiment at a 5% scale was conducted. Laboratory scale agitation rates of 1400 RPM and 150 RPM were selected for this experiment because they provided similar bulk mixing to commercial scale agitation rates of 300 RPM and 60 RPM respectively. This reproduced the particle size growth behavior previously observed for an experimental full production scale batch that was manufactured to investigate the consequences of extending the process hold time limits filed in the application. The results are graphically presented in **Figure 6**, which shows three sublots (each at 5% of full scale) used to independently assess the effects of agitation and temperature control on particle size growth during the holding period for the bulk compounded product:

- Sublot 1 was mixed at high speed with no temperature control (i.e., the temperature was allowed to rise via the mechanical energy imparted through mixing).
- Sublot 2 was mixed at the same high speed, but with the bulk temperature controlled at $24 \pm 3^{\circ}\text{C}$ (i.e., the same temperature control that is applied at Patheon during the Final Compounding step for the commercial process).
- Sublot 3 was mixed at low speed with the bulk temperature controlled at $24 \pm 3^{\circ}\text{C}$.



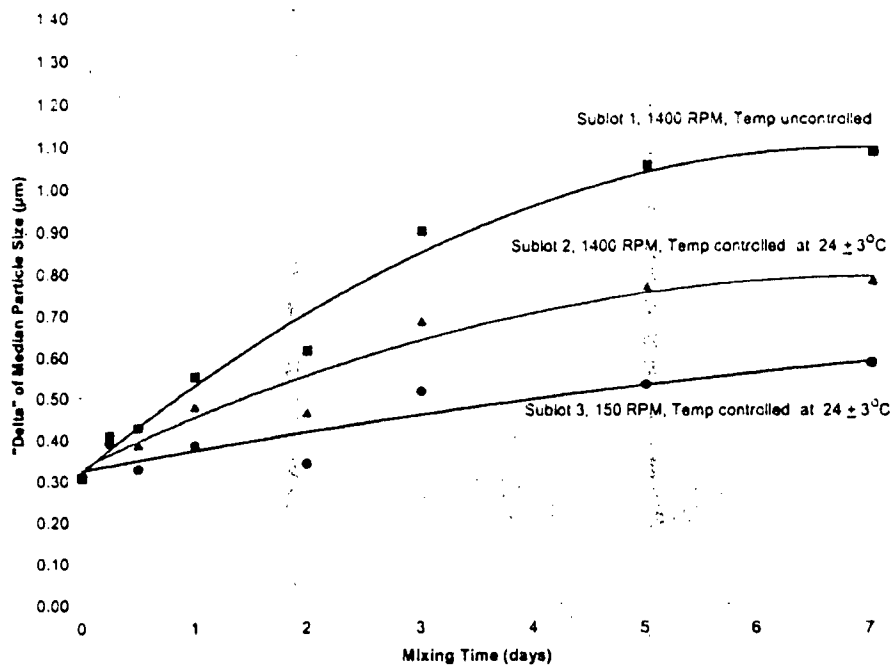


Figure 6 Changes in the calculated Δ values of the bulk compounded product over time – Batch 82163-063

The results of the study clearly demonstrated the impact over time of both temperature and agitation rate on particle size growth. It was concluded from this study that:

- The degree of particle size growth was shown to be proportional to both agitation speed and batch temperature.
- By controlling the batch temperature while maintaining a "high" agitation speed, the degree of particle size growth was significantly reduced.
- By reducing the mixing to a "low" agitation speed and also controlling the batch temperature, the degree of particle size growth was further reduced

Since this product is a suspension, one potential impact of reducing the agitation speed during the bulk compounded product hold is inhomogeneity that might result from particle settling. Posaconazole Oral Suspension has been demonstrated to be a very stable suspension. Formulation studies have been

performed to characterize the settling behavior of the packaged suspension, which have demonstrated that the product settles very slowly and remains homogeneous for up to 14 days upon standing (as discussed in NDA **Section 3.2.P.2.2.3.2**, Settling Rate). This result was also confirmed in a separate experiment on a sample of bulk product after final compounding where the suspension was held without any mixing at all. Therefore the bulk product is expected to remain homogeneous during the holding period, even with reduced agitation speeds.

As a result of this investigative study, we will implement two minor process modifications to minimize the rate of particle size growth during the bulk compounded product holding period:

- The first of these actions is to extend the temperature control ($24 \pm 3^{\circ}\text{C}$) used during the Final Compounding operation to also include the bulk compounded product holding period.
- The second action is to reduce the agitation speed (from 300 to 60 RPM) following completion of the Final Compounding step.

These two actions will reduce the variability of the process and provide final product particle size results consistent with those seen during development at Kenilworth. In the context of the revised finished product specification of $1.5 - 2.0 \mu\text{m}$, the summary provided in **Table 6** shows that all Kenilworth batches meet the proposed new specification. The detailed batch analysis table can be found in NDA **Section 3.2.P.5.4**. **Table 6** also shows that, with the anticipated reduction in median particle size of 0.1 to $0.2 \mu\text{m}$ for Patheon batches as a result of these process changes, these will also meet the revised specification. One batch has been produced at Patheon with an unexpectedly high median particle size ($2.4 \mu\text{m}$, $\Delta = 0.9 \mu\text{m}$). No definitive root cause for this one result could be identified, but is attributed to process variability arising from a lack of temperature control during the final compounded drug product hold period.

To provide an even higher level of assurance that commercial production will consistently meet the revised specification, we are also reducing the upper limit of the in-process control specification for the Particle Size Reduction step from $1.3 - 1.6 \mu\text{m}$ to $1.3 - 1.5 \mu\text{m}$. Reducing this in-process control limit by $0.1 \mu\text{m}$ will reduce



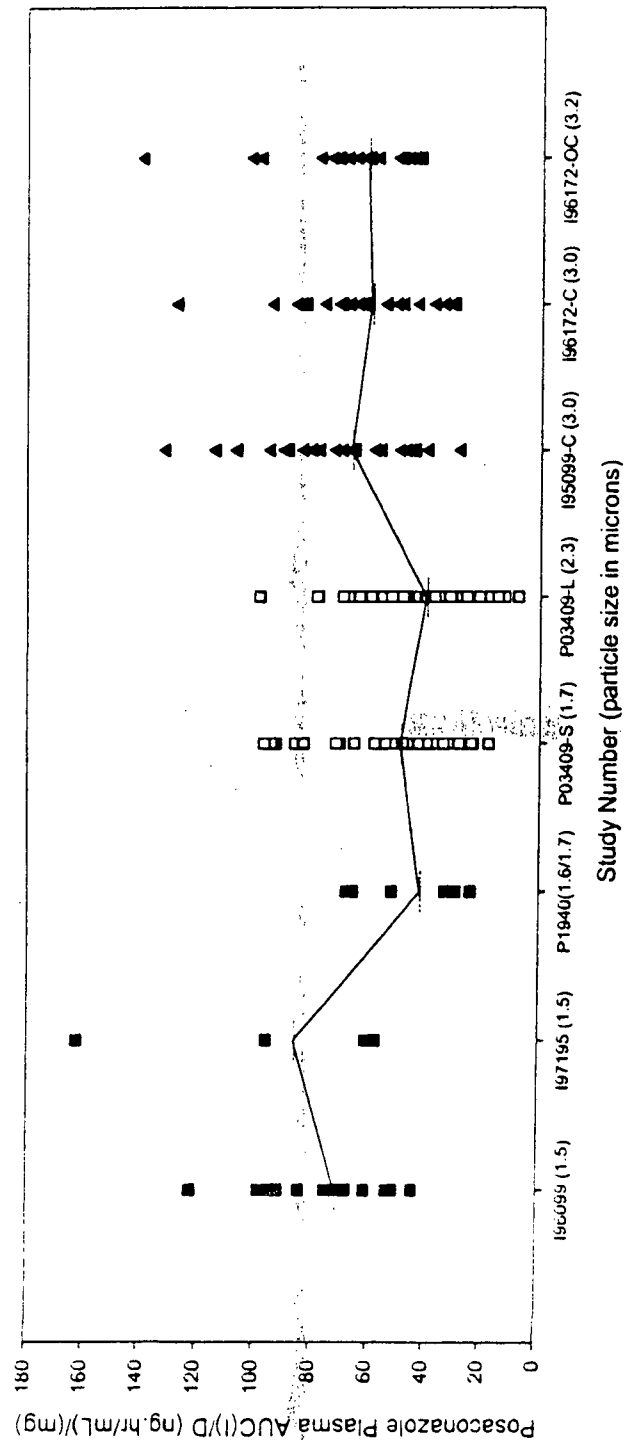


Figure 2 Dose-normalized (to 1 mg) AUC values from single-dose oral administration of posaconazole to fed subjects in Phase I studies with varying particle sizes. The solid squares represent the suspension formulation and the solid triangles represent capsule formulations [P03409 (S, 1.7 µm particle size; L, 2.3 µm particle size); 195099 and 196172 (C, capsule formulation # 3151); (OC, capsule formulation # 3227). Horizontal dotted lines on plot denote geometric means and the solid line connects the geometric means.]

the final drug product particle size median by a similar amount. **Table 7** summarizes the cumulative effects of various parts of the process. Particle size data at these various intermediate points are not routinely gathered during production, and so these data are derived from investigative studies on both laboratory and full scale batches.

Table 7 Particle size Δ values for various stages in the drug product manufacturing process			
Process Stage	Kenilworth	Patheon	Investigative Studies
Δ Concentrate hold	Not studied	Not studied	0.1
Δ Final compounding	Not studied	Not studied	0.1 – 0.2
Δ Bulk compounded product hold	Not studied	Not studied	0.1 ^a
Δ total (μm)	Ave = 0.2 Range = 0.1 – 0.5	Ave = 0.4 Range = 0.3 – 0.6 ^b	0.3 – 0.4
a: Proposed hold conditions for commercial manufacture, i.e., low agitation speed (60 RPM) and controlled temperature at $24 \pm 3^\circ\text{C}$. b: One Patheon batch showed an unexpectedly high Δ value of 0.9 μm. No definitive root cause for this one result could be identified, but it is attributed to process variability arising from a lack of temperature control during the final compounded drug product hold period.			

Based on the data shown in **Table 7**, we anticipate the process modifications will result in a typical reduction in Δ for commercial site (Patheon) batches of about 0.1 μm . These data also indicate that an in-process result at the upper end of our specification, 1.5 μm , will yield a maximum result of 1.9 μm at release. A result at the low end of the in-process specification, 1.3 μm , could yield a result of 1.5 μm at release for batches that have minimal hold times. Therefore, the revised release specification range of 1.5 – 2.0 μm provides a margin that allows for the variability in both the analytical methods and the process. The specifications for % less than 3 μm and % less than 10 μm remain unchanged. The revised drug product particle size specification is provided in the attached specification page. Also provided are the temperature controls used during the bulk compounded product holding period in the drug product Method of Manufacture (**Section 3.2.P.3.3**) and revised in-process

controls (**Section 3.2.P.3.4**). The manufacturing batch records will be revised to include the revised in-process control, temperature and appropriate agitation speed.

Based on the data and rationale outlined above, the additional controls in the manufacturing process will consistently produce drug product meeting the revised release specifications; the finished product particle size results will be similar to those previously observed for the Kenilworth batches.

4. CONCLUSION

The recent bioequivalence clinical study results show posaconazole drug product batches of particle sizes 1.7 and 2.3 μm are not bioequivalent. Based on the BE study results and the capability of the process, the finished product median particle size specification has been revised to 1.5 - 2.0 μm . This tightened range continues to bracket the median particle size range of 1.6 - 1.8 μm used in the pivotal clinical efficacy trial. This revised specification is justified by:

- calculation of theoretical absorption based on Fick's law, which shows that, even if the assumptions inherent in this worst case analysis were true, suspension with a particle size of 2.0 μm would have (at a minimum) 85% of the absorption of a 1.7 μm suspension, which is within the range of the variability seen between different clinical studies using drug product batches of similar particle size.
- review of previous studies (both single dose and steady state AUCs vs. particle size) which, although not definitive, shows that there is significant variability within each group and that there is no strong relationship between absorption and particle size across the range of 1.5 to 3.2 μm .
- the capability of the manufacturing process which, following introduction of two minor modifications to the process and a reduction in the upper limit of the in-process particle size specification at the end of microfluidization, will be able to consistently meet the revised specification.
- the data presented in the histograms in **Figure 4** and **Figure 5** which show that, following implementation of the changes discussed in **Section 5**, the particle size values for the majority of final product batches will be consistent with the range used in the pivotal clinical study.

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Based on the above arguments, we consider the revised median particle size specification for the drug product to be appropriate.



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Appendix 1

Table 1 Summary of Demographic Characteristics for Study No. P03409	
Sex (n,%)	
Female	27 (64)
Male	15 (36)
Race (n,%)	
Caucasian	30 (71)
Non-Caucasian	12 (29)
Asian	1 (2)
Black	7 (17)
Other	4 (10)
Age (yrs)	
Mean (SD)	25.0 (4.9)
Median (Range)	23.0 (18 – 36)
Weight (kg)	
Mean (SD)	64.1 (10.84)
Median (Range)	64.8 (50.0 - 88.3)
Height (cm)	
Mean (SD)	169.3 (7.60)
Median (Range)	168.5 (152.0 – 184.0)
n = number of subjects	



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Table 2 Summary Statistics of Posaconazole Pharmacokinetic Parameters After Single-Dose Oral Administration of 1.7 μ m Particle Size Suspension (Balanced Data, Treatment S, Study No. P03409)

Parameter	Unit	n	Mean	CV(%)	Min	Max	Median	Geometric Mean
AUC(l)	ng.hr/mL	38 ^a	21808	45	6919	41929	20497	19590
AUC(tf)	ng.hr/mL	39	21054	45	6611	41175	19884	18913
Cmax	ng/mL	39	620	39	180	1270	592	574
Tmax	hr	39	5.72	31	4.00	12.00	5.00	N/A
t1/2	hr	38 ^a	22.8	24	13.70	36.92	21.28	N/A
Tlag	hr	39	0.14	161	0.00	0.50	0.00	N/A

n = number of subjects

N/A = not applicable

a: half-life and AUC(l) could not be determined in one subject

Table 3 Summary Statistics of Posaconazole Pharmacokinetic Parameters After Single-Dose Oral Administration of 2.3 μ m Particle Size Suspension (Balanced Data, Treatment L, Study No. P03409)

Parameter	Unit	n	Mean	CV(%)	Min	Max	Median	Geometric Mean
AUC(l)	ng.hr/mL	38 ^a	17688	54	2258	43562	15912	14932
AUC(tf)	ng.hr/mL	39	17203	54	1979	42928	15467	14495
Cmax	ng/mL	39	534	54	82.9	1510	514	456
Tmax	hr	39	6.26	122	4.00	72.00	5.00	N/A
t1/2	hr	38 ^a	22.6	24	15.3	43.4	21.1	N/A
Tlag	hr	39	0.28	91	0.00	0.50	0.50	N/A

n = number of subjects

N/A = not applicable

a: half-life and AUC(l) could not be determined in one subject



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Activity of posaconazole in the treatment of central nervous system fungal infections

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Objectives: A multinational, multicentre, open-label clinical trial was conducted to evaluate the safety and efficacy of posaconazole, an extended-spectrum triazole antifungal agent, in subjects with invasive fungal infections who had refractory disease or who were intolerant of standard antifungal therapy. In this subanalysis, we report on those subjects in this trial who had a fungal infection that involved the CNS.

Methods: Subjects received posaconazole oral suspension 800 mg/day in divided doses for up to 1 year; however, subjects could receive additional therapy as part of a treatment-use extension protocol. A blinded, third-party data review committee determined subject eligibility and outcome.

Results: Of the 330 subjects who enrolled in the study, 53 had infections of the CNS, of which 39 were considered evaluable for efficacy. Most had refractory disease (37 of 39) and underlying HIV infection (29 of 39). Twenty-nine subjects had cryptococcal infections, and 10 had infections caused by other fungal pathogens [*Aspergillus* spp. (four), *Pseudallescheria boydii* (two), *Coccidioides immitis* (one), *Histoplasma capsulatum* (one), *Ramichloridium mackenziei* (one), and *Apophysomyces elegans* plus a *Basidiomycetes* sp. (one)]. Successful outcomes were observed in 14 of 29 (48%) subjects with cryptococcal meningitis and five of 10 (50%) subjects with CNS infections due to other fungal pathogens. Posaconazole was well tolerated.

Conclusions: These data suggest that posaconazole, as an oral medication, has clinical activity against fungal infections of the CNS and may provide a valuable alternative to parenteral therapy in patients failing existing antifungal agents.

Keywords: salvage treatment, cryptococcal infections, triazoles, invasive

Introduction

Morbidity and mortality associated with fungal infections of the CNS remains unacceptably high despite treatment with available antifungal therapy.^{1,2} Most patients with CNS fungal infections are immunocompromised, adding to the challenge of treating these opportunistic infections.^{2–5} *Cryptococcus neoformans* is the most common fungal infection in patients infected with HIV and often represents the AIDS-defining opportunistic infection.^{6,7} Other fungal pathogens that can invade the CNS include *Aspergillus* spp., *Coccidioides immitis*, *Histoplasma capsulatum*,

Pseudallescheria boydii, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis* and *Zygomycetes*. Zygomycosis may occur in patients who have significant metabolic abnormalities that affect phagocytic responses (e.g. diabetic ketoacidosis, iron overload states and malnutrition).⁸ Additionally, these fungal pathogens, especially the endemic mycoses such as histoplasmosis and coccidioidomycosis, may also cause severe disease in immunocompetent persons exposed to a large inoculum of conidia.

The most recent treatment guidelines for cryptococcal meningitis recommend therapy with amphotericin B with or without flucytosine,¹ however, clinical response rates in HIV-infected

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patients are low. After 2 weeks of therapy, negative CSF cultures were observed in eight of 12 patients with AIDS treated with amphotericin B and flucytosine, and two of 14 patients treated with amphotericin B alone.⁹ Fluconazole was shown to be less effective than amphotericin B for induction therapy, but effective as step-down therapy in patients infected with HIV. Prolonged maintenance therapy with fluconazole is recommended to prevent recurrence in patients with HIV; however, some patients experience relapses during therapy.¹ Other patients develop immune reconstitution inflammatory syndrome (IRIS) during HAART. Patients with IRIS may develop an inflammatory meningitis, characterized by increased white blood cell counts in the CSF despite negative CSF cultures and declining *C. neoformans* CSF titres.¹⁰

Infections in the CNS are more difficult to treat than those outside of the CNS owing to the added complexity of drug penetration across the meninges. The standard of care for CNS infections caused by moulds has been amphotericin B; however, low efficacy rates and long-term tolerability issues make it less than optimal. Fluconazole is currently the recommended treatment for coccidioidomycosis involving the CNS.¹¹ Voriconazole has demonstrated promising activity in the CNS,^{12,13} but reports of adverse events and its lack of activity against Zygomycetes^{14,15} may limit its usefulness as an alternative to amphotericin B. Thus, there remains interest in newer antifungal therapies that have potent activity against a wide range of fungal pathogens in the CNS, are well tolerated and can be administered to seriously ill patients over long periods of time.

Posaconazole is a new extended-spectrum triazole antifungal agent with *in vitro* activity against many yeasts and moulds that have a predilection for the CNS. These include Zygomycetes, *Aspergillus* spp., *Cryptococcus* spp., *P. boydii*/*Scedosporium apiospermum*, *C. immitis*, *H. capsulatum*, and *B. dermatitidis*.^{14,16–22} In experimental animal models of CNS fungal disease, including infections with *P. boydii*, *Cladophialophora bantiana*, *Wangiella dermatitidis* and *Aspergillus fumigatus*, posaconazole substantially reduced fungal burden in the brain and improved survival.^{23–26} In addition, oral posaconazole therapy resulted in negative CSF titres in a rabbit model of cryptococcal meningitis²⁷ and in successful treatment of a CNS infection in a chimpanzee with disseminated coccidioidomycosis.²⁸ Posaconazole, in combination with amphotericin B, was also shown to significantly reduce fungal burden in brain tissue and improve survival in a murine model of cryptococcosis.²⁹ No significant antagonism between these agents was observed.²⁹ These data provide support for the fact that posaconazole, a lipophilic compound, has therapeutic activity in the CNS after oral administration.

Posaconazole is bioavailable following oral administration and is well tolerated in both healthy volunteers and in patients with invasive fungal disease. In a model-dependent pharmacokinetic analysis of patients with refractory invasive fungal infection or febrile neutropenia, posaconazole bioavailability was greatest when administered in divided doses, with average plasma concentrations of 758 and 259 ng/mL following 400 mg twice-daily and 800 mg once-daily dosing, respectively.³⁰ Posaconazole has a large volume of distribution indicating extensive distribution in body tissues.³¹ Preliminary clinical studies suggest that posaconazole has a favourable safety and tolerability profile during short- and long-term (6–12 month) treatment.^{32–35} Case reports of patients with invasive fungal infection who required lengthy antifungal therapy to control or cure the disease suggest that posaconazole is well tolerated over long-term administration.^{36,37}

The safety and efficacy of posaconazole were evaluated in a large, multinational (11 countries), multicentre (72 centres), open-label study of patients with invasive fungal infections who were intolerant of or who had disease refractory to available antifungal therapy. Herein, we report on those subjects in this trial who had a fungal infection that involved the CNS.

Patients and methods

Study population

Subjects, aged 13 years or older, with a proven or probable invasive fungal infection (adapted from the European Organisation for Research and Treatment of Cancer/Mycosis Study Group criteria)³⁸ who were intolerant of or had disease refractory to standard therapies were eligible for study participation. Subjects were considered intolerant of available antifungal therapies if they had: (i) history of serious, severe or life-threatening toxicity while receiving antifungal therapy [i.e. organ toxicity (grade 3 or greater); nephrotoxicity defined as persistent serum creatinine greater than 2× the upper limit of normal (ULN) or recurrent serum creatinine greater than 2× the ULN after resumption of amphotericin B therapy; or idiosyncratic or anaphylactic reaction to other antifungal agents]; (ii) preexisting organ dysfunction precluding the use of standard antifungal therapies, including preexisting renal insufficiency; (iii) high risk for toxicity because of underlying disease or concomitant medications; or (iv) previous major idiosyncratic or hypersensitivity reaction to antifungal agents. Subjects were considered to have disease refractory to standard therapies (amphotericin B, other azoles or flucytosine) if they experienced disease progression or if their conditions did not improve clinically, while receiving antifungal therapy given for an adequate period of time (10–14 days for cryptococcal meningitis, 7 days for infections caused by *Aspergillus*, 14 days for non-*Aspergillus* moulds, 30 days for histoplasmosis and 180 days for coccidioidomycosis).

Subjects were excluded from participation if they were pregnant or lactating; had not undergone surgical sterilization and were using inadequate contraception (women only); had concurrent progressive neurological disease (e.g. seizures, demyelinating syndromes, unstable multiple sclerosis, peripheral neuropathy); required artificial ventilation but were unlikely to undergo extubation within 24 h of study entry; had a history of serious or severe hypersensitivity to azole antifungals; received medications known to interact with azoles (i.e. quinidine) within 10 days of study entry or drugs that lower azole serum concentrations (i.e. rifampicin, phenytoin) within 7 days of study entry; had electrocardiography results showing prolonged QTc interval (≥20% above normal) within 7 days of study entry; needed concomitant systemic antifungal agents during the study period; had abnormal hepatic function test results (alanine aminotransferase or aspartate transferase greater than 10× ULN); or were expected to live <72 h.

Each subject or a legal representative provided written, informed consent in accordance with the World Medical Association Declaration of Helsinki before receiving the first dose of study drug. The study protocol was reviewed and approved by an institutional review board or an independent ethics committee in accordance with good clinical practices.

Study design and treatment regimen

Subjects were treated with posaconazole oral suspension (40 mg/mL) (Schering-Plough Research Institute, Kenilworth, NJ, USA) at a daily dose of 800 mg in divided doses (200 mg four times daily or 400 mg twice daily). Doses were to be administered with food or a liquid nutritional supplement whenever possible. Subjects were treated for a minimum of 28 days and for at least 7 days after complete resolution

of symptoms. The protocol-defined treatment duration was a maximum of 12 months; however, subjects who required longer therapy could be enrolled in compassionate use programmes to continue maintenance therapy.

An independent, blinded, data review committee (DRC) consisting of 15 experts in the diagnosis and treatment of fungal infection or in the radiography of invasive fungal infections, reviewed each case to ensure that predetermined eligibility criteria were satisfied. Teams consisting of three DRC members determined eligibility and outcomes using protocol-defined criteria (see 'Efficacy and safety definitions'). They reviewed treatment outcomes until the end of therapy or 12 months of treatment, whichever came first.

Efficacy and safety definitions

The primary efficacy endpoint was global response at the end of treatment as assessed by the DRC according to the following definitions. A successful outcome was defined as the resolution of all attributable symptoms, signs and radiographic abnormalities (complete response) or clinically meaningful improvement in attributable symptoms, signs and radiographic abnormalities (partial response). A non-successful outcome was defined as no improvement in attributable symptoms, signs and radiographic abnormalities (stable disease) or deterioration in attributable clinical or radiographic abnormalities that necessitated alternative antifungal therapy or resulted in death (failure). If for any reason the response could not be assessed, the subject was considered to have a non-successful outcome.

Safety and tolerability were evaluated throughout the study period and are reported herein on all subjects who enrolled in the clinical trial ($n = 330$). Adverse events were recorded and categorized using the National Institute of Allergy and Infectious Diseases AIDS grading system, when possible. Alternatively, adverse events were scored as mild, moderate, severe or life threatening. Investigators also assessed the relationship of each adverse event to study medication (unlikely, possible or probable) and whether the event necessitated premature discontinuation of therapy or hospitalization. Blood samples were collected at each visit during treatment and at the final post-therapy visit for routine haematological and chemistry evaluations. In addition, a standard 12-lead electrocardiogram was obtained at the baseline visit and at week 4 to determine QTc changes.

Results

Fifty-three subjects with CNS infections were enrolled in the study; 43 subjects had cryptococcal infections and 10 subjects had infections caused by other fungal pathogens. Fourteen of 43 subjects with cryptococcal meningitis were deemed ineligible by the DRC for the efficacy analysis. Of these, 13 subjects with proven or probable cryptococcal meningitis did not meet the protocol-defined criteria for refractory disease or intolerance to antifungal therapies, and one subject did not meet the protocol-defined criteria for a proven or probable CNS infection or the criteria for refractory disease or intolerance to antifungal therapies. Of the remaining 29 subjects with cryptococcal meningitis, 26 had refractory infections (25 had failed to improve after a reasonable trial of standard antifungal therapy and one had progressive disease), two were intolerant of other antifungal therapies and one subject met both criteria. All 10 subjects with other fungal CNS infections were evaluable for efficacy; eight subjects had disease refractory to current therapies, and two subjects met both criteria for intolerance and refractory disease. Overall, 39 subjects treated with oral posaconazole were considered evaluable for efficacy.

In the efficacy population, most subjects were men (87.2%) and ranged in age from 18 to 74 years (Tables 1–3). The cohort with cryptococcal meningitis was generally younger (mean age 33 years) than the subpopulation with CNS infections caused by other invasive fungal pathogens (mean age 45 years). Most evaluable subjects (29 of 39; 74%) had underlying HIV infection. Twenty-eight of the 29 HIV-infected subjects were in the cryptococcal cohort. The subject with cryptococcal disease without HIV infection (subject 20) had hypogammaglobulinaemia as the only known risk factor for invasive fungal infection. Additional underlying immunodeficiencies in subjects with other fungal CNS infections included acute lymphoblastic leukaemia ($n = 2$), solid organ transplantation (kidney) with end-stage renal disease ($n = 2$), non-Hodgkin lymphoma ($n = 2$), bilateral lung transplantation ($n = 1$) and diabetes mellitus ($n = 1$). One subject in this cohort had no identifiable risk factor for fungal disease (subject 30).

Before posaconazole therapy was initiated, subjects had been treated with amphotericin B and/or azoles for varying durations (Tables 1–3). Subjects with cryptococcal meningitis received antifungal therapy with amphotericin B therapy (range 2–91 days) or fluconazole therapy (range 1–101 days). Similarly, subjects with other fungal CNS infection received one or more different antifungal agents before enrolment (Table 3). Two of these subjects (subjects 30 and 33) had received antifungal therapy for 2–7 years.

Outcomes in subjects with cryptococcal meningitis

Clinical success occurred in 14 of 29 (48%) subjects with cryptococcal meningitis. Complete responses were reported for four subjects, all of whom had not responded to previous standard antifungal therapy. Partial response occurred in 10 subjects, eight of whom had disease refractory to previous antifungal therapy (Table 1). Of the 15 subjects who had a non-successful outcome, eight were treatment failures and six had stable disease; the status of one subject was undetermined (Table 2). Twelve subjects died; six deaths were due to progression of the fungal infection and six deaths occurred after stopping posaconazole and were considered unrelated to treatment. The mean duration of posaconazole therapy in the 29 eligible subjects with cryptococcal meningitis was 81 days (range 4–195 days).

Outcomes in subjects with CNS infections caused by other invasive fungal pathogens

CNS infections were due to *Aspergillus* spp. ($n = 4$), *P. boydii* (the teleomorph of *S. apiospermum*) ($n = 2$), *C. immitis* ($n = 1$), *H. capsulatum* ($n = 1$), *Ramichloridium mackenziei* ($n = 1$) and *Apophysomyces elegans* plus a *Basidiomycetes* sp. ($n = 1$), and all infections were refractory to standard antifungal therapy. The case of infection caused by *R. mackenziei* is discussed in more detail elsewhere.³⁹ Posaconazole resulted in a successful outcome in five of 10 (50%) subjects after 6–12 months of therapy (Table 3). Two of the five subjects had complete responses and three had partial responses.

Among the five subjects who did not have a successful outcome, four died (two with aspergillosis, one with zygomycosis and one with pseudallescheriasis), each within ~2 weeks of the initiation of salvage therapy with posaconazole. All four subjects had advanced disease and severe underlying medical conditions (kidney or lung transplantation, allogeneic bone marrow transplantation with graft-versus-host disease, or lymphoma) when posaconazole therapy was initiated.

Table 1. Summary of subjects with cryptococcal meningitis who had successful outcomes at the end of posaconazole therapy

Subject characteristics				Previous antifungal therapy		Posaconazole therapy		
subject No.	age (years)	sex	refractory (R) or intolerant (I)	drug	duration (days)	duration (days)	response	death (day)
1	37	M	R	FLC	1	169	complete	-
2	54	M	failure to improve	AMB	9	142	CSF culture negative	151
3	31	M	R	AMB	13	142	complete	chronic diarrhoea
			failure to improve	FLC	76	35	CSF culture negative	-
			failure to improve	AMB	76		complete	-
4	27	M	R	AMB	*	62	CSF culture negative, India ink positive	-
			failure to improve	FLC			complete	-
5	28	M	I	FLC	6	195	CSF culture negative	-
				AMB	4		partial	-
				LIP-AMB	5		serum cryptococcal antigen positive	-
6	35	M	R	AMB	33	68	partial	70
			failure to improve	FLC	14		CSF culture negative, meningeal symptoms ongoing	chronic diarrhoea, ascitis syndrome, AIDS, suspected TB/MAI
7	31	M	R	AMB	20	42	partial	-
			failure to improve	FLC	12		CSF culture negative, India ink positive	-
8	26	M	R	AMB	50	42	partial	48
			failure to improve				CSF culture negative	disseminated Kaposi's sarcoma, severe dyspnoea
9	48	M	R	LIP-AMB	2	70	partial	-
			failure to improve				CSF culture negative, India ink positive	-
10	32	M	R	AMB	36	43	partial	-
			failure to improve				CSF culture negative, India ink positive	-
11	30	M	I	AMB	7	169	partial	206
							CSF culture negative, India ink positive	diarrhoea, mycobacterium infection
12	30	M	R	AMB	12	139	partial	-
			failure to improve				CSF culture negative, headache remained, moderate fever and mild cerebellar dysfunction developed	-
13	22	M	R	AMB	4	168	partial	-
			failure to improve				CSF culture negative, intermittent nausea, headache, vertigo, and blurred vision	-
14	33	M	R	AMB	9	14	partial	-
			failure to improve				CSF culture negative, India ink positive, mild ongoing headache	-

M, male; F, female; AMB, amphotericin B; CSF, cerebrospinal fluid; LIP-AMB, lipid formulation of AMB; FLC, fluconazole; MAI, mycobacterium avium intracellulare; TB, tuberculosis.

*Subject received amphotericin B from days -42 to -16 and fluconazole from -15 to -3 before starting treatment with posaconazole, but the investigator did not make the diagnosis of invasive fungal infection until day -2.

Table 2. Summary of subjects with cryptococcal meningitis who had non-successful outcomes at the end of posaconazole therapy

Subject characteristics				Previous antifungal therapy		Posaconazole therapy		
subject No.	age (years)	sex	refractory (R) or intolerant (I)	drug	duration (days)	duration (days)	response	death (day)
15	28	M	R failure to improve	AMB	31	54	failure	125 cardio-respiratory failure, cryptococcal meningitis, AIDS
16	40	M	R failure to improve	AMB FLC	34 7	190	stable disease	-
17	29	M	R failure to improve	AMB FLC	91 29	60	failure	64 disseminated TB
18	46	M	R and I failure to improve	AMB LIP-AMB	31 1	152	failure	181 sepsis, disseminated cryptococcosis
19	32	M	R failure to improve	FLC	15	59	stable disease	-
20	25	M	R failure to improve	AMB	60	11	stable disease	-
21	34	M	R failure to improve	FLC	101	43	failure	71 progressive cryptococcal meningitis
22	26	M	R failure to improve	AMB FLC	38 85	42	failure	51 intracranial hypertension, cryptococcal meningitis
23	40	F	R failure to improve	AMB	15	28	stable disease	-
24	34	F	R progression of IFI	AMB	17	27	stable disease	71 disseminated cryptococcosis
25	41	M	R failure to improve	AMB	11	104	failure	-
26	36	M	R failure to improve	AMB	10	168	stable disease	-
27	35	M	R failure to improve	AMB FLC	18 3	5	failure	6 recurrent cryptococcal meningitis, AIDS
28	35	M	R failure to improve	AMB	10	4	undetermined ^a	-
29	21	F	R failure to improve	AMB FLC	13 14	40	failure	40 recurrent cryptococcosis

M, male; F, female; AMB, amphotericin B; IFI, infectious fungal infection; LIP-AMB, lipid formulation of AMB; FLC, fluconazole; TB, tuberculosis.

^aAsymptomatic but treatment taken for only 4 days.

Safety and tolerability

Based on the analysis of the safety population ($n = 330$) from the open-label trial of posaconazole in subjects with invasive fungal infections, the most common drug-related adverse events included nausea (9%), vomiting (6%), headache (5%), abdominal pain (5%), diarrhoea (3%), increased serum glutamic-pyruvic transaminase (alanine aminotransferase) levels (3%), increased serum glutamic-oxaloacetic transaminase (aspartate aminotransferase) levels (3%) and rash (3%). Mild, transient increases in liver function test values were noted in 2–3% of subjects, but they rarely necessitated that posaconazole therapy be discontinued. Posaconazole did not cause QTc prolongation or negative cardiac inotropic effects. Long-term administration (>6 months) of posaconazole did not produce a

safety profile different from that for short-term administration (<6 months).

Discussion

This analysis of subjects with proven CNS fungal infections receiving oral posaconazole for salvage therapy suggests that the new extended-spectrum antifungal agent may be an alternative for treatment of these serious and difficult to treat infections. In the cohort of subjects in this trial with CNS fungal infections, oral posaconazole therapy resulted in an overall success rate of 49% (19 of 39 subjects). Specifically, 50% of subjects with non-cryptococcal CNS infections and 48% of subjects with

Table 3. Summary of subjects with other fungal CNS infections and outcomes at the end of posaconazole therapy

Subject characteristics				Prior antifungal therapy			Posaconazole therapy			
subject No.	age (years)	sex	pathogen	refractory (R) or intolerant (I)	CNS infection	drug	duration (days)	duration (days)	outcome (response)	death (day)
30	57	M	<i>H. capsulatum</i>	R	intermittently positive CSF cultures/serology.	AMB LIP-AMB ITC	117 514 249	241	success (complete)	-
31	40	M	<i>P. boydii</i>	R	CT scan showed enlarging cerebral abscess in region of left lateral ventricle with oedema and right cerebellar lesion. Part of enhancing rim of medial aspect of lesion remained with significant residual oedema of left cerebrum and ventricular shift. Cerebellar lesion unchanged. MRI showed development of new lesions in right occipital and right posterior temporal lobes.	AMB KTC	13 9	336	success (complete)	-
32	74	M	<i>A. fumigatus</i>	R	MRI and CT scan showed several large cerebral abscesses and invasive sinusitis with destruction of right orbital wall.	LIP-AMB	24	189	success (partial)	218 progression of cerebral aspergillosis
33	43	F	<i>C. immitis</i>	R	obstructive hydrocephalus due to chronic coccidioidal meningitis 1.5 years prior—lumboperitoneal shunt placed. Approximately 30 days prior, disconnected lumboperitoneal shunt revised. CSF positive for <i>Coccidioides</i> . CT scan showed mild ventricular enlargement.	FLC	2553	372	success (partial)	-
34	62	M	<i>R. mackenziei</i>	R	MRI showed large frontoparietal abscess with satellite lesions.	LIP-AMB ITC	82 93	609	success (partial)	-

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35	51	F	<i>P. boydii</i>	R	MRI showed two frontal/parietal lesions consistent with <i>Aspergillus</i> . Bilateral craniotomies to debulk largest lesions performed prior to posaconazole therapy.	LIP-AMB ITC	2 2	7	non-success (failure)	8 progressive fungal brain abscess
36	22	M	<i>A. fumigatus</i>	R and I	left eye redness and swelling with bilateral endophthalmitis and MRI showed bilateral chorioretinitis and leptomeningeal process. Necrotizing encephalitis and fungal vasculitis of left parietal lobe noted on CT scan.	ITC LIP-AMB AMB	27 27 1	10	non-success (failure)	11 cardio- pulmonary arrest
37	52	M	<i>A. elegans</i> plus a <i>Basidiomycetes</i> sp.	R and I	abscess in the right parietal region noted on CT scan.	LIP-AMB FLC	11 1	4	non-success (failure)	5 sepsis, hypotension, ventricular tachycardia 16 apnoea
38	35	M	<i>A. fumigatus</i>	R	MRI showed six lesions that increased in size with oedema. Neurosurgical debridement performed prior to posaconazole therapy.	ITC AMB	23 30	15	non-success (failure)	
39	18	M	<i>Aspergillus</i> sp.	R	MRI showed changes at craniocervical junction of brain suggestive of infection. Slight interval progression of lesion noted and leptomeningeal biopsy showed granulomatous fungal patchy meningitis. Progressive disease, lesion extension and mild coning noted on MRI.	AMB	48	168	non-success (undetermined ^a)	

M, male; F, female; AMB, amphotericin B; FLC, fluconazole; ITC, itraconazole; KTC, ketoconazole; LIP-AMB, liposomal amphotericin B; CT, computed tomography; MRI, magnetic resonance imaging.
^aMeningeal biopsy culture and histology showed no evidence of fungus, but chronic non-specific leptomeningitis evident.

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cryptococcal meningitis had successful clinical outcomes, with a 52% survival rate at the end of therapy for subjects with cryptococcal meningitis. Importantly, these success rates were obtained in a select group of seriously ill subjects, most with underlying immunodeficiencies, who were refractory to other antifungal therapy. Moreover, these success rates were obtained using an oral therapy. Thus, posaconazole may provide an alternative to parenteral therapy for subjects who are refractory to, or intolerant of, intravenous therapy with amphotericin B.

The cases of cryptococcal meningitis presented here were part of a larger study that included several other fungal infections, and therefore response definitions were adapted for the entire study population and not for each fungal infection type. Although our definitions of success (complete or partial response) were not based on absence or presence of positive cultures and may not be optimal for cryptococcal meningitis, the data presented here are useful in determining the efficacy of posaconazole. Response definitions for cryptococcal meningitis vary amongst the large number of antifungal treatment studies. A recent study of voriconazole for refractory fungal infections including cryptococcosis, and a study of triple combination therapy with amphotericin B, flucytosine and itraconazole for cryptococcal meningitis, had similar success definitions to those in this study,^{40,41} while a number of studies categorized response into clinical success (resolution of signs and symptoms) or mycological success (negative blood/CSF cultures).⁴²⁻⁴⁶ Conversely, other studies considered patients as successes only if they displayed both resolution of signs and symptoms, and negative blood or CSF cultures.^{5,47-51} Although the definitions of success in these previous studies are somewhat different from those in the present study, and a large majority of these patients entered the trial as a consequence of failure to improve on prior antifungal therapy, it should be noted that all patients presented here who were considered successes also had negative CSF cultures. In addition, failure of posaconazole therapy also included patients with stable disease and survival of >30 days, indicating that the response criteria in the present study were remarkably stringent.

Cryptococcal antigen testing of CSF has been documented by several researchers to be an effective and reliable diagnostic tool for cryptococcal meningitis.^{52,53} However, *Cryptococcus* antigen testing was not performed in all instances in this study, since a large proportion of the cases were seen in geographic areas where antigen testing was not readily available.

For cryptococcal meningitis in patients infected with HIV, clinical success rates with induction therapy of amphotericin B plus flucytosine are 40-60%.^{5,47} Although fluconazole is effective for maintenance therapy, it is less effective than amphotericin B for initial therapy.^{1,49} Thus, nearly 50% of patients with cryptococcal meningitis require salvage therapy due to refractory disease. Although evaluated in a small number of patients, salvage therapy with amphotericin B lipid complex for cryptococcal meningitis resulted in a 50% clinical success rate, and with voriconazole, <39% of patients had a complete or partial response.^{40,54}

Causes for failure of primary therapy may be related to predictors of poor response to therapy such as extraneural sites of infection, abnormal mental status, hyponatraemia and low CSF white blood cell counts,⁴⁷ although it is unlikely that failures are due to the development of resistant *C. neoformans* strains.⁵⁵ posaconazole MIC₉₀s for *C. neoformans* range from 0.25 to 0.5 mg/L, while those for itraconazole and fluconazole are 0.5 and 8-16 mg/L, respectively.^{16,56,57} Failure of posaconazole therapy may be related

to the predictors of poor response and non-compliance with medications, and due to the fact that posaconazole was administered as salvage therapy to extremely sick patients with refractory infections. However, it should be emphasized that the response rate seen for posaconazole is very encouraging in patients in whom other antifungals have failed, and given the underlying conditions and refractory disease status of these patients. In addition, some of these patients were also intolerant to prior antifungal therapy; therefore, posaconazole provides an important antifungal treatment option.

Data from this report also demonstrate that posaconazole has a wide spectrum of clinical activity against clinically significant fungal pathogens other than *C. neoformans*. Posaconazole therapy resulted in a 50% success rate and 60% survival rate in the treatment of advanced CNS infections caused by rare moulds, supporting its extended spectrum of activity. Posaconazole demonstrated clinical activity against *R. mackenziei*, a dematiaceous fungus exclusively found in the Middle East that has an a predilection for the CNS and a high mortality rate.⁵⁸ One of three subjects with cerebral aspergillosis achieved a partial clinical response during posaconazole therapy. More research is needed to determine the role of posaconazole as a first-line therapy for the treatment of these invasive and deadly fungal diseases.

Conclusions

In summary, the success rate of posaconazole as salvage therapy for subjects with CNS fungal infections intolerant of or refractory to standard antifungal therapy approached 50%. The activity of posaconazole salvage therapy against cryptococcal meningitis seems to be comparable to that of amphotericin B lipid complex and voriconazole when examining results reported from uncontrolled trials, although direct comparisons cannot be made. With increasing reports of breakthrough fungal disease during long-term maintenance for cryptococcal meningitis with fluconazole in patients with HIV, alternative therapies are needed. Results of this study suggest that posaconazole, as an oral medication, has clinical activity against fungal infections of the CNS and may provide a valuable alternative to parenteral therapy in patients in whom existing antifungal agents have failed.

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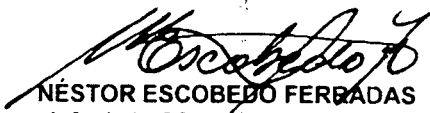
Registro de la Propiedad Industrial

Oficina de Invenciones y Nuevas Tecnologías

TITULO N° 4424

La Oficina de Invenciones y Nuevas Tecnologías del Indecopi certifica que por mandato de la Resolución N° 001094-2006/OIN-INDECOPI de fecha 27 de septiembre de 2006, ha quedado inscrita en el Registro de Patentes de Invención, la siguiente invención:

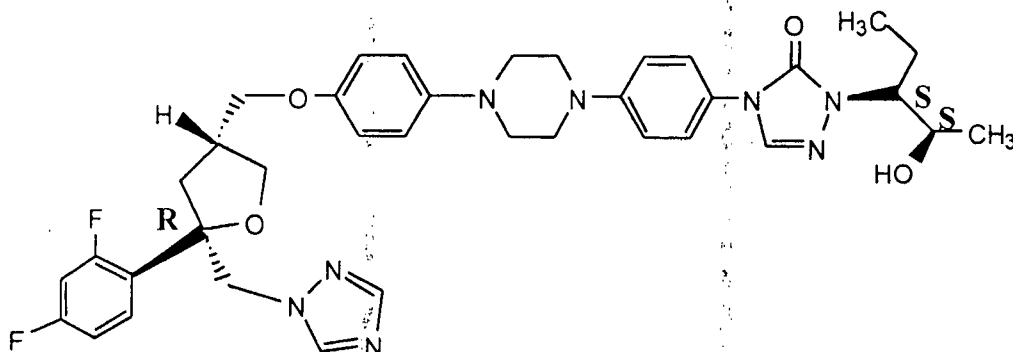
Denominación	: COMPOSICION ANTIFUNGAL CON AUMENTADA BIODISPONIBILIDAD
Clasificación	: A61K 9/08; A61K 47/34; A61K 31/495; A61P 31/10
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Titular	: SCHERING CORPORATION
País	: Estados Unidos de América
Inventores	: STEFAN SHARPE; JOEL SEQUEIRA; DAVID HARRIS; SHASHANK MAHASHABDE
Vigencia	: 02 de abril de 2022


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INDECOPI

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We claim:

1. A liquid suspension comprising an antifungally effective amount of the
 5 micronized compound represented by the chemical structural formula I comprising:



wherein the micronized compound of formula I has a mean particle size of 1000 nm to 1800 nm, a thickening agent, a non-ionic surfactant, and a pharmaceutically acceptable liquid carrier.

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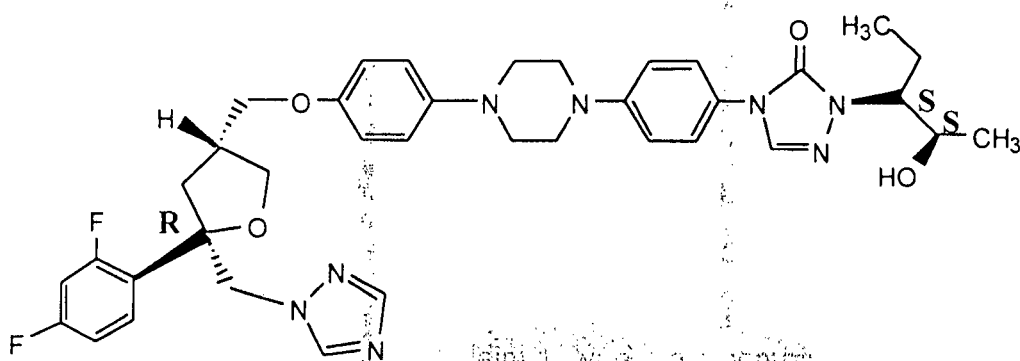
2. The liquid suspension of claim 1, wherein the non-ionic surfactant is a block copolymer of ethylene oxide and propylene oxide, glycol or glyceryl esters of saturated or unsaturated C₈ to C₂₀ acids, polyoxyethylene esters of saturated or unsaturated C₈ to C₂₀ acids, polyoxyethylene ethers of saturated or unsaturated C₈ to C₂₀ acids,
 15 polyvinylalcohols or sorbitan esters of saturated or unsaturated C₁₀ to C₂₀ acids.

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3. The liquid suspension of claim 1, wherein the non-ionic surfactant is a polyoxyethylene derivative of a sorbitan ester of a saturated or unsaturated C₁₀ to C₂₀ acid.
4. The liquid suspension of claim 1, wherein the thickening agent is selected from xanthan gum, liquid sugars, starches, celluloses and mixtures thereof.

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5. The liquid suspension of claim 1, wherein a combination of xanthan gum and a liquid sugar is used as the thickening agent.
6. The liquid suspension of claim 1, wherein the micronized compound of formula I has a mean particle size of 1200 nm to 1600 nm.
7. A liquid suspension comprising:
 - (a) an antifungally effective amount of the micronized compound represented by the chemical structural formula I:



wherein the micronized compound of formula I has a mean particle size of 1000 nm to 1800 nm;

- (b) an effective amount of a thickening agent;
 - (c) an amount of a buffer system effective to maintain the pH of the system in the range of 4.0 to 6.0;
 - (d) an effective amount of a non-ionic surfactant; and
 - (e) a pharmaceutically acceptable liquid carrier.
8. The liquid suspension of claim 7, wherein the non-ionic surfactant is a polyoxyethylene derivative of a sorbitan ester of a saturated or unsaturated C₁₀ to C₂₀ acid.

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9. The liquid suspension of claim 7, wherein the sorbitan ester is one or more fatty acid ester of sorbitan selected from the group consisting of sorbitan monolaurate, sorbitan monooleate, sorbitan sesquioleate, sorbitan trioleate, sorbitan monopalmitate, sorbitan monostearate and sorbitan tristearate.

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10. The liquid suspension of claim 7, wherein the thickening agent is selected from gums, liquid sugars, starches, cellulose and mixtures thereof.

11. The liquid suspension of claim 7, wherein a combination of a xanthan gum and a liquid sugar is used as the thickening agent.

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12. The liquid suspension of claim 7, wherein a combination of a xanthan gum and a liquid glucose is used as the thickening agent.

13. The liquid suspension of claim 7, wherein the buffer system comprises sodium citrate and citric acid.

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14. The liquid suspension of claim 7, wherein the pharmaceutically acceptable liquid carrier is a combination of purified water, glucose and glycerin.

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15. The liquid suspension of claim 7, wherein the micronized compound of formula I has a mean particle size of 1200 nm to 1600 nm.

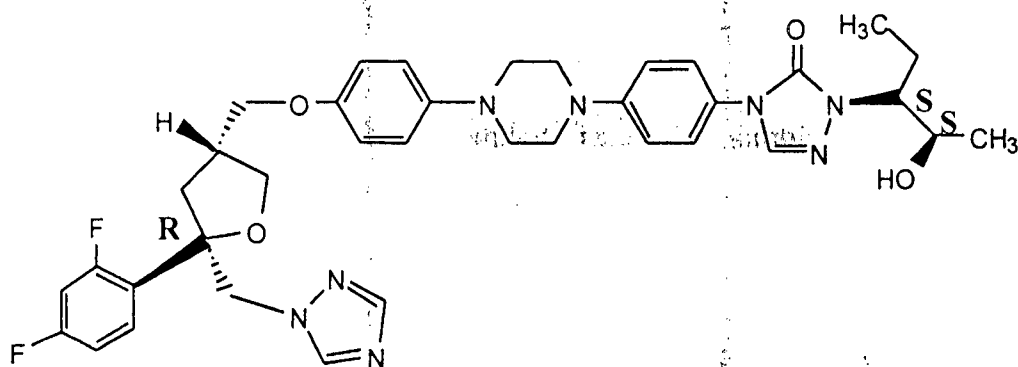
16. A liquid suspension comprising:

(a) an antifungally effective amount of the micronized compound comprising chemical structural formula I:

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wherein the micronized compound of formula I has a mean particle size in the range of 1000 nm to 1800 nm,

- (b) an effective amount of a polyoxyethylene derivative of sorbitan esters of saturated or unsaturated C₁₂ to C₁₈ acids;
- (c) an effective amount of a buffer system sufficient to maintain a pH in the range of 4.0 to 6.0;
- (d) an effective amount of a combination of two thickening agents, wherein one is a liquid sugar; and
- (e) a pharmaceutically acceptable liquid carrier.

18. The liquid suspension of claim 16, wherein the micronized compound of formula I has a mean particle size in the range of 1200 nm to 1600 nm.

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LETTERS PATENT

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STANDARD PATENT

I, Fatima Beattie, the Commissioner of Patents, grant a Standard Patent with the following particulars:

Name and Address of Patentee(s):

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Name of Actual Inventor(s):

Sequeira, Joel; Mahashabde, Shashank; Harris, David and Sharpe, Stefan.

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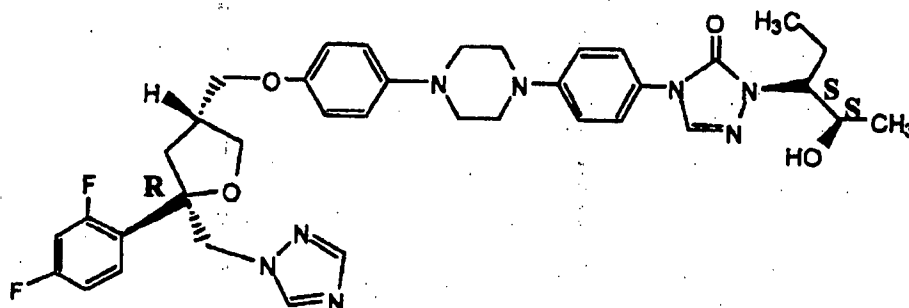
(56) Related Art
US5661151
US5834472

PD0996K AV
granted claims

- 25 -

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A liquid suspension comprising an antifungally effective amount of the micronized compound represented by the chemical structural formula I:



wherein the micronized compound of formula I has a mean particle size of about 1000 nm to about 1800 nm, and a pharmaceutically acceptable liquid carrier.

2. The liquid suspension of Claim 1, further comprising at least one thickening agent and at least one non-ionic surfactant.
3. The liquid suspension of Claim 2, wherein the at least one non-ionic surfactant is a block copolymer of ethylene oxide and propylene oxide, glycol or glyceryl esters of saturated or unsaturated C_8 to C_{20} acids, polyoxyethylene esters of saturated or unsaturated C_8 to C_{20} acids, polyoxyethylene ethers of saturated or unsaturated C_8 to C_{20} acids, polyvinylalcohols or sorbitan esters of saturated or unsaturated C_{10} to C_{20} acids.
4. The liquid suspension of Claim 2, wherein the at least one non-ionic surfactant is a polyoxyethylene derivative of a sorbitan ester of a saturated or unsaturated C_{10} to C_{20} acid.

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5. The liquid suspension of Claim 2, wherein the at least one thickening agent is selected from xanthan gum, liquid sugars, starches, celluloses, and mixtures of two or more thereof.
- 5 6. The liquid suspension of Claim 2, wherein a combination of xanthan gum and a liquid sugar is used as the at least one thickening agent.
7. The liquid suspension of Claim 2, wherein the micronized compound of formula I has a mean particle size of about 1200 nm to about 1600 nm.
- 10 8. The liquid suspension of Claim 1, further comprising an amount of a buffer system effective to maintain the pH of the system in the range of about 4.0 to about 6.0.
- 15 9. The liquid suspension of Claim 8, further comprising:
- an effective amount of at least one thickening agent; and
 - an effective amount of at least one non-ionic surfactant.
- 20 10. The liquid suspension of Claim, 9, wherein the at least one non-ionic surfactant is a polyoxyethylene derivative of a sorbitan ester of a saturated or unsaturated C₁₀ to C₂₀ acid.
- 25 11. The liquid suspension of Claim 10, wherein the sorbitan ester is one or more fatty acid ester of sorbitan selected from the group consisting of sorbitan monolaurate, sorbitan monooleate, sorbitan sesquileate, sorbitan trioleate, sorbitan monopalmitate, sorbitan monostearate and sorbitan tristearate.
- 30 12. The liquid suspension of Claim 9, wherein the at least one thickening agent is selected from gums, liquid sugars, starches, celluloses, and mixtures of two or more thereof.
13. The liquid suspension of Claim 9, wherein a combination of a xanthan gum and a liquid sugar is used as the at least one thickening agent.

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14. The liquid suspension of Claim 9, wherein a combination of a xanthan gum and a liquid glucose is used as the thickening agent.
15. The liquid suspension of Claim 9, wherein the buffer system comprises sodium citrate and citric acid.
16. The liquid suspension of Claim 9, wherein the pharmaceutically acceptable liquid carrier is a combination of purified water, glucose, and glycerin.
17. The liquid suspension of Claim 9, wherein the micronized compound of formula I has a mean particle size of about 1200 nm to about 1600 nm.
18. The liquid suspension of Claim 1, further comprising:
- an effective amount of a polyoxyethylene derivative of sorbitan esters of saturated or unsaturated C₁₂ to C₁₈ acids;
 - an effective amount of a buffer system sufficient to maintain a pH in the range of about 4.0 to about 6.0;
 - an effective amount of a combination of two thickening agents, wherein one is a liquid sugar.
19. The liquid suspension of Claim 18, wherein the micronized compound of formula I has a mean particle size in the range of about 1200 nm to about 1600 nm.
20. The liquid suspension of Claim 1, further comprising at least one thickening agent, and in which at least one thickening agent is xanthan gum.
21. The liquid suspension of Claim 20, further comprising at least one non-ionic surfactant, in which at least one non-ionic surfactant is polyoxyethylene sorbitan monooleate.

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22. The liquid suspension of Claim 1, further comprising xanthan gum and a liquid sugar, and at least one non-ionic surfactant.
23. The liquid suspension of Claim 1, further comprising xanthan gum and a liquid sugar, and a polyoxyethylene sorbitan monooleate.
24. The liquid suspension of Claim 1, further comprising a combination of xanthan gum and a liquid sugar, a polyoxyethylene sorbitan monooleate, and a buffer system effective to maintain the pH of the system in the range of about 4.0 to about 6.0.
25. The liquid suspension of Claim 24, wherein the buffer system comprises sodium citrate and citric acid.
26. Use of a liquid suspension according to Claim 1, for preparing a medicament in the form of an oral suspension for the prevention of a fungal infection in a mammal.
27. Use of a liquid suspension according to Claim 2, for preparing a medicament in the form of an oral suspension for the prevention of a fungal infection in a mammal.
28. The use of Claim 27, which provides a maximum concentration (C_{max}) of posaconazole in the plasma serum of about 485 ng/ml to about 512 ng/ml post-single dose administration.
29. The use of Claim 28, wherein the C_{max} is measured at a time of maximum concentration (T_{max}) in the range of about 4 to about 5 hours post-single dose administration.
30. The use of Claim 28, wherein a plot of the plasma concentration of posaconazole over time yields an Area Under the Curve over 72 hours [AUC(0-72 hrs)] value of posaconazole of about 13141 ng-hr/ml to about 13885 ng-hr/ml.

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31. The use of Claim 28, wherein the Cmax of posaconazole is about 485 ng/ml, and an Area Under the Curve over 72 hours [AUC(0-72 hrs)] value of posaconazole is about 13141 ng-hr/ml.
- 5 32. The use of Claim 28, wherein the Cmax of posaconazole is about 512 ng/ml, and an Area Under the Curve over 72 hours [AUC(0-72 hrs)] value of posaconazole is about 13885 ng-hr/ml.
- 10 33. The use of Claim 28, wherein said liquid, oral suspension is administered with a high-fat meal to deliver 200 mg of posaconazole.
34. The use of Claim 27, which provides a maximum concentration (Cmax) of posaconazole in the plasma serum that is about 2.6 to about 4 times greater
15 when administered with a high-fat meal or a non-fat meal as compared to when administered following a 10 hr fast.
35. The use of Claim 34, wherein the Cmax of posaconazole in the plasma serum is about 4 times greater when administered with a high-fat meal as
20 compared to when administered following a 10 hr fast.
36. The use of Claim 34, wherein said liquid, oral suspension is administered to deliver 200 mg of posaconazole.
- 25 37. A method of preventing a fungal infection in a mammal comprising administering to a mammal in need thereof a liquid, oral suspension comprising the liquid suspension of Claim 1.
38. A method of preventing a fungal infection in a mammal comprising
30 administering to a mammal in need thereof a liquid, oral suspension comprising the liquid suspension of Claim 2.

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39. The method of Claim 38, which provides a maximum concentration (Cmax) of posaconazole in the plasma serum of about 485 ng/ml to about 512 ng/ml post-single dose administration.
- 5 40. The method of Claim 38, wherein the Cmax is measured at a time of maximum concentration (Tmax) in the range of about 4 to about 5 hours post-single dose administration.
- 10 41. The method of Claim 38, wherein a plot of the plasma concentration of posaconazole over time yields an Area Under the Curve over 72 hours [AUC(0-72 hrs)] value of posaconazole of about 13141 ng-hr/ml to about 13885 ng-hr/ml.
- 15 42. The method of Claim 38, wherein the Cmax of posaconazole is about 485 ng/ml, and an Area Under the Curve over 72 hours [AUC(0-72 hrs)] value of posaconazole is about 13141 ng-hr/ml.
- 20 43. The method of Claim 38, wherein the Cmax of posaconazole is about 512 ng/ml, and an Area Under the Curve over 72 hours [AUC(0-72 hrs)] value of posaconazole is about 13885 ng-hr/ml.
44. The method of Claim 38, wherein said liquid, oral suspension is administered with a high-fat meal to deliver 200 mg of posaconazole.
- 25 45. The method of Claim 37, which provides a maximum concentration (Cmax) of posaconazole in the plasma serum that is about 2.6 to about 4 times greater when administered with a high-fat meal or a non-fat meal as compared to when administered following a 10 hr fast.
- 30 46. The method of Claim 45, wherein the Cmax of posaconazole in the plasma serum is about 4 times greater when administered with a high-fat meal as compared to when administered following a 10 hr fast.

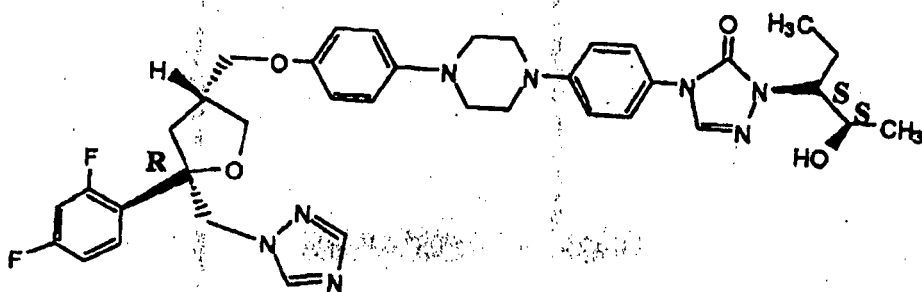
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47. The method of Claim 45, wherein said liquid, oral suspension is administered to deliver 200 mg of posaconazole.

48. A liquid suspension comprising an antifungally effective amount of the micronised compound represented by the chemical structure of formula I:



substantially as herein described with reference to Example 1 or Example 2.

49. A method according to Claim 37 or 38 substantially as herein described with reference to Treatment A, Treatment B or Treatment C in the Examples.

Dated this 16th day of January 2006

Schering Corporation

By its Patent Attorneys

GRIFFITH HACK

LETTERS PATENT

Number 528363

ELIZABETH THE SECOND, by the Grace of God Queen of New Zealand and Her Other Realms and Territories, Head of the Commonwealth, Defender of the Faith; To all to whom these presents shall come, Greeting:

WHEREAS pursuant to the Patents Act 1953 an application has been made for a patent of an invention for

Antifungal composition with enhanced bioavailability

(more particularly described in the complete specification relating to the application)

AND WHEREAS

SCHERING CORPORATION, 2000 Galloping Hill Road, Kenilworth, New Jersey 07033-0530, United States of America

(hereinafter together with his or their successors and assigns or any of them called "the patentee") is entitled to be registered as the proprietor of the patent hereinafter granted:

Address for service: A J PARK, 6th Floor, Huddart Parker Building, 1 Post Office Square, Wellington, New Zealand

NOW, THEREFORE, We by these letters patent give and grant to the patentee our special licence, full power, sole privilege, and authority, that the patentee by himself, his agents, or licensees and no others, may subject to the provisions of any statute or regulation for the time being in force make, use, exercise and vend the said invention within New Zealand and its dependencies during a term of twenty years from 1 April 2002 and that the patentee shall have and enjoy the whole profit and advantage from time to time accruing by reason of the said invention during the said term:

AND WE strictly command all our subjects whomsoever within New Zealand and its dependencies that they do not at any time during said term either directly or indirectly make use of or put into practice the said invention, nor in any way imitate the said invention without the consent, licence, or agreement of the patentee in writing under his hand, on pain of incurring such penalties as are prescribed by law and of being answerable to the patentee according to law for his damages thereby occasioned:

PROVIDED ALWAYS:

- (1) That these letters patent shall determine and become void if the patentee does not from time to time pay the renewal fees prescribed by law in respect of the patent;
- (2) That these letters patent are revocable on any of the grounds prescribed by the Patents Act 1953 as grounds for revoking letters patent;
- (3) That nothing in these letters patent shall prevent the granting of licences in the manner in which and for the considerations on which they may by law be granted;
- (4) That these letters patent shall be construed in the most beneficial sense for the advantage of the patentee.

IN WITNESS whereof We have caused these letters patent to be signed and sealed on 11 August 2005 with effect from 1 April 2002.

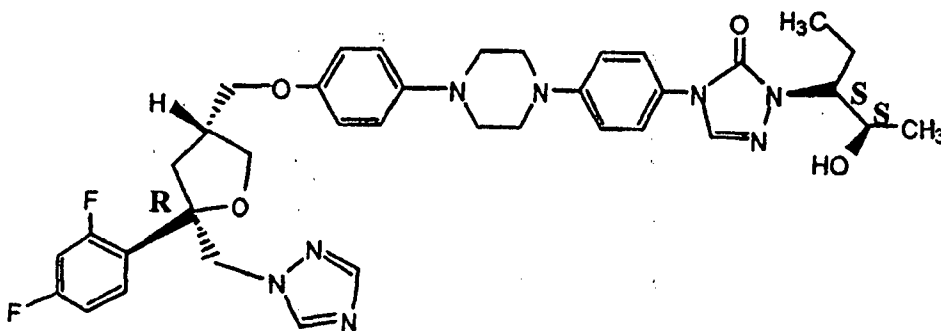


Neville Harris

Neville Harris
Commissioner of Patents, Trade Marks and Designs

WHAT WE CLAIM IS:

1. A liquid suspension comprising an antifungally effective amount of the micronized compound represented by the chemical structural formula I;



at least one thickening agent, at least one non-ionic surfactant, and a pharmaceutically acceptable liquid carrier.

2. The liquid suspension of claim 1, wherein the at least one non-ionic surfactant is a block copolymer of ethylene oxide and propylene oxide, glycol or glyceryl esters of saturated or unsaturated C₈ to C₂₀ acids, polyoxyethylene esters of saturated or unsaturated C₈ to C₂₀ acids, polyoxyethylene ethers of saturated or unsaturated C₈ to C₂₀ acids, polyvinylalcohols or sorbitan esters of saturated or unsaturated C₁₀ to C₂₀ acids.
3. The liquid suspension of claim 1, wherein the at least one non-ionic surfactant is a polyoxyethylene derivative of a sorbitan ester of a saturated or unsaturated C₁₀ to C₂₀ acid.

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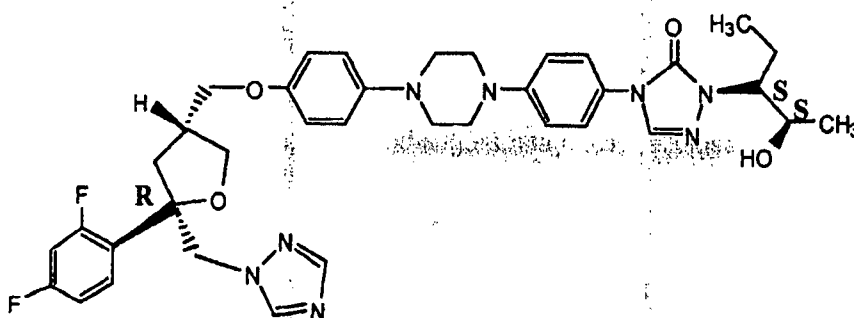
4. The liquid suspension of claim 1, wherein the at least one thickening agent is selected from xanthan gum, liquid sugars, starches, celluloses and mixtures thereof.

5. The liquid suspension of claim 1, wherein a combination of xanthan gum and a liquid sugar are used as the at least one thickening agent.

6. The liquid suspension of claim 1, wherein the micronized compound of formula I has a mean particle size of about 1200 nm to about 1600 nm.

7. A liquid suspension comprising:

- (a) an antifungally effective amount of the micronized compound represented by the chemical structural formula I:



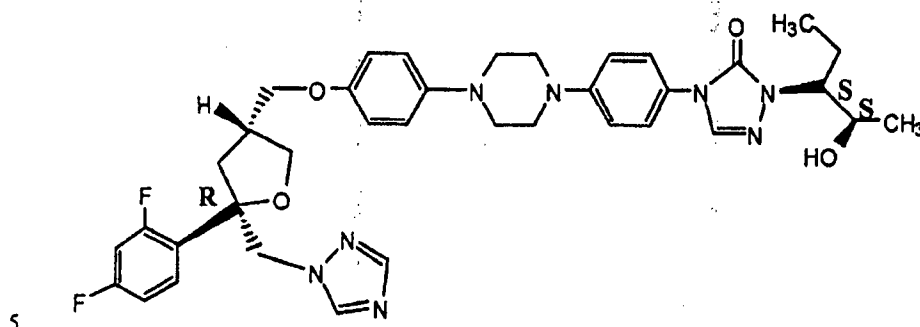
- 15 (b) an effective amount of at least one thickening agent;
- (c) an amount of a buffer system effective to maintain the pH of the system in the range of about 4.0 to about 6.0;
- (d) an effective amount of at least one non-ionic surfactant; and
- (e) a pharmaceutically acceptable liquid carrier.

8. The liquid suspension of claim 7, wherein the at least one non-ionic surfactant is a polyoxyethylene derivative of a sorbitan ester of a saturated or unsaturated C₁₀ to C₂₀ acid.
9. The liquid suspension of claim 7, wherein the sorbitan ester is a fatty acid ester of sorbitan selected from sorbitan monolaurate, sorbitan monooleate, sorbitan sesquioleate, sorbitan trioleate, sorbitan monopalmitate, sorbitan monostearate and sorbitan tristearate, or mixtures thereof.
10. The liquid suspension of claim 7, wherein the at least one thickening agent is selected from gums, liquid sugars, starches, cellulose and mixtures thereof.
- 10 11. The liquid suspension of claim 7, wherein a combination of a xanthan gum and a liquid sugar is used as the at least one thickening agent.
12. The liquid suspension of claim 7, wherein a combination of a xanthan gum and a liquid glucose is used as the thickening agent.
13. The liquid suspension of claim 7, wherein the buffer system comprises sodium citrate and citric acid.
- 15 14. The liquid suspension of claim 7, wherein the pharmaceutically acceptable liquid carrier is a combination of purified water, glucose and glycerin.
15. The liquid suspension of claim 7, wherein the micronized compound of formula I has a mean particle size of about 1200 nm to about 1600 nm.

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16. A liquid suspension comprising
- (a) an antifungally effective amount of the micronized compound comprising chemical structural formula I:



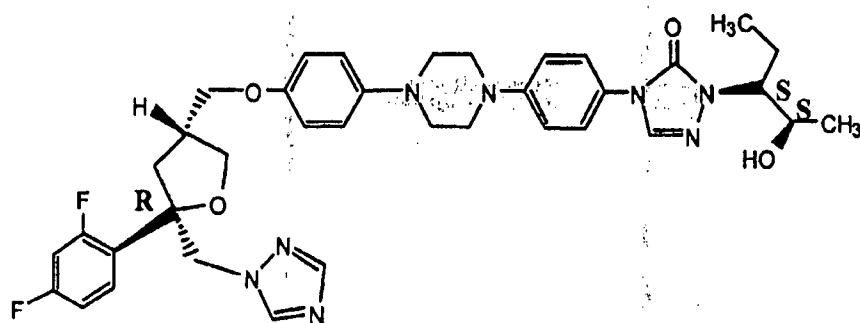
wherein the micronized compound of formula I has a mean particle size in the range of about 1200 nm to about 1600 nm,

- 10 (b) an effective amount of a polyoxyethylene derivative of sorbitan esters of saturated or unsaturated C₁₂ to C₁₈ acids;
- (c) an effective amount of a buffer system sufficient to maintain a pH in the range of about 4.0 to about 6.0;
- (d) an effective amount of a combination of two thickening agents,
- 15 wherein one is a liquid sugar; and
- (e) a pharmaceutically acceptable liquid carrier.

17. The use of micronized particles of the compound of chemical structural formula I:

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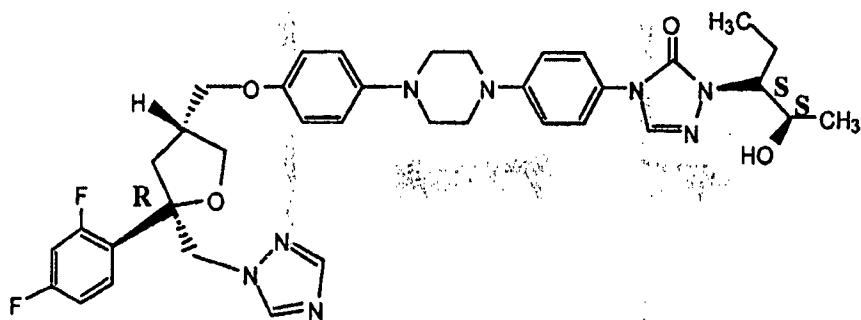
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for preparation of a medicament in the form of an oral suspension for the treatment and/or prevention of a fungal infection in a mammal comprising an antifungally effective amount of the compound of formula I and at least one thickening agent

5 and a non-ionic surfactant in a pharmaceutically acceptable carrier.

18. A suspension comprising an antifungally effective amount of the micronized particles of the compound comprising chemical structural formula I:



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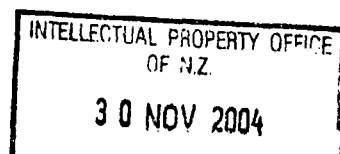
at least one thickening agent and a non-ionic surfactant in a pharmaceutically acceptable liquid carrier.

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19. A liquid suspension as defined in claim 1 or claim 7, or as claimed in claim 16, substantially as herein described with reference to any example thereof and with or without reference to the accompanying figures.

20. A use as claimed in claim 17, substantially as herein described with reference to any example thereof and with or without reference to the accompanying figures.

21. A suspension as claimed in claim 18, substantially as herein described with reference to any example thereof and with or without reference to the accompanying figures.



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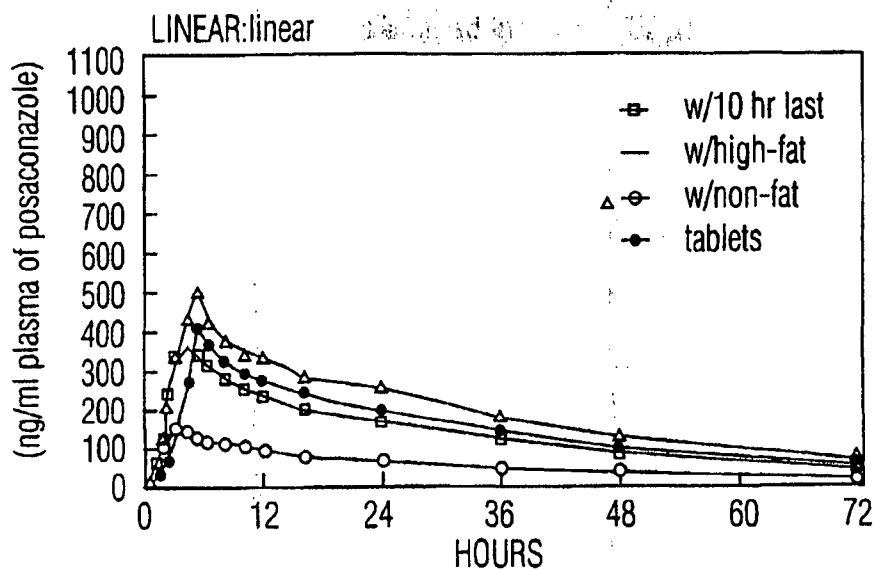


FIG. 1

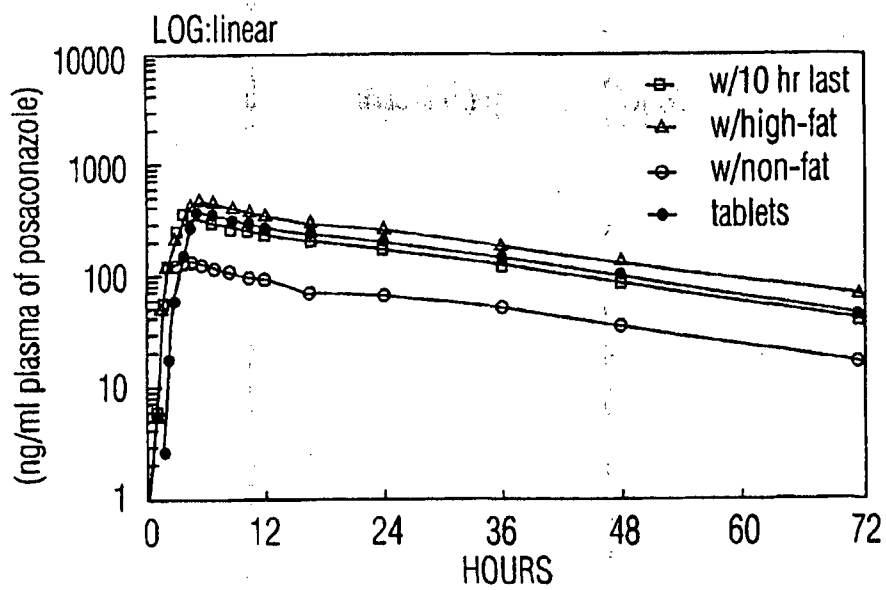


FIG. 2

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GEOGRAPHICAL INDICATIONS



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GOVERNMENT OF INDIA
THE PATENT OFFICE
PATENT
(RULE - 74)

No. D - CHE/1780

No. 203528

of

01/04/2002

Whereas SCHERING CORPORATION, 2000 GALLOPING HILL ROAD, KENILWORTH, NEW JERSEY 07033-0530, USA; A US CORPORATION

has/have declared that he is/they are in possession of an invention for
A LIQUID SUSPENSION

and that he is/they are the true and first inventor(s) thereof (or the legal representatives(s) or assignee(s) of the true and the first inventor(s)) and that he is/they are entitled to a patent for the said invention, having regard to the provisions of the Patents Act, 1970, as amended and that there is no objection to the grant of a patent to him/them.

And whereas he has/they have, by an application, requested that a patent may be granted to him/them for the said invention;

And whereas he has/they have by and in his/their complete specification particularly described the said invention and the manner in which the same is to be performed;

Now, these present(s) that the above-said applicant(s) (including his/their legal representative(s) and assignee(s) or any of them) shall, subject to the provisions of the Patents Act, 1970, as amended and the conditions specified in Section 47 of the said Act, and to the conditions and provisions specified by any other law for the time being in force, has/have the exclusive right to prevent third parties from making, using, offering for sale, selling or importing for those purposes the product in India/using the process and using, offering for sale, selling or importing for those purposes the product obtained, if any, directly by that process in India, for a term of twenty years from the 1st APRIL 2002 and of authorizing any other person to do so, subject to the conditions that the validity of this patent is not guaranteed and that the fee prescribed for the continuance of this patent is duly paid.

In witness thereof, the Controller has caused this patent to be granted as of the TWENTY FIRST day of NOVEMBER, 2006.

Date of Grant : 21/11/2006

Controller of Patents

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NOTICE

The renewal fees payable to keep a patent in force under Section 53 of the Patents Act, 1970 (39 of 1970) are as follows :

Before expiration of	In respect of	Fees in Rs./Year For natural Persons(s)	Fees in Rs./Year For other than natural person(s) either alone or jointly with natural person(s)
" 2nd year	" 3rd year	Rs. 500/-	Rs. 2000/-
" 3rd year	" 4th year		
" 4th year	" 5th year		
" 5th year	" 6th year		
" 6th year	" 7th year	Rs. 1500/-	Rs. 6000/-
" 7th year	" 8th year		
" 8th year	" 9th year		
" 9th year	" 10th year		
" 10th year	" 11th year	Rs. 3000/-	Rs. 12000/-
" 11th year	" 12th year		
" 12th year	" 13th year		
" 13th year	" 14th year		
" 14th year	" 15th year	Rs. 5000/-	Rs. 20000/-
" 15th year	" 16th year		
" 16th year	" 17th year		
" 17th year	" 18th year		
" 18th year	" 19th year		
" 19th year	" 20th year		

- (i) The above Schedule of renewal fees has come into force on 01/01/2005
(ii) The fees for two or more years may be paid in advance.

(1)

When tendering the renewal fee full particulars regarding the number and date of the patent and the name of the grantee should be given

(2)

If the renewal fee is not paid within the prescribed time, an extension of time upto 6 months can be obtained on payment of additional fee of Rs. 300/- for natural person(s) and Rs. 1,200/- for other than natural person(s) either alone or jointly with natural person(s) per month.
Request for extension of time should be made on Form 4.

(3)

Deeds of assignments of patents and other documents affecting the right proprietorship of patents such as licences, mortgages etc. should be entered in the Register of Patents as otherwise the rights or interests created by such documents may not be recognized.

(4)

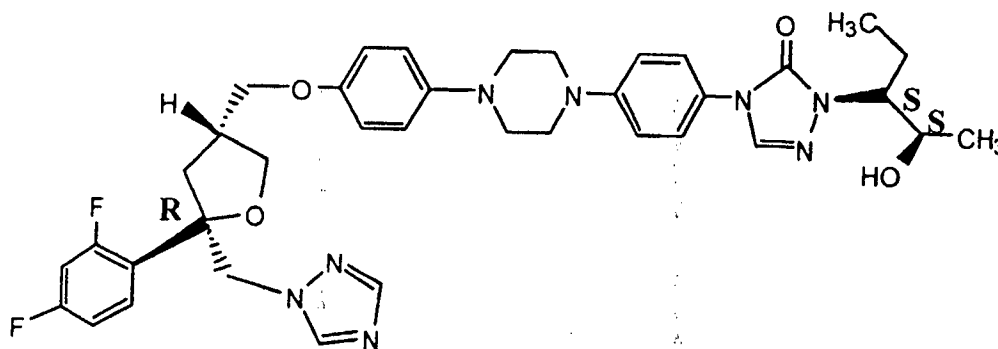
It is obligatory on the part of the patentee to supply information under Section 146 (2) read with Rules 131(1) & 131(2), failure of which will penalty provisions under the Patents Act, 1970. You are, therefore requested to file the said information within the prescribed time every year.

(5)

For further details, The Patents Rules, 2003 may be referred to.

We claim:

1. A liquid suspension comprising an antifungally effective amount of the micronized compound represented by the chemical structural formula I comprising:



at least one thickening agent, at least one non-ionic surfactant, and a pharmaceutically acceptable liquid carrier.

2. The liquid suspension of claim 1, wherein the at least one non-ionic surfactant is a block copolymer of ethylene oxide and propylene oxide, glycol or glyceryl esters of saturated or unsaturated C_8 to C_{20} acids, polyoxyethylene esters of saturated or unsaturated C_8 to C_{20} acids, polyoxyethylene ethers of saturated or unsaturated C_8 to C_{20} acids, polyvinylalcohols or sorbitan esters of saturated or unsaturated C_{10} to C_{20} acids.
- 15 3. The liquid suspension of claim 1, wherein the at least one non-ionic surfactant is a polyoxyethylene derivative of a sorbitan ester of a saturated or unsaturated C_{10} to C_{20} acid.

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4. The liquid suspension of claim 1, wherein the at least one thickening agent is selected from xanthan gum, liquid sugars, starches, celluloses and mixtures thereof.

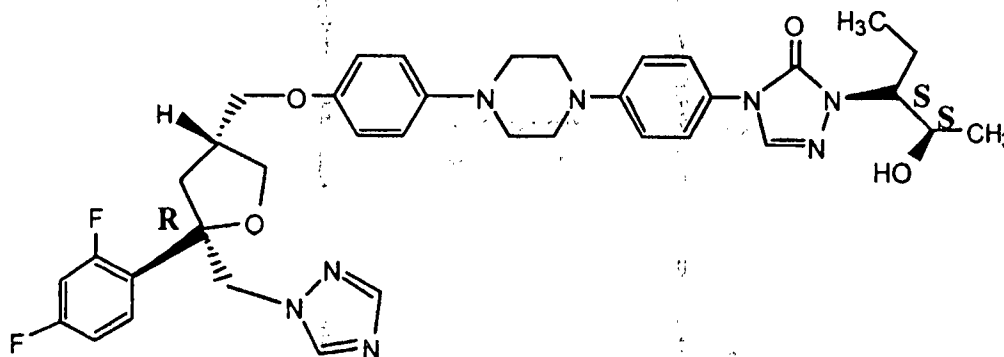
5. The liquid suspension of claim 1, wherein a combination of xanthan gum and a liquid sugar are used as the at least one thickening agent.

6. The liquid suspension of claim 1, wherein the micronized compound of formula I has a mean particle size of about 1200 nm to about 1600 nm.

7. A liquid suspension comprising:

(a) an antifungally effective amount of the micronized compound

represented by the chemical structural formula I:



- 15 (b) an effective amount of at least one thickening agent;
- (c) an amount of a buffer system effective to maintain the pH of the system in the range of about 4.0 to about 6.0;
- (d) an effective amount of at least one non-ionic surfactant; and
- (e) a pharmaceutically acceptable liquid carrier.

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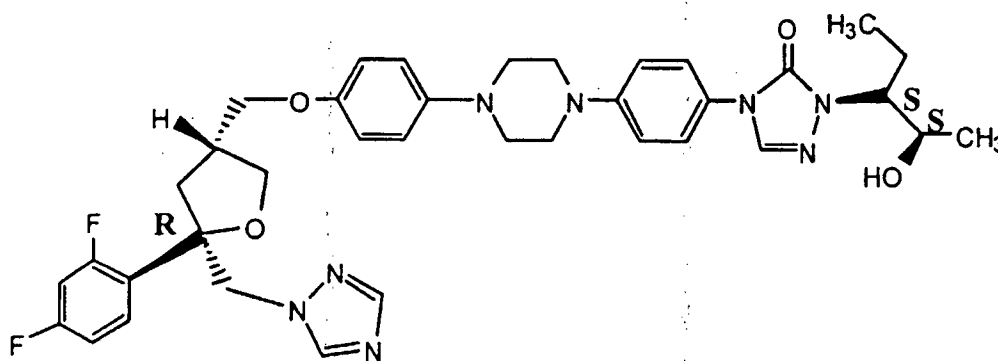
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8. The liquid suspension of claim 7, wherein the at least one non-ionic surfactant is a polyoxyethylene derivative of a sorbitan ester of a saturated or unsaturated C₁₀ to C₂₀ acid.
9. The liquid suspension of claim 7, wherein the sorbitan ester is a fatty acid ester of sorbitan selected from sorbitan monolaurate, sorbitan monooleate, sorbitan sesquioleate, sorbitan trioleate, sorbitan monopalmitate, sorbitan monostearate and sorbitan tristearate, or mixtures thereof.
10. The liquid suspension of claim 7, wherein the at least one thickening agent is selected from gums, liquid sugars, starches, cellulose and mixtures thereof.
- 10 11. The liquid suspension of claim 7, wherein a combination of a xanthan gum and a liquid sugar is used as the at least one thickening agent.
12. The liquid suspension of claim 7, wherein a combination of a xanthan gum and a liquid glucose is used as the thickening agent.
13. The liquid suspension of claim 7, wherein the buffer system comprises sodium citrate and citric acid.
- 15 14. The liquid suspension of claim 7, wherein the pharmaceutically acceptable liquid carrier is a combination of purified water, glucose and glycerin.
15. The liquid suspension of claim 7, wherein the micronized compound of formula I has a mean particle size of about 1200 nm to about 1600 nm.

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16. A liquid suspension comprising:

- (a) an antifungally effective amount of the micronized compound comprising chemical structural formula I:



wherein the micronized compound of formula I has a mean particle size in the range of about 1200 nm to about 1600 nm,

- (b) an effective amount of a polyoxyethylene derivative of sorbitan esters of saturated or unsaturated C₁₂ to C₁₈ acids;

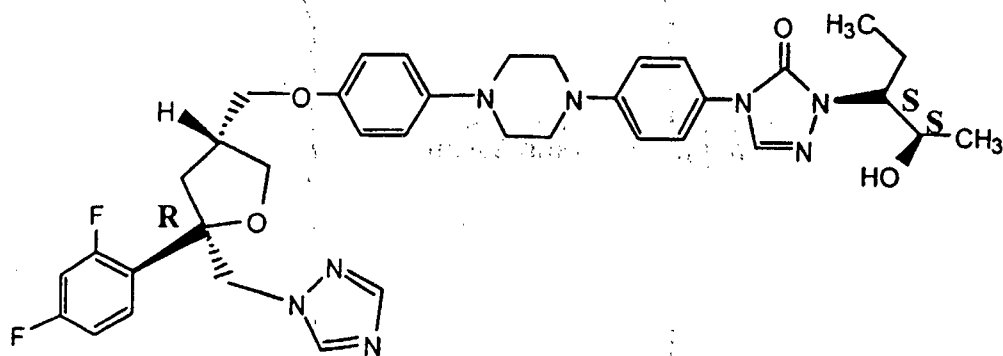
(c) an effective amount of a buffer system sufficient to maintain a pH in the range of about 4.0 to about 6.0;

(d) an effective amount of a combination of two thickening agents,

wherein one is a liquid sugar; and

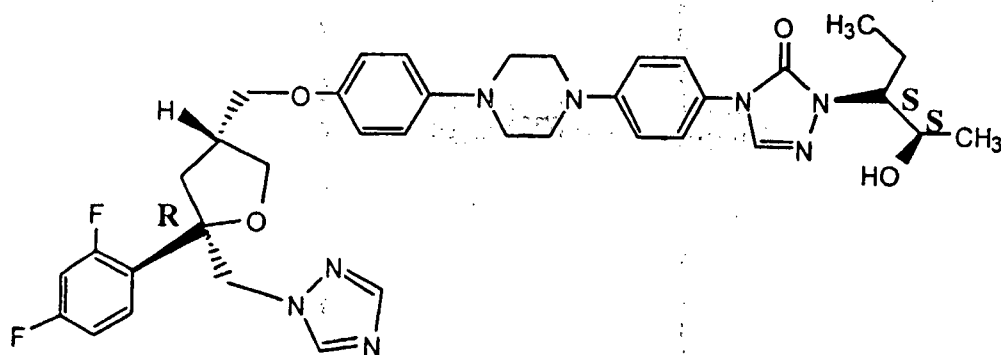
(e) a pharmaceutically acceptable liquid carrier.

17. The use of micronized particles of the compound of chemical structural formula I:



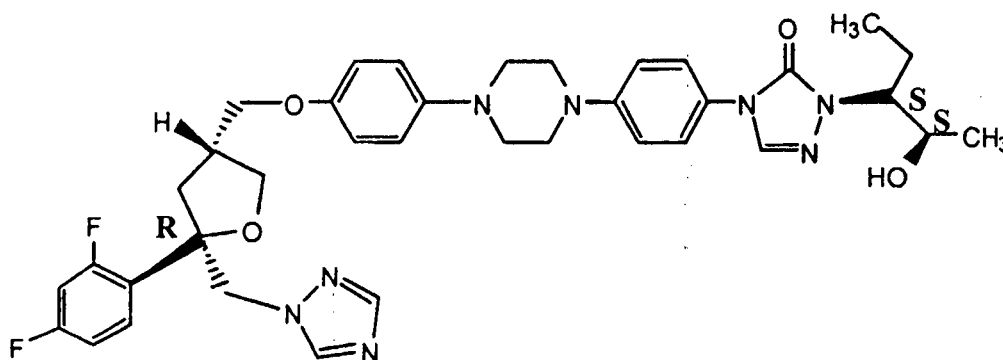
for preparation of a medicament in the form of an oral suspension for the treatment and/or prevention of a fungal infection in a mammal comprising an antifungally effective amount of the compound of formula I and at least one thickening agent and a non-ionic surfactant in a pharmaceutically acceptable carrier.

18. A suspension comprising an antifungally effective amount of the micronized particles of the compound comprising chemical structural formula I:



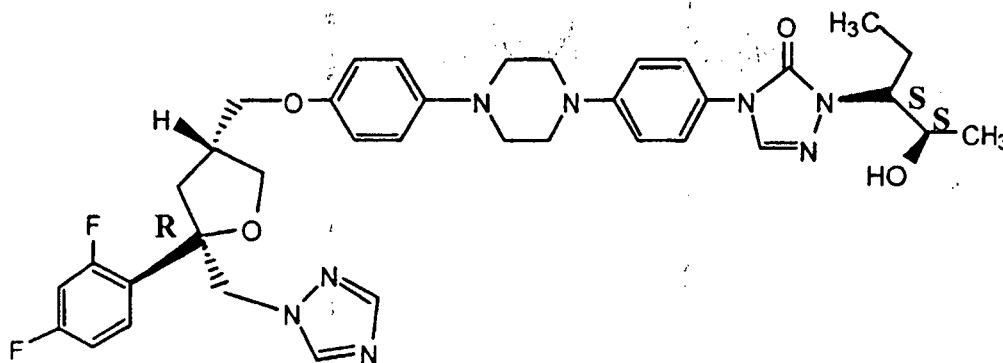
at least one thickening agent and a non-ionic surfactant in a pharmaceutically acceptable liquid carrier.

19. A composition of matter comprising micronized particles of the compound represented by structural formula I



and at least one non-ionic surfactant.

20. A composition of matter comprising micronized particles of the compound represented by chemical structural formula I:



in suspension.