



No. 13-108961- -00009-0000

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Act. 412 REPOSPRESRECU

Folios: 72

Señor Superintendente
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
E. S. D.

Trámite : 011
Evento : 378
Actuación : 412

Referencia : Expediente No. **13.108.961**
Asunto : Solicitud de Patente para: **"HIDRATO DE TARTRATO DE 1-
{(2S)-2-AMINO-4-[2,4-BIS(TRIFLUOROMETIL)-5,8-DI-
HIDROPIRIDO[3,4-d]PIRIMIDIN-7(6H)-IL-]-4-OXOBUTIL}-5,5-
DIFLUORO-PIPERIDIN-2-ONA"**
Solicitante : LG LIFE SCIENCES, LTD.
Fecha de solicitud : 30 de abril de 2013.

I. RECURSO VÍA GUBERNATIVA

Yo, Luz Clemencia DE PÁEZ, en mi condición de apoderada de la sociedad **LG LIFE SCIENCES, LTD.** solicitante de la patente de la referencia, por el presente escrito atentamente manifiesto a usted que debidamente notificada de la Resolución No. **11320** del 13 de Marzo de 2015, interpongo en su contra el recurso de **REPOSICIÓN**, para que sea revocada y en consecuencia se conceda el privilegio de patente de invención para **"HIDRATO DE TARTRATO DE 1-{(2S)-2-AMINO-4-[2,4-BIS(TRIFLUOROMETIL)-5,8-DI-HIDROPIRIDO[3,4-d]PIRIMIDIN-7(6H)-IL-]-4-OXOBUTIL}-5,5-DIFLUORO-PIPERIDIN-2-ONA"** a favor de **LG LIFE SCIENCES, LTD.**

II. OPORTUNIDAD DE LA IMPUGNACIÓN

La Resolución No. **11320** del 13 de Marzo de 2015, tiene fecha de publicación en listado 17 de Marzo de 2015, fecha de vencimiento 17 de Abril de 2015, y fecha del término legal 4 de Mayo de 2015.

"EXCELENCIA LEGAL EN UN MUNDO SIN FRONTERAS"

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III. LA DECISIÓN RECURRIDA

3.1. Mediante la Resolución objeto de la presente impugnación, el Superintendente de Industria y Comercio decidió:

3.1.1. Denegar la patente de invención a la creación titulada: **“HIDRATO DE TARTRATO DE 1-((2S)-2-AMINO-4-[2,4-BIS(TRIFLUOROMETIL)-5,8-DI-HIDROPIRIDO[3,4-d]PIRIMIDIN-7(6H)-IL-]-4-OXOBUTIL)-5,5-DIFLUORO-PIPERIDIN-2-ONA”**.

3.2. En la parte motiva de la providencia, se mencionaron los siguientes fundamentos:

3.2.1. No se acepta el contenido de agua como una característica esencial del compuesto reivindicado ya que se considera que éste es un resultado a alcanzar.

3.2.2. Se considera que la definición del sólido cristalino no es clara, ya que se incluyen únicamente seis picos del espectro de difracción de rayos X, mientras que menos de diez picos son insuficientes para caracterizar de manera inequívoca un sólido cristalino de un compuesto dado.

3.2.3. Teniendo en cuenta lo anterior, se determinó que las reivindicaciones carecen de nivel inventivo debido a que la modificación de las propiedades de un compuesto mediante la obtención de polimorfos del mismo es una práctica común en el campo técnica, y a que no se presenta evidencia de ventajas reales del sólido reivindicado frente al compuesto divulgado en el documento D1.

IV. OBJETO DEL RECURSO

De acuerdo con los argumentos que expongo a continuación, solicito atentamente a su Despacho lo siguiente:

4.1. Revocar la Resolución No. **11320** del 13 de Marzo de 2015.

4.2. Conceder el privilegio de patente de invención para “HIDRATO DE TARTRATO DE 1-{(2S)-2-AMINO-4-[2,4-BIS(TRIFLUOROMETIL)-5,8-DI-HIDROPIRIDO[3,4-d]PIRIMIDIN-7(6H)-IL-]-4-OXOBUTIL}-5,5-DIFLUORO-PIPERIDIN-2-ONA” a favor de LG LIFE SCIENCES, LTD..

V. FUNDAMENTOS DEL RECURSO

Respetuosamente me permito disentir de lo expuesto por ese Despacho en la Resolución objeto del presente recurso, toda vez que la presente solicitud sí cumple con los requerimientos de novedad y nivel inventivo exigidos en la Decisión 486, como se demostrará a continuación:

5.1. Modificación al pliego reivindicatorio

De acuerdo con el artículo 34 de la Decisión 486 de la Comisión de la Comunidad Andina, donde se establece que la modificación de una solicitud es posible en cualquier momento del trámite mientras no implique una ampliación de la solicitud inicial, me permito presentar un nuevo pliego reivindicatorio conformado por 3 reivindicaciones.

El nuevo pliego reivindicatorio es esencialmente igual al anterior, pero se han incluido cuatro picos adicionales en el difractograma de XRD, con el fin de sumar un total de diez picos, como lo sugiere el Señor Examinador.

5.2. Con respecto a la claridad del pliego reivindicatorio

En primer lugar, es preciso mencionar que el contenido de agua del hidrato reivindicado no corresponde a un resultado a alcanzar, sino a un parámetro del mismo. Este parámetro le permite a una persona versada en la materia reproducir la invención inequívocamente obteniendo todos los resultados técnicos mencionados en la solicitud.

Con respecto a lo anterior, se hace referencia al numeral 2.6.5.9 de la "GUÍA PARA EXAMEN DE SOLICITUDES DE PATENTE DE INVENCION Y MODELO DE UTILIDAD" de su Despacho, el cual establece que:

"Una reivindicación de producto, por ejemplo un compuesto químico, se puede caracterizar por su estructura y elementos, por su fórmula química, como un producto de un proceso, o excepcionalmente por sus parámetros.

(...)

El examinador no permitirá la caracterización de un compuesto químico solamente por sus parámetros, a menos que la invención no se pueda definir de otra manera. En cualquier caso, el parámetro tiene que poder ser determinado y medido sin ambigüedad por métodos estándar conocidos en el campo en cuestión, o descritos claramente en la solicitud".

En este caso, y en vista de lo anterior, la invención sí puede ser definida mediante el parámetro "resistencia a la extracción" ya que:

- La invención no está definida solamente por sus parámetros;
- La invención no puede ser definida adecuadamente de otra manera;
- El parámetro puede ser medido sin ambigüedad por métodos estándar conocidos en el campo técnico.

Consecuentemente, la reivindicación 1 cumple con lo dispuesto en la "GUÍA PARA EXAMEN DE SOLICITUDES DE PATENTE DE INVENCION Y MODELO DE UTILIDAD" de su Despacho, por lo que el contenido de agua en el hidrato debe ser considerado un parámetro que sí puede definir la invención, y por lo tanto debe ser considerado una característica técnica esencial de dicha reivindicación.

Por otro lado, y de acuerdo con las sugerencias del Señor Examinador, se han incluido cuatro picos adicionales (2θ) en el difractograma de XRD, definiendo así la invención mediante diez picos en el patrón de difracción de Rayos X en polvo. De esta forma, se ha cumplido con la exigencia establecida en la Resolución No. **11320** del 13 de Marzo de 2015 con respecto a la definición de polimorfos.

En consecuencia de lo anterior, se considera que las objeciones formuladas en cuanto a la falta de claridad de las reivindicaciones han sido superadas.

5.3. Con respecto al nivel inventivo de la presente solicitud

Mediante el Radicado No. 13-108961- -00005-0000 se presentó, en respuesta al oficio técnico 10462 notificado por fijación en lista el 26 de Agosto de 2014, la siguiente tabla con resultados experimentales comparativos con respecto a la estabilidad térmica de la base libre y la sal de tartrato:

Condición	Tiempo (Semanas)	Base libre (%)	Sal de tartrato (%)
40°C/75%RH	0	96.24	98.58
	2	96.22	98.12
	4	94.27	97.75
	6	-	97.74

También se explicó en dicho memorial que la base libre del ejemplo 83 de D1 tiene propiedades físicas que dificultan el desarrollo de un fármaco que lo incluya, ya que es difícil de cristalizar y tiene poca estabilidad térmica.

Con respecto a lo anterior el Señor Examinador afirmó, en la Resolución No. **11320** del 13 de Marzo de 2015, que habría resultado obvio para una persona versada en la materia preparar hidratos u otras formas cristalinas de los compuestos activos con el fin de mejorar sus propiedades fisicoquímicas.

Sin embargo, no es posible afirmar que los hidratos exhiben en general una mayor estabilidad que sus otras contrapartes. Ni siquiera una persona altamente versada en la materia habría podido predecir las propiedades de un hidrato y un anhidrato. Para soportar lo anterior se adjuntan tres Referencias:

- Referencia 1 (R1): Joel Bernstein, *Polymorphism in Molecular Crystals*, Clarendon Press, Oxford, 2002, pp. 241-249

- Referencia 2 (R2): Abu T. M. Serajuddin, et al., *Journal of Pharmaceutical Sciences*, Vol. 75, No. 5, Mayo 1986, pp. 492-496
- Referencia 3 (R3): WO 96/039406 A1 (12 de Diciembre de 1996)

R1 confirma repetidamente que las propiedades de los compuestos de acuerdo con las formas sólidas no pueden ser predichas y tienen que ser determinadas experimentalmente. Así, no es posible predecir cuál de las formas es más estable (compuesto libre, anhidrato, hidrato) y qué cantidad de hidratación es preferida para la estabilidad del mismo.

Por su parte, R2 se relaciona con el farmacéutico α -pentil-1-3-(2-quinolinilmetoxi)benzenometanol (REV 5901), que es muy poco soluble en agua. En R2, la sal clorhidrato del REV 5901 existe en las formas anhidra y monohidrato. La forma hidrato puede ser convertida en hidrato a una humedad relativa mayor al 40%. Sin embargo, el hidrato es inestable ya que éste pierde agua a una temperatura relativamente baja (ver página 495 de R2). En R2 se selecciona la forma libre (base) debido a que la estabilidad de ésta es mayor que la del hidrato, y también debido a la variabilidad de hidratación de la sal y la ausencia de una mejora en la disolubilidad de la sal.

R3 está relacionada con una sal de ácido metanosulfónico de derivados de ácido carboxílico naftiridina, y afirma que dicha sal tiene una estabilidad ventajosa para su formulación como un agente antibacteriano (abstract de R3). Esta referencia enseña que el anhidrato es más estable que el hidrato.

Las tres referencias aquí citadas son una prueba clara de que no es posible predecir las propiedades de un hidrato sin la experimentación requerida.

Por otro lado, se debe mencionar que el método problema-solución, utilizado para determinar si una reivindicación es inventiva o no, está basado en determinar si una persona normalmente versada en la materia habría podido derivar de forma obvia dicha reivindicación al enfrentarse al problema técnico resuelto por ésta, en vista de las enseñanzas del arte previo. Ya que es imposible prever las propiedades físico-químicas

de un solvato-morfo, el nivel inventivo de la reivindicación 1 de la presente solicitud debe ser reconocido.

Ahora con respecto a la afirmación incluida en la Resolución No. **11320** del 13 de Marzo de 2015 según la cual: *"si bien la solicitante identificó una aplicación farmacotécnica para la forma 1.5 hidrato obtenida, esto no representa evidencia de ventajas reales de dicho sólido, toda vez que no existe ningún efecto asociado a una mejora en las propiedades farmacológicas o en la actividad de dicho agente, más aun teniendo en cuenta que es conocido que las formas de hidrato la mayor parte de las veces son menos solubles en medio acuoso"*, es preciso mencionar que ésta es una apreciación personal del Examinador, ya que, como se mencionó anteriormente, es imposible prever de forma general las propiedades de los hidratos de forma general. Así, la aseveración mencionada carece completamente de sustento, y es contraria a los hallazgos de los inventores, según los cuales la Forma I (1.5 hidrato) de la presente invención tiene una estabilidad térmica, estabilidad ponderada y densidad aparente mayores que la Forma cristalina II (0.5 hidrato).

Con respecto a lo anterior, el numeral 2.13.5 de la "GUÍA PARA EXAMEN DE SOLICITUDES DE PATENTE DE INVENCION Y MODELO DE UTILIDAD" de su Despacho establece que "El examinador no debe basarse en apreciaciones personales, toda objeción referente a falta de nivel inventivo debe probarse con documentos del estado de la técnica". Por lo tanto, las objeciones plasmadas en la Resolución No. **11320** del 13 de Marzo de 2015 carecen de validez y, por lo tanto, no pueden afectar el nivel inventivo de la presente solicitud.

Como consecuencia de lo anterior, la reivindicación independiente 1 es inventiva sobre el estado del arte citado. Consecuentemente, las reivindicaciones 2 y 3 son también inventivas en virtud de su dependencia de la reivindicación 1.

5.4. Concesiones internacionales

En adición a los argumentos presentados, es relevante mencionar que el mérito investigativo de la presente solicitud ha sido reconocido por otras oficinas de patentes del

mundo, las cuales han otorgado patentes para invenciones equivalentes. Tal es el caso de los Estados Unidos de América (Patente US 8,859,567 adjunta) y la Unión Europea (Se adjunta la intención de concesión y las reivindicaciones concedidas).

Si bien es cierto que cada país es autónomo en conceder o negar el privilegio de patente para una solicitud, también es cierto que los requisitos de concesión de dichos privilegios son universales, esto es, novedad, nivel inventivo y aplicación industrial, como también la forma de evaluarlos. Así las cosas, el hecho de que la misma solicitud cumpla con los mencionados requisitos en diferentes países, como se mencionó anteriormente, es un claro indicio del merecimiento de la patente en Colombia.

5.5. Conclusión

Los documentos D1 y D2 no resultan anterioridades válidas capaces de afectar la novedad y el nivel inventivo del hidrato de la presente solicitud.

Por todo lo anterior, se considera que la presente solicitud cumple a cabalidad con los requerimientos de patentabilidad exigidos por la legislación actual, por lo que atentamente solicitamos en consideración lo siguiente:

VI. PETICIÓN

Por todo lo anterior, solicito a ese Despacho lo siguiente:

6.1. Que al considerar las razones precedentes se revoque la decisión contenida en la Resolución No. **11320** del 13 de Marzo de 2015, en cuanto resuelve negar el privilegio de patente de invención.

6.2. Que se otorgue el privilegio de patente de invención para “**HIDRATO DE TARTRATO DE 1-({2S)-2-AMINO-4-[2,4-BIS(TRIFLUOROMETIL)-5,8-DI-HIDROPIRIDO[3,4-**

d]PIRIMIDIN-7(6H)-IL-]-4-OXOBUTIL}-5,5-DIFLUORO-PIPERIDIN-2-ONA” a favor de LG LIFE SCIENCES, LTD.

VII. NOMBRE Y DIRECCIÓN

El nombre y la dirección del solicitante recurrente son los siguientes:

LG LIFE SCIENCES, LTD.

LG Gwanghwamun BLDG., 92 Sinmunno

2-ga, Jongno-gu, Seoul 110-062

Seúl

Corea

VIII. ANEXOS

- Formulario de modificaciones en solicitud pendiente
- Recibo de pago de \$132.000 por concepto de modificaciones voluntarias a una solicitud de patente
- Nuevo pliego reivindicatorio conformado por 3 reivindicaciones
- Referencia 1 (R1): Joel Bernstein, *Polymorphism in Molecular Crystals*, Clarendon Press, Oxford, 2002, pp. 241-249
- Referencia 2 (R2): Abu T. M. Serajuddin, et al., *Journal of Pharmaceutical Sciences*, Vol. 75, No. 5, Mayo 1986, pp. 492-496
- Referencia 3 (R3): WO 96/039406 A1 (12 de Diciembre de 1996)
- Patente US 8,859,567
- Intención de concesión de la Oficina Europea de patentes y reivindicaciones concedidas

IX. NOTIFICACIONES

Atentamente manifiesto a usted que recibiré notificaciones en la Secretaria de su Despacho o en mi oficina de abogada situada en la Carrera 4ª. No. 72-35, piso 4º, de esta ciudad de Bogotá, D.C.

Señor Superintendente,


LUZ CLEMENCIA DE PÁEZ

C.C. 35.456.344 de Usaquén

T.P.A. No. 23.555

mem

COLO-13548-0841-0012-1
\\SML\\ID-279868\\WPC13548
No. M-2015-279868\\

 Industria y Comercio SUPERINTENDENCIA	Espacio reservado para el adhesivo de radicación
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DIRECCIÓN DE NUEVAS CREACIONES
MODIFICACIONES Y/O CORRECCIONES

1. Identificación del Trámite

☐ Error material

☒ Patente de Invención

☐ Patente de Modelo de Utilidad

☐ Registro de Diseño Industrial

☐ Esquema de Trazado de Circuitos Integrados

☒ Modificación a una Solicitud en trámite

2. Datos del solicitante

Persona Natural

Persona Jurídica

LG LIFE SCIENCES, LTD.

Nombre o denominación / Nombre o razón social completa

Nombre representante legal

Tipo de empresa

Micro

Pequeña

Mediana

Otra

Documento de identificación:

C.C.

C.E.

NIT

Otro Número

Nacionalidad/País de constitución	Dirección y domicilio del titular	Ciudad
Corea	LG Gwanghwamun Bldg., 92, Sinmunno, 2-ga, Jongno-gu, Seoul 110-062, Corea.	Seoul
Dirección electrónica	No. Fax	Número telefónico
clientes@cavelier.com	2 11 86 50	3 47 36 11

☐

Autorizo expresamente a la Superintendencia de Industria y Comercio para que todas las comunicaciones sean enviadas a través de la dirección de correo electrónico

3. Datos del apoderado:

Apellidos y Nombre

Documento de identificación

Tarjeta profesional

De Páez Luz Clemencia

C.C. 35.456.344

T.P.A. 23.555

Dirección y domicilio	Ciudad	
Carrera 4ª No. 72 –35	Bogotá	
Dirección electrónica	No. Fax	Número telefónico
cavelier@cavelier.com	2 11 86 50	3 47 36 11

En caso de haber presentado poder general para asuntos que se adelanten ante la Delegatura de Propiedad Industrial, sírvase indicar el número de su radicación o protocolo de poder general

4. Datos del trámite

Corrección:

Expediente No 13.108.961

Aparece

Debe ser

Modificación:

☒ **Modificación al capítulo reivindicatorio.**
Presento nuevo capítulo reivindicatorio compuesto por 3 reivindicaciones y pido respetuosamente que el examen de patentabilidad sea realizado sobre estas nuevas reivindicaciones presentadas. Respetuosamente manifiesto que a pesar de eliminar materia del pliego reivindicatorio originalmente presentado, mi representada no renuncia a la protección que pueda obtener sobre dicha materia y además se reserva el derecho de presentar una solicitud divisional que la contenga durante el trámite de la presente solicitud, beneficiándose de la fecha de prioridad inicialmente reivindicada.

☐ **Modificación al capítulo descriptivo.**

☐ **Modificación al resumen.**

☐ **Modificación a los dibujos.**

5. Anexos

- ☒ Comprobante de pago de la tasa para la presentación de la corrección y/o modificación por \$132.000.
- ☐ Poder, si fuere el caso.
- ☒ Documento que contiene las modificaciones o correcciones.

6. Firma	
Nombre y apellidos	Firma
LUZ CLEMENCIA DE PAEZ	<i>LUZ CLEMENCIA DE PAEZ</i>
C.C. 35.456.344 DE Usaquéen	Tarjeta Profesional 23.555

REIVINDICACIONES

1. Un 1,5 hidrato de sal de tartrato de 1-((2S)-2-amino-4-[2,4-bis(trifluorometil)-5,8-dihidro-pirido[3,4-d]pirimidin-7(6H)-il]-4-oxobutil)-5,5-difluoropiperidin-2-ona que tiene un contenido de agua en el rango que oscila entre 3,5 y 5,5 %, en donde los valores característicos del pico (2θ) en el difractograma de XRD son de 10.6, 14.6, 15.4, 16.6, 18, 20, 21, 23, 25.7 y 28.9°.

2. Un proceso para preparar el 1,5 hidrato de acuerdo con la reivindicación 1, caracterizado porque la sal de tartrato de 1-((2S)-2-amino-4-[2,4-bis(trifluorometil)-5,8-dihidropirido[3,4-d]pirimidin-7(6H)-il]-4-oxobutil)-5,5-difluoropiperidin-2-ona se recrystaliza a partir de solvente de agua, acetonitrilo/agua, etanol/agua, etanol/hexano o acetato de etilo/hexano.

3. El proceso de acuerdo con la reivindicación 2, caracterizado porque el solvente de recrystalización es agua.

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Polymorphism in Molecular Crystals

JOEL BERNSTEIN

*Department of Chemistry
Ben-Gurion University of the Negev*



CLARENDON PRESS • OXFORD
2002

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7.2 Occurrence of polymorphism in pharmaceuticals

7.2.1 Drug substances

The development of a new drug from a promising lead compound to a marketed product is a long and expensive process, with odds of success estimated at 1 in 10 000 (Yevich 1991). The strict quality control requirements and the intellectual property implications of the drug industry lead to thorough and intensive investigations of the formation and properties of solid substances intended for the use in pharmaceutical formulations, both active ingredients and excipients. These efforts, often extending over long periods of time and with many potential experimental and environmental variables, can create conditions that can lead to the appearance of polymorphic forms, intentionally or serendipitously. While it may not be surprising that many pharmaceutically important materials have been found to be polymorphic, or that any particular compound may turn out to be polymorphic, every compound is essentially a new situation, and the state of our knowledge and understanding of the phenomenon of polymorphism is still such that we cannot predict with any degree of confidence if a compound will be polymorphic, prescribe how to make possible (unknown) polymorphs, or predict what their properties might be (Beyer *et al.* 2001).

There have been a few attempts to compile instances of polymorphism in pharmaceutically important materials. Since even the definition of what comprises 'pharmaceutically important materials' is itself subject to debate it is difficult to judge how comprehensive such compilations might be. However, generally they serve as useful references and are given here. One of the first organized attempts at such a compilation for steroids, sulphonamides and barbiturates was by Kuhnert-Brandstätter (1965) (see also Kuhnert-Brandstätter and Martinek 1965). Much of those data may be found in her subsequent book (Kuhnert-Brandstätter 1971), which also contains a compilation of many of the thermal studies of pharmaceutical compounds that revealed polymorphic behaviour. Numerous additional reports of studies by the Innsbruck school of polymorphic pharmaceutical compounds (using thermomicroscopy and IR spectroscopy) have appeared in the literature since the middle 1960s; many of those have been listed by Byrn *et al.* (1999). Borka and Halebian (1990) compiled a list of over 500 references to reports of polymorphism in over 470 pharmaceutically important compounds.¹ This was shortly followed (Borka 1991) by a review of polymorphic substances included in Fasciculae 1–12 of the European Pharmacopoeia (EP), including a comparison of melting points in the EP and the original literature. The latter review was subsequently updated in 1995 by including EP entries for Fasciculae 13–19 (Borka 1995).

Griesser and Burger (1999) compiled the information regarding 559 polymorphic forms, solvates (including hydrates) of drug solids at 25 °C in the 1997 edition of

¹ Dr Borka has communicated with this author that he and Dr Halebian did not receive galley proofs of this paper, which unfortunately contains 'numerous printing errors'. The list of errata actually contains 48 of them. Even with that cautionary note, it is a useful compilation.

18
16

the EP. They also noted that of the 10 330 compounds in the 1997 edition Merck Index only 140 (1.4 per cent) are specifically noted as polymorphic, 540 (5 per cent) are noted as hydrates and 55 (0.5 per cent) have been specified as solvates. These numbers reflect a failure to report or to include these phenomena rather than representative statistics, and may suggest the current state of awareness of polymorphism on the part of compilers of such compendia and reference works.

A survey by this author of the 1 October 2000 release of the CSD (~225 000 entries) yielded 6353 hits for the qualifier 'form', 1045 hits for the qualifier 'phase' 528 hits for the qualifier 'polymorph', 201 hits for the qualifier 'modification', 28 342 hits for the qualifier 'solvate' and 21 132 hits for the qualifier 'hydrate'. Of course, the last two numbers give no indication of whether the materials are polymorphic and none of these statistics indicate the instances for which the structures of more than one form have been determined. The data for hydrates and solvates from the CSD may be considered quite reliable, since molecules of solvation are usually positively identified in the course of a crystal structure determination. The frequency of polymorphs, however, is likely to be underestimated, since many crystal structures of polymorphic systems, or what later are discovered to be polymorphic systems, are reported without making note of that fact. If the structure of only one member of a polymorphic family has been reported, then there may not be any reference to the polymorphism in the CSD. References to drugs discovered prior to 1971 that form solvates have been compiled, along with a separate summary of the thermomicroscopic behaviour of drug hydrates by Byrn *et al.* (1999).

Because of the rather select nature of the sample set and the distinct possibility that not all forms are always reported caution must be exercised in drawing conclusions from such statistical surveys. Nevertheless, according to Griesser and Burger (1999) there may be some apparent tendencies which should be monitored as data continue to accumulate: polymorphism seems to be more common for compounds with molecular weight below 350; polymorphism seems to be more common for compounds with low solubility in water; for organic salts, the formation of hydrates appears to be more common among larger molecules; organic solvates appear to be more common among neutral compounds with higher molecular weights.

7.2.2 Excipients

Pharmaceutical formulations contain the active drug ingredient(s) as well as excipients that serve a variety of purposes: fillers, stabilizers, coatings, drying agents, etc. As solid materials excipients exhibit varying degrees of crystallinity, from the highly crystalline calcium hydrogen phosphate to nearly amorphous derivatives of cellulose. These materials can also exhibit polymorphism which may influence their performance in the formulation. Giron (1995, 1997) has listed many of the excipients that are known to exhibit a number of forms (polymorphs, solvates and amorphous). They include many of those that are widely used: lactose, sorbitol, glucose, sucrose, magnesium stearate, various calcium phosphates and mannitol (Burger *et al.* 2000).

The solid nature of the excipient may influence the final physical form of the tablet (Byrn *et al.* 2001), such as a tendency to stick (Schmid *et al.* 2000), or may induce a polymorphic conversion of the active ingredient (Kitamura *et al.* 1994). Hence, there have been attempts to develop protocols for the selection of compatible active ingredient–excipient compositions (Serajuddin *et al.* 1999). For instance, nuclear magnetic resonance spectroscopy has been employed to study the structural changes in epichlorohydrin cross-linked high amylose starch excipient (Shiftan *et al.* 2000), and has also been used to discriminate between two polymorphs of prednisolone present in tablets with excipients, even at low concentrations (5 per cent w/w) of the active ingredient (Saindon *et al.* 1993). The characterization of excipients by thermal methods has also been reviewed by Giron (1997).

7.3 Importance of polymorphism in pharmaceuticals

Polymorphism can influence every aspect of the solid state properties of a drug. Many of the examples given in preceding chapters on the preparation of different crystal modifications, on analytical methods to determine the existence of polymorphs and to characterize them and to study structure/property relations, were taken from the pharmaceutical industry, in part because there is a vast and growing body of literature to provide examples. One of the important aspects of polymorphism in pharmaceuticals is the possibility of interconversion among polymorphic forms, whether by design or happenstance. This topic has also been recently reviewed (Byrn *et al.* 1999, especially Chapter 13) and will not be covered here. Rather, in this section, we will present some additional examples of the variation of properties relevant to the use, efficacy, stability, etc. of pharmaceutically important compounds that have been shown to vary among different crystal modifications.

7.3.1 Dissolution rate and solubility

The dissolution properties and solubility are often crucial factors in the choice of the crystalline form for formulation of a drug product (Carstensen 1977). In general, these two factors play a major, if not over-riding role in determining the bioavailability of the drug substance (see also Section 7.3.2). The physiological absorption of a solid dosage form usually involves the dissolution of the solid in the stomach and the rate and extent of that dissolution is often the rate-determining step in the overall absorption process. Since different crystalline forms can exhibit different dissolution kinetics and limits, these properties are routinely studied in great detail for any drug substance, whether polymorphic or not; clearly, characterization for polymorphic substances is even more critical. As a result, there is extensive literature covering such studies. An early review contained a compilation of references to many of the previously studied materials (Kuhnert-Brandstätter 1973).

The fundamentals of the measurements of solubility and dissolution properties of pharmaceuticals are given elsewhere in considerable detail (Vachon and Grant 1987;

Byrn *et al.* 1999, esp. Chapter 6; Grant and Brittain 1995). Reviews of the physical principles of dissolution and solubility behaviour of organic materials (Grant and Higuchi 1990) and pharmaceuticals (Grant and Brittain 1995) have been given and, particularly relevant to this chapter, typical examples of the variation of these processes among crystalline forms of pharmaceuticals are comprehensively described by Brittain and Grant (1999).

7.3.2 Bioavailability

The rate and extent of the physiological absorption of an active drug substance are decisive factors in its overall efficacy (e.g. carbamazepine, Zannikos *et al.* 1991). These can vary among different crystal modifications, and they have become an important scientific and regulatory issue (Ahr *et al.* 2000).

While a number of studies of the connection between the crystal modification and bioavailability have been published, it is reasonable to assume that many more remain the intellectual property of pharmaceutical companies or in confidential documentation submitted to regulatory agencies. Also many *in vitro* studies, especially those of extent or rate of dissolution, are used to extrapolate to expected bioavailabilities (e.g. Chikaraishi *et al.* 1995; Shah *et al.* 1999), which is indeed proven in some cases such as the tetramorphic tolbutamide (Kimura *et al.* 1999). We cite here a few additional examples from the open literature, limiting ourselves (except for one example below) to cases in which the bioavailability does differ among crystal modifications.

The two polymorphs of the barbiturate pentobarbital exhibit significantly different rates of absorption and area under the curve for oral administration (Fig. 7.1) (Draguet-Brughmans *et al.* 1979). Comparison of rectal absorption of suppositories containing the two polymorphs of indomethacin showed higher levels for the α

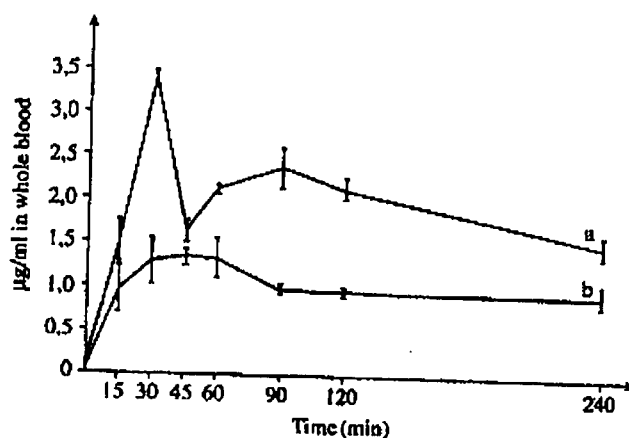


Fig. 7.1 Rate of absorption of the two polymorphs of orally administered pentobarbital. a, Form I; b, Form II. (From Draguet-Brughmans *et al.* 1979, with permission.)

form than for the γ form (Yokoyama *et al.* 1979). For the trimorphic antileukemic mercaptopurine Form III was found to be 6–7 times more soluble than Form I, but the bioavailability (in rabbits) was approximately 1.5 times greater for Form III than for Form I (Yokoyama *et al.* 1980). The antianxiety agent nabilone has at least four polymorphs, designated A, B, C and D, which appear to be equally hydrophobic and insoluble. Forms B and D are bioavailable in dogs, while A and C are not. On prolonged storage, heating or grinding, all convert to the nonbioavailable Form A (Thakar *et al.* 1977).

The bioavailability can also vary between crystalline and amorphous modifications (Section 7.7), as well as among polymorphic forms. The amorphous form of the antibacterial azlocillin sodium has more activity than the crystalline form (Kalinkova and Stoeva 1996). Similarly, an antinematode drug, code named PF1022A exists in four modifications, designated α (amorphous) and crystalline I, II and III. α and III have higher solubility and are more effective than I and II against tissue-dwelling nematodes (Kachi *et al.* 1998). An amorphous form of another code-name drug (YM022), generated by spray drying, has enhanced bioavailability over two crystalline forms (Yanu *et al.* 1996).

The degree of hydration of different modifications also plays a role in the bioavailability. One of the most studied systems in this regard is the anhydrate/trihydrate of the antibiotic ampicillin, although the results have not always led to consistent conclusions. Early *in vitro* solubilities (Poole *et al.* 1968) and rates of dissolution (Poole and Bahal 1968) were shown to differ. *In vivo* the anhydrous form reaches a maximum concentration. *In vivo* bioavailability studies by Ali and Farouk (1972) (Fig. 7.2)

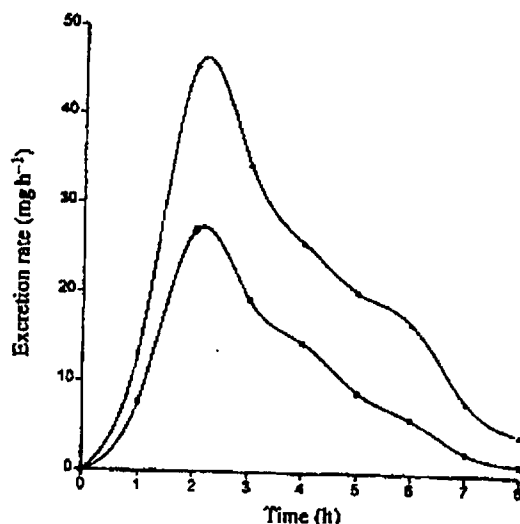


Fig. 7.2 Urinary excretion rates following administration of the anhydrate (Δ) and trihydrate (■) forms of ampicillin. (From Brittain and Grant 1999, with permission.)

clearly indicated differences between the two. Other studies, however, indicated similar bioavailabilities (Cabana *et al.* 1969; Mayersohn and Endrenyi 1973; Hill *et al.* 1975). Brittain and Grant (1999) have suggested that the differences reported by various groups indicate that the bioavailability in the case of ampicillin is strongly influenced by the nature of the formulation of the dosage form.

There are also some examples of solvates (as opposed to hydrates) that have been reported to exhibit differing bioavailability. For instance, for implants of the methanol solvate of prednisolone *tert*-butyl acetate the mean absorption rate was found to be 4.7 times that of the anhydrous form which in turn was similar to the hemiacetone solvate (Ballard and Biles 1964). The same authors also reported that the mean absorption rate for all solvates of cortisol *tert*-butyl acetate were significantly different from that of the anhydrous form.

While the rate of absorption may differ, the extent of absorption, may still be equivalent. Such is the case for sulphameter (sulphamethoxydiazine). The different polymorphic forms have been shown to exhibit different equilibrium solubilities and dissolution rates (Moustafa *et al.* 1971). Form II is thermodynamically less stable and has an absorption rate about 1.4 times that of Form III, which is more stable in water (Khalil *et al.* 1972). This group determined that the two forms have different absorption, but using 72-hour excretion data showed that the extent of the absorption of the two forms was equivalent (Khalafallah *et al.* 1974).

Perhaps the classic example of the dependence of bioavailability on polymorphic form is chloramphenicol palmitate. Chloramphenicol is a broad spectrum antibiotic and antirickettsial which was developed in the 1960s and had a significant portion of the market until the appearance of side effects limited its use to topical application. The exceedingly bitter taste of the active chloramphenicol led to its formulation as an oral suspension of the tasteless 3-palmitate (CAPP). The early physical and physiological studies on the material were summarized by Aguiar *et al.* (1967) (see also Mitra *et al.* 1993). There are three polymorphic forms (A, B and C) in addition to an amorphous form. The characterization of the various forms by melting point and IR analyses proved problematic, even inconclusive, due to polymorphic transitions during grinding for sample preparation (Borka and Backe-Hansen 1968).

The A form is the most stable, but only the B and amorphous forms are biologically active. Aguiar *et al.* determined the physiological absorption rate as a function of the A and B polymorphs, as shown in Fig. 7.3. The suspension containing only the metastable B form gives higher blood levels following oral doses than those containing only Form A, by nearly an order of magnitude. Since particle size was shown to have little effect on blood levels, it was concluded that the structure of the solid plays an intimate role in determining the physiological absorption rate. As a result of this finding, the mechanism of this absorption and its connection with the polymorphism were investigated in considerable detail.

CAPP is nearly insoluble in water; hence, it must be hydrolysed by enzymes in the small intestine before absorption can take place. According to one possible proposed mechanism (Aguiar *et al.* 1967), the first and rate determining step in the total process

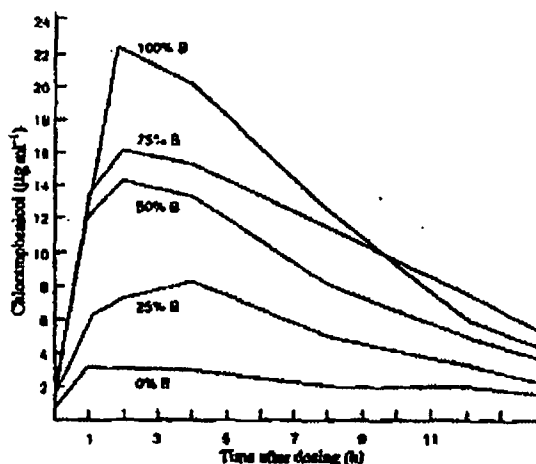


Fig. 7.3 Peak blood serum levels of chloramphenicol following dosing for pure polymorphs A and B and various mixtures for a single oral dose equivalent to 1.5 g of chloramphenicol palmitate. (From Haleblan and McCrone 1969 (after Aguilar *et al.* 1967) with permission.)

is a dissolution of the ester followed by enzymatic hydrolysis of CAPP. Such a mechanism is consistent with the generally accepted dissolution/absorption mechanism for most solid drugs. However, Andersgaard *et al.* (1974) proposed a second mechanism in which solid CAPP is enzymatically attacked in the small intestine. If dissolution is the first and rate determining step of the total process, then there should be a close relationship between the rates of dissolution and the rates of enzymatic hydrolysis of polymorphs A and B. On the other hand, no relationship of this sort is expected if CAPP is attacked in the undissolved state.

The rates of dissolution on the one hand and *in vitro* hydrolysis of the solid by the enzyme pancreatic lipase on the other hand are given in Fig. 7.4. If dissolution is the first step in the total hydrolysis process, the reaction scheme may be written as



Since the rate of the second step of this process must be the same for Forms A and B of CAPP, this model for the absorption process leads to the conclusion that any differences in the rate of formation of hydrolysed CAPP must be due to a difference in the rate of dissolution of the two polymorphs. The data presented in Fig. 7.4 are not compatible with the assumption that dissolution is the first and rate determining step, since the slopes at time zero are significantly different from those expected for such a mechanism. Rather, Andersgaard *et al.* claim that it is more reasonable to assume that CAPP is attacked in the undissolved state, probably by pancreatic lipase, which is known to act on substances insoluble in water (Waki 1970). Further studies on CAPP and the analogous stearate (Cameron *et al.* 1976) apparently corroborate

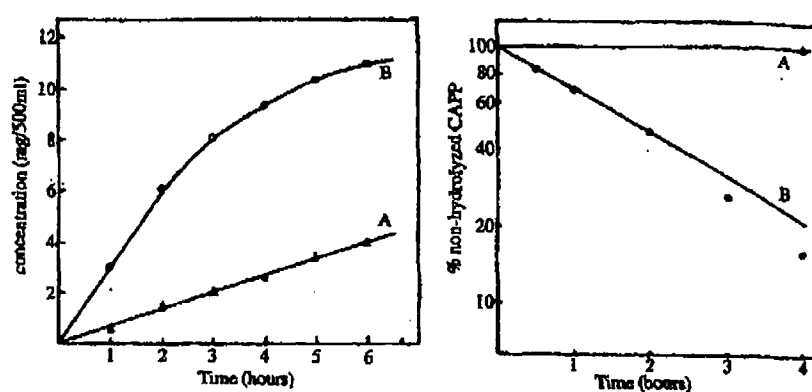


Fig. 7.4 Left: Rates of dissolution of polymorphs A and B of CAPP. Right: Rates of *in vitro* enzymatic hydrolysis by pancreatic lipase of polymorphs A and B of CAPP. (After Andersgaard *et al.* 1974, with permission.)

this assumption, indicating that there is a direct connection between the solid state structure and the enzymatic hydrolysis of chloramphenicol palmitate. It has been found that storage of the B form at the relatively high temperature of 75 °C leads to conversion to the inactive A form in a matter of hours, suggesting that extended storage at higher temperatures could lead to reduced efficacy of a formulated product (Devilliers *et al.* 1991).

The effect of the transformation of crystal form after ingestion has been of increasing interest in the past decade (see also Chapter 10). In a recent study of the two anhydrous Forms (I and III) and a dihydrate of carbamazepine (Kobayashi *et al.* 2000), it was shown that the initial *in vitro* dissolution rate was of the order III > I > dihydrate, with Form III being transformed more rapidly to dihydrate than Form I. The solubilities of the two anhydrous forms were 1.5–1.6 times that of the hydrated form. When dosed to dogs at 40 mg per body weight there were no significant differences between for the area under the curve. However, at doses of 200 mg per body weight significant differences in plasma concentration versus time were observed. It was suggested that the difference was due to rapid transformation from Form III to dihydrate in the gastrointestinal fluids.

Two recent examples involving commercially successful drugs indicate the diversity in bioavailability among different polymorphic systems. At one extreme, the two polymorphic forms of ranitidine hydrochloride (Glaxo SmithKline's H₂-antagonist Zantac®) have been shown to be bioequivalent, which is one of the reasons that ethical and generic companies were involved in litigations over the two forms (see Section 10.2). At the other end of the spectrum is Abbot's protease inhibitor Norvir®, generically ritonavir. After approximately two years on the market a new thermodynamically stable polymorph began to precipitate out of the semisolid formulated product. This proved to have lower solubility with greatly reduced bioavailability.

resulting in removal of the drug from the market for almost a year, until a new gel capsule (i.e. 'solution') formulation could be developed (Chemburkar *et al.* 2000). The apparently greater stability of a newly prepared crystal form of glibenclamide has also been attributed to reduction in dissolution and bioavailability of its tablets (Panagopoulou-Kaplanis and Malamataris 2000).

7.4 Microscopy and thermomicroscopy of pharmaceuticals

In Chapter 4, we noted the efficiency, simplicity, and utility of microscopy and thermomicroscopy in the characterization and study of polymorphs. Perhaps the most widespread and systematic application of these techniques has been in the field of pharmaceutical materials, as promoted particularly by the Institute of Pharmacognosy at the University of Innsbruck, and summarized in the (now out of print) book by Kuhnert-Brandstätter (1971). In addition to introductory chapters on techniques of hot stage microscopy, the book contains a compilation of the results of hot stage studies on approximately 1000 pharmaceutically important compounds. Many of these results were reported in more detail in an extensive series of papers beginning in the 1960s (Kuhnert-Brandstätter 1962) and continuing through the 1980s by Kuhnert-Brandstätter and her late successor, A. Burger. Later contributions often dealt with compounds actually listed in a pharmacopoeia (e.g. Burger *et al.* 1986).

Recent descriptions of microscopy and thermomicroscopy applied to pharmaceutically important compounds, along with quite comprehensive references to standard texts on the fundamentals and apparatus of the technique may be found in Byrn *et al.* (1999) and Brittain (1999b).

Prior to the development of routine and increasingly sophisticated analytical methods, hot stage microscopy competed as one of the principal tools for polymorph characterization and classification. As noted in Chapter 4, successful strategies for the investigation of polymorphs require the application of as many analytical techniques as possible, and hot stage microscopy should be considered as one of the first, if not the first to be employed in a comprehensive characterization of a compound (Morris *et al.* 1998). In spite of our current ability to generate a great deal of precise analytical data, there is often no substitute for physically observing solid materials and their behaviour as a function of temperature, preferably on the polarizing microscope. Evidence for the success of this approach has been provided in a number of recent studies.

In some cases, new phases that may not be detectable by other methods may be detected optically (Chang *et al.* 1995). Solid state conversions and their monotropic (Burger *et al.* 1997) or enantiotropic nature (Henck *et al.* 2000), or the products of desolvations may be easily recognized (Schinzer *et al.* 1997). Intimate processes of polymorphic behaviour, such as nucleation, crystal growth, habit transformation, sublimation and properties of the melt (e.g. degradation) may be readily observed and video recorded (de Wet *et al.* 1998).

As noted at the conclusion of Chapter 4, the amalgamation of a number of analytical techniques into a single instrument considerably expands the possibilities for

Preformulation Study of a Poorly Water-Soluble Drug, α -Pentyl-3-(2-quinolinylmethoxy)benzenemethanol: Selection of the Base for Dosage Form Design

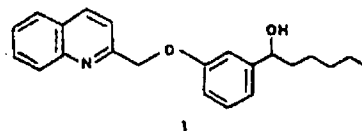
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Abstract □ The physicochemical properties of the base and hydrochloride salt of the poorly water-soluble drug α -pentyl-3-(2-quinolinylmethoxy)benzenemethanol (REV 5901) were investigated in order to select an appropriate form of the drug for dosage form development. The pH-solubility profiles of both the base and the salt at 37°C were identical and were in agreement with a pK_a value of 3.67 determined by the UV spectral method. The solubility of the drug (~0.002 mg/mL at pH 6) increased gradually with a decrease in pH and reached a value of 0.95 mg/mL at pH 1; at pH values <1, the solubility decreased due to the common-ion effect. The pH_{max} , i.e., the pH of maximum solubility of the drug was, therefore, 1.0. The role of the pH_{max} in the selection of a salt or base form of a compound was investigated. Due to the conversion of the salt to the base at the surface of the dissolving solid at pH values > pH_{max} , the dissolution rates of both the base and the salt were identical. In the solid state, the salt existed in anhydrous and monohydrate forms; the anhydrous salt converted to the hydrate at >40% relative humidity, and the hydrate lost water at 40–60°C. The thermal properties of the salt were indicative of its potential instability, which was confirmed by accelerated stability studies. The base existed in a stable crystalline solid form, and also in an oily liquid form which converted to crystals on standing. In consideration of the better stability of the base, the variability in the hydration of the salt, and the absence of a higher dissolution rate of the salt, the base was selected for the dosage form design.

Various methods of increasing the bioavailability of poorly water-soluble drugs have been reported in the literature. Among them are: micronization, salt formation, polymorphic transformation, formation of a solid solution, solubilization, coadministration with fatty meals, etc.^{1,2} Most of these methods increase the dissolution rate and/or solubility of drugs. However, the applicability of any technique in the design of a bioavailable dosage form depends on the physicochemical properties of the drug itself. No general procedure can be adopted for increasing the bioavailability of all poorly water-soluble drugs. For example, while salt formation was reported to increase the dissolution rate^{3,4} and the absorption of drugs,⁵ in some cases it retards the dissolution rate^{6,7} and does not increase the bioavailability of solid dosage forms.^{8,9}

Selection of a suitable physicochemical form of a drug at an early stage of development is important because, as indicated by Kaplan,¹⁰ subsequent modifications may require the biological and the clinical studies to be repeated. In the present study, the physicochemical properties of the base and hydrochloride salt forms of α -pentyl-3-(2-quinolinylmethoxy)benzenemethanol (1) were studied to select one of the two forms for dosage form design. The compound is a novel 5-lipoxygenase enzyme inhibitor in the arachidonic acid cascade in the body; its biological profiles were reported earlier.¹¹ It was originally synthesized as the hydrochloride salt. Due to variable physicochemical and biopharmaceutical properties of the salt during its initial biochemical, pharmacological, and drug safety screenings, the present study was undertaken.



Experimental Section

Materials—The base, α -pentyl-3-(2-quinolinylmethoxy)benzenemethanol (1) (mol. wt. 335.45), and its hydrochloride salt (mol. wt. 371.90) were synthesized by Revlon Health Care Research, Tuckahoe, NY.¹² The formation of the salt of 1 with acids weaker than hydrochloric acid was not satisfactory. The lot numbers of 1 used were 2 and 9, and those of the salt were 2 and 3. The purity of these lots of materials, when calculated on the basis of dry (anhydrous) weights, was >99.5%. The hydrochloride salt may exist as an anhydrous form or as a monohydrate. The anhydrous form was used for the determination of solubility; however, due to its conversion to a hydrate in the presence of water, the solubility profile represents the values in equilibrium with a monohydrate. The monohydrate was used for the determination of the intrinsic dissolution rate. All other reagents and chemicals used were of spectrophotometric or analytical grade.

pH-Solubility Profile—The pH-solubility profiles of 1 and its hydrochloride salt were determined at 37°C by the phase-solubility technique of Dittert et al.¹³ The Burrell wrist-action shaker was used. Solubilities at pH values less than that of the saturated solution in water were determined by stepwise titration with 0.1 or 1.0 M HCl, as necessary. After each addition, the suspension was equilibrated for 6–8 h, the pH was recorded with a Beckman model 3560 digital pH meter, and an aliquot was removed (1–2 mL). Solubilities at pH values higher than that of a saturated solution in water were determined by the stepwise addition of 0.1 M NaOH. Preliminary experiments showed that the solutions were saturated in <6 h; no significant change in solubility was observed when the equilibration was continued up to 24 h. Each aliquot was filtered through a Millipore 0.45- μ m filter immediately after collection and was diluted with a suitable solvent for UV spectral analysis. There was no significant loss of drug due to possible adsorption to the filters.

Determination of pK_a —The apparent pK_a of 1 was determined by the UV spectral method. Solutions of 1 (2.5 μ g/mL) in the pH range from 1.1 to 7.0 were used. These solutions were prepared by diluting 0.5 mL of a 0.5-mg/mL methanolic stock solution to 50 mL with water and adjusting the pH with HCl or NaOH solution. To prevent any possible precipitation of 1 at higher pH values, the spectrum was recorded within 15 min of preparation of each solution. The average value of the apparent pK_a was calculated from the absorbances at a particular wavelength using the following equation:

$$pK_a = pH - \log \left[\frac{A_{obs} - A_{BH^+}}{A_B - A_{obs}} \right] \quad (1)$$

where A_{BH^+} and A_B represent the absorbances of the ionized and the un-ionized species, respectively, and A_{obs} is the observed absorbance at a certain pH.

Intrinsic Dissolution Rate—The dissolution rates of 1 and the hydrochloride salt from a constant surface area of 0.95 cm² were studied using the apparatus designed by Wood et al.¹⁴ About 600 mg of a compound was compressed by a 10-ton hydraulic press to form a pellet at 3600 kg of pressure. The apparatus containing the compressed pellet was submerged to one-half the height of the die in 300 mL each of 0.1 and 0.01 M HCl solutions in a 600-mL beaker which was rotated at 200 rpm. The temperature of each medium was maintained at 37 ± 0.2°C by equilibration in a water bath, and the pH was monitored by using a long, thin electrode. The pH of the 0.1 and 0.01 M HCl solutions remained unchanged at 1.1 and 2.05, respectively, throughout the dissolution period. Two milliliter samples were collected at each time interval and analyzed spectrophotometrically.

Analysis of Drug—The aliquots were diluted appropriately with acidified methanol (1 M HCl:CH₃OH, 1:9 v/v) and analyzed spectrophotometrically at a wavelength of 239 nm by using a Beckman DU-8 spectrophotometer.

Conversion of the Hydrochloride Salt to the Base—The stability of the undissolved drug as the salt form in aqueous media and the possible conversion of the salt to the base were studied. For this purpose, 400 mg of the salt was dissolved in 20 mL of methanol and the solution was added to 250, 500, or 1,000 mL of water. The pH values of the resultant emulsion-type liquids were recorded. Initial dissolution of the salt in methanol facilitated its conversion to the base. Otherwise, an oily layer formed at the surface of undissolved crystals which prevented further conversion to the base. Emulsions were also formed when a smaller volume of methanol was used, or if other water-miscible solvents were substituted for methanol. When the emulsion (or the separated oil) was left overnight at room temperature, the oil globules converted to solid crystals. The crystals were dried under reduced pressure and characterized by differential scanning calorimetry, hot stage microscopy by using the Mettler FP5 apparatus, IR spectroscopy, and elemental analysis.

Thermal Properties—For the determination of differential scanning calorimetric thermograms, the samples, contained in aluminum pans, were heated with a DuPont 990 thermal analyzer at the rate of 5°C/min and at a sensitivity of 0.08 mcal/s · cm. The environment of a sample was varied by heating it in: (a) an uncovered aluminum pan; (b) an uncovered aluminum pan under a flow of nitrogen at the rate of 50 mL/min; (c) a crimped aluminum pan; or (d) a hermetically sealed aluminum pan. Thermogravimetric studies were also conducted with the same equipment using appropriate accessories. Moisture analysis was by the Karl Fischer method.

Solid State Stability—The samples were packaged in 45-mL Marlex high-density polyethylene bottles with a 28-mm vinyl-lined cap, except for the samples placed on open Petri dishes in the light cabinet. The samples were stored under various atmospheric conditions and temperatures for different intervals of time and analyzed by a stability-indicating HPLC assay.

Results and Discussion

pH-Solubility Profiles and pK_a—The pH-solubility profiles of 1 and its hydrochloride salt at 37°C are given in Fig. 1. A saturated solution of 1 in water had a pH of 6.0 and a drug concentration of ~0.002 mg/mL. Due to various problems associated with the solubility determination of sparingly soluble compounds,¹⁵ only an approximate value of such a low solubility was obtained. The solubility of 1 increased gradually with the decrease in pH by the addition of HCl, and a solubility of 0.95 mg/mL was reached at pH 1.0. A phase conversion of the undissolved 1, in equilibrium with the solution, to the hydrochloride salt was observed at pH values <1, and the solubility decreased with a decrease in pH.

The pH of a saturated solution of the hydrochloride salt in water was ~3.4 and the solubility ranged between 0.003 and 0.004 mg/mL. On lowering the pH of this solution, the solubility of the salt, as shown in Fig. 1, followed a profile identical to that of the base. There was no significant chemical degradation of 1 during the equilibration period at any of the pH conditions studied. Additionally, a stability-indicating HPLC assay confirmed that the compound was stable at pH ~1 for >7 d.

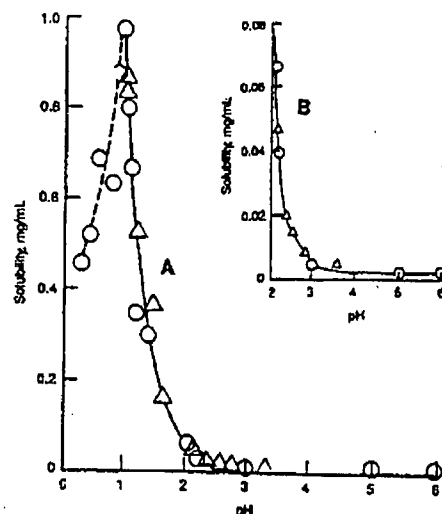


Figure 1—(A) pH-solubility profiles of α -pentyl-3-(2-quinolinylmethoxy)benzenemethanol, 1 (O) and its hydrochloride salt (Δ) at 37°C. Solubilities are expressed as base equivalents. (B) pH-solubility profile: at pH > 2 are shown with an expanded scale. The solid curves were fitted theoretically according to eq. 2 using 0.002 mg/mL as the solubility of un-ionized 1 and 3.67 as the apparent pK_a.

The UV spectra of 1 recorded under various pH conditions (Fig. 2) showed isobestic points at wavelengths of 284, 247, and 234 nm, indicating that only two species, ionized and un-ionized, determined the spectral changes. The maximum difference in the absorbance of ionized and un-ionized species was observed at 239 nm, and the apparent pK_a calculated at this wavelength was 3.67.

Several investigators¹⁶⁻²⁰ reported that the pH of maximum solubility, the pH_{max}, exists in the pH-solubility profile of a base and its hydrochloride salt; the base is the equilibrium species at pH values > pH_{max} and a phase conversion from base to salt occurs when the pH is lowered below the pH_{max}. In the present investigation, the pH_{max} was 1.0 (Fig. 1). Good agreement between the solubility profile and the pK_a value was observed when the data at pH values > pH_{max}

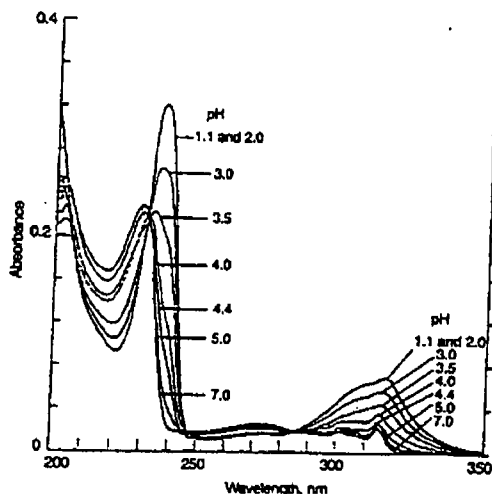


Figure 2—Changes in the UV spectrum of a solution of 1 in aqueous medium (2.5 µg/mL) with the change in pH at room temperature.

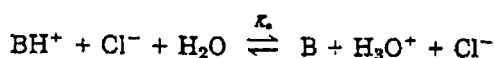
in Fig. 1 were fitted theoretically¹⁷ using the following equation:¹⁷

$$S_T = [BH^+] + [B]_s = [B]_s \left(1 + \frac{[H_3O^+]}{K_a} \right) \quad (2)$$

where $[BH^+]$ and $[B]$ are the concentrations of the ionized and un-ionized species, respectively, the subscript s indicates a saturated species, and K_a is the apparent dissociation constant. The decrease in the solubility of the drug at pH values $< pH_{max}$ (Fig. 1) was due to the common-ion effect. The theoretical pH-solubility profile¹⁸ at such a pH was not calculated because of the difficulty in determining the chloride ion activity and the solubility product from the experimental data.

Conversion of the Hydrochloride Salt to the Base—An emulsion was formed when the hydrochloride salt of 1, initially dissolved in a small volume of methanol, was mixed with water, and the oil globules of the emulsion subsequently converted to a solid. The identity of the solid was confirmed to be the free base. These results, therefore, show that 1 can exist as both an oily liquid and a solid, and the conversion of the hydrochloride salt to the solid form of 1 occurs via the metastable liquid phase.

When a hydrochloride salt is dissolved in water, the following equilibrium exists:



where B represents 1. The conversion of the hydrochloride salt of 1 to the base is in agreement with this relationship. For example, 400 mg of anhydrous salt contains 1.075 mmol of HCl. This amount of acid, when mixed with 250, 500, and 1000 mL of water, would theoretically give pH values of 2.37, 2.67, and 2.97, respectively. The pH values observed experimentally were 2.40, 2.73, and 3.02, respectively. Thus, the agreement between the theoretical and the experimental pH values shows that the decreased hydrogen ion concentration of the emulsions formed were due to almost complete conversion of salt to free base; the base in excess of its saturation solubility in water separated from solution as oil globules.

In the present investigation, the pH values of the saturated solutions of the hydrochloride salt in water and dilute HCl (e.g., 0.1 and 0.01 M) were higher than the pH_{max} values. This is contrary to earlier reports that pH values of saturated solutions of some hydrochloride salts in water were lower than their respective pH_{max} values.^{15,19,20} The observation of a pH above the pH_{max} indicates that there is a potential for conversion of the undissolved salt to the base form. Such a conversion can occur in the presence of water or moisture during processing (e.g., wet granulation), storage, and dissolution of solid dosage forms, thus affecting the stability and bioavailability of the drug. No phase conversion would, however, take place if the pH were lower than the pH_{max} , because the salt is the equilibrium species at such a pH.^{15,19,20} These results also explain why strong acids (e.g., HCl, H_2SO_4) were necessary to prepare a salt of 1; without a strong acid, the pH would not decrease below 1.0 and the salt would not nucleate.

Intrinsic Dissolution Rates—The dissolution profiles of 1 and the hydrochloride salt from a constant surface area of 0.95 cm² at pH 1.10 and 2.05 are shown in Fig. 3. The dissolution rates of the base and the salt in 0.1 M HCl (pH 1.1) at 200 rpm calculated from the slopes of the graphs in Fig. 3 were 0.098 and 0.088 mg/(cm² · min), respectively. In 0.01 M HCl (pH 2.05), the dissolution rates are 0.0096 and 0.0079 mg/(cm² · min), respectively.²¹ Thus, the dissolution rates of the base and its hydrochloride salt at a particular pH

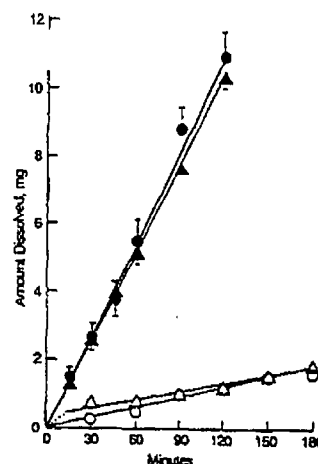


Figure 3—Dissolution profiles of 1 and its hydrochloride salt at 37°C and 200 rpm from a surface area of 0.95 cm² under pH-stat conditions. Key: (●) 1, pH 1.1; (○) pH 2.05; (▲) salt, pH 1.1; (△) pH 2.05.

were almost the same. This is contrary to earlier observations^{15,16} that the dissolution rates of a base and its hydrochloride salt at a particular pH differ greatly due to the difference in the self-buffering action of the two forms in their diffusion layers.

The similarity in the dissolution profiles of the base and the salt is due to the conversion of the salt to the base at the surface of the dissolving pellet. Due to its oily nature and poor solubility, the base coats the surface of the salt completely, and the dissolution rate of the salt is essentially that of the oily form of the base. The results also do not indicate any significant difference between the dissolution rates of the liquid and solid forms of the base.

The nature of the interfacial film may exert a significant effect on the dissolution rate of a salt. Earlier, when the precipitation of the base was observed by Serajuddin and Jarowski¹⁶ during the dissolution of phenazopyridine hydrochloride at pH 5 and 7, the film formed was solid and porous in nature. The dissolution rate of phenazopyridine hydrochloride, although retarded, was higher than that of the base. Since the oily film in the present study was nonporous, no improvement in the dissolution rate of the salt was observed.

Thermal Properties—To elucidate the mechanism of thermal change the differential scanning calorimetric (DSC) thermograms of 1 and its hydrochloride salt were recorded at various atmospheric conditions. These are shown in Fig. 4. The base shows a sharp melting endotherm with the onset at 63°C and the peak at 67°C (thermogram 1, Fig. 4), and there is no other thermal change with heating up to 200°C. The melt did not crystallize when cooled to ambient temperature but formed a gummy mass. Identical thermograms were obtained under all experimental conditions.

Thermograms 2–4 in Fig. 4 show that the onsets and the nature of the melting endotherm of the anhydrous hydrochloride salt varied with a change in atmosphere surrounding the samples. Even more pronounced differences were noticed when the monohydrate form of the salt was heated (thermograms 5–8, Fig. 4). Endotherm A in thermogram 5 was due to dehydration. The occurrence of dehydration was confirmed by Karl Fischer moisture analysis of a sample initially and after the completion of endotherm A. The melting range of the compound corresponds to endotherm B which was confirmed by hot-stage microscopy. Heating the monohydrate in closed pans (thermograms 7 and 8) shifted the onset of the dehydration endotherm (endotherm A) to a higher temperature; this endotherm was followed by an exotherm not found

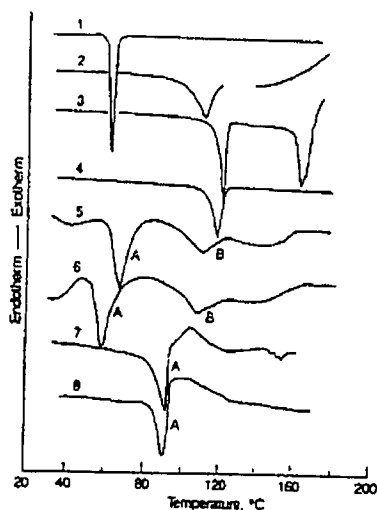


Figure 4—Differential scanning calorimetric thermograms of 1 and its hydrochloride salt recorded by subjecting the samples to various atmospheric conditions. The thermogram of 1 (thermogram 1) did not change significantly with the change in the atmospheric condition of the sample. Thermograms of the anhydrous salt were recorded by placing the sample on an open pan without purging with N_2 (thermogram 2), in a pan closed by crimping (thermogram 3), and in a hermetically sealed pan (thermogram 4). Thermograms of the monohydrate of the salt were recorded by placing the sample on an open pan without purging N_2 (thermogram 5), on an open pan with purging of N_2 50 mL/min (thermogram 6), in a pan closed by crimping (thermogram 7), and in a hermetically sealed pan (thermogram 8). The letters A and B represent dehydration and melting endotherms of the monohydrate, respectively.

a previous thermograms. The presence of this exotherm indicates the occurrence of a possible chemical reaction which, as will be described later, might influence the stability of the compound.

The results of thermogravimetric analysis of 1 and of both the anhydrous and hydrated forms of its hydrochloride salt are shown in Fig. 5. The free base form of the drug (thermogram 1) did not lose weight during melting at 67°C and at temperatures up to 150°C. Conversely, the monohydrate salt (thermogram 3) shows a weight loss of 6.5% at temperatures between 25 and 64°C. Loss of 1 mol of bound water would amount to a 4.6% weight loss, while the additional loss (-1.9%) may be due to evaporation of unbound water. Some additional samples of the hydrate were prepared by exposing the salt to different humidity conditions. Their equilibrium water contents varied between 5.5 and 6.5%. The anhydrous salt (thermogram 2) also showed a weight loss of 1.1% at 60°C; this may be due to evaporation of unbound and, possibly, a small amount of bound water absorbed by the sample during storage and sample handling. The moisture content of the anhydrous sample increased gradually on exposure to ambient atmospheric conditions. The conversions of anhydrous salt to a monohydrate and vice versa are shown in thermograms 1-4 in Fig. 6. These thermograms, together with the thermogravimetric (Fig. 5) and moisture analyses, confirmed that the conversion from the anhydrous form to the hydrate was complete in 24 h when the sample was exposed to >60% relative humidity at 22°C. At ambient humidity (~40% relative humidity), the conversion took 72 h to be complete. The monohydrate formed also loses water at a relatively low temperature (thermograms 3 and 4, Fig. 6). Since moisture and heat may be involved in processing and storage of a solid dosage form, these results indicate that the persistence of either an anhydrous or a monohydrate form of the salt in such a dosage form cannot be ensured.

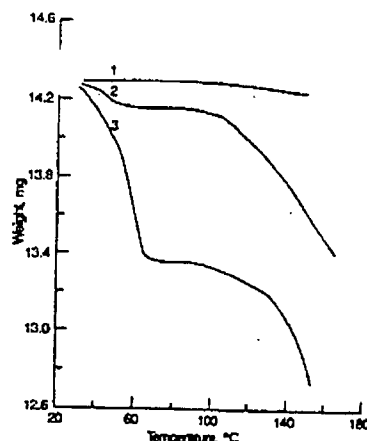


Figure 5—Thermogravimetric scans of 1 (thermogram 1) and anhydrous (thermogram 2) and monohydrate (thermogram 3) forms of its hydrochloride salt.

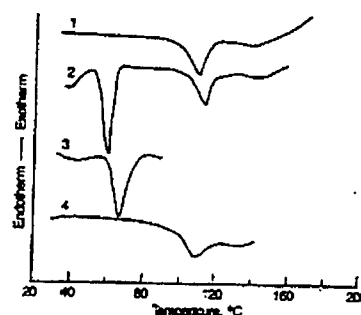


Figure 6—Differential scanning calorimetric thermograms showing the conversion of anhydrous salt (thermogram 1) to a monohydrate (thermogram 2) by subjecting the sample to 100% relative humidity for 24 h, and the conversion of monohydrate (thermogram 3) to an anhydrous form (thermogram 4) by heating the sample past its dehydration endotherm.

If a reaction involves the evolution or absorption of gas, e.g.,



the onset temperature, peak temperature, and shape of the DSC thermogram are affected by the gas pressure of the system.²³ Thus, according to the van't Hoff equation:

$$\ln \frac{(K_p)_2}{(K_p)_1} = \ln \frac{(P_c)_2}{(P_c)_1} = \frac{\Delta H(T_2 - T_1)}{R(T_2 T_1)} \quad (3)$$

where K_p , P_m , and T are the equilibrium constant for the reaction, partial pressure of C_{gas} , and temperature, respectively; the subscripts 1 and 2 represent two different temperature conditions; H is the enthalpy; and R is the gas constant. The variations observed in the onsets of the dehydration endotherms (Fig. 4) are in agreement with eq. 3. The onset temperature of dehydration decreased (thermogram 6, Fig. 4) when the partial pressure of water vapor was lowered by purging the system with nitrogen. The partial pressure of water vapor and, as a result, the dehydration temperature increased when the salt (hydrate) was heated in crimped and hermetically sealed pans (thermograms 7 and 8, Fig. 4). Since the base 1 was anhydrous, no gas evolved during its melting; therefore, its thermogram did not change with a

ge in the atmospheric conditions surrounding the sample. This study gives a simple method to determine, by changing the experimental conditions and observing changes in thermograms, whether a gaseous product is being evolved.

Stability of Solids—The physical stability of 1 and the hydrochloride salt were studied for 2 and 3 months, respectively. The storage conditions were 25°C, 35°C, 35°C/85% relative humidity (open and closed bottles), 45°C, 55°C, and (3,500 lux, closed bottle and open Petri dish). The color consistency of the powder and the general appearance of a high density polyethylene bottle were recorded. There was no physical change in 1 over 3 months. Chemical analysis of the samples by HPLC also did not show any significant chemical degradation of 1.

Like 1, major physical changes of the salt were observed under accelerated stability conditions in 2 months. At 45 and 55°C, the color of the powder turned from white to orange red, consistency of powder changed from a free-flowing powder to agglomerates (45°C) or a sticky mass (55°C), and the interior of the polyethylene bottle was stained yellow. Increased humidity also affected the physical stability of the compound. While there was no change in color of the powder in the bottle at 35°C, both the powder and the interior of the bottle turned yellow when stored at 35°C/85% relative humidity. No significant physical changes of the salt were observed at 25°C or in the presence of light at room temperature. In view of the marked change in physical appearance under accelerated conditions, the samples of the salt were not used chemically.

Correlation between Thermal Properties and Stability—A correlation between the changes of DSC thermograms and the stability of the hydrochloride salt was also observed in the present study. Variation in both the onset and the magnitude of the melting endotherm of the anhydrous salt suggest that a reaction involving gaseous component(s) occurred during the melting of the compound. The loss of weight in thermogravimetric curves at temperatures >90°C indicates evolution of a gas. The evolved gas was not chemically analyzed in the present study; however, it is probable that the gas liberated from the salt is hydrogen chloride. In a separate study, a decrease of ~6% in the weight of the salt was observed after storage at 65°C for 3 months.

The liberation of hydrogen chloride and partial conversion to an oily base may thus be responsible for the formation of lumps and the stickiness of the powder at 45 and 55°C.

The liberation of corrosive hydrogen chloride gas may be responsible for the discoloration and browning of the powder observed at elevated temperatures. It was later found that the salt corrodes the tablet tooling when left in product contact, especially in the presence of water. Recently, Narurkar et al.²⁴ showed that such corrosion was due to the evolution of hydrogen chloride.

In contrast to the thermograms of the anhydrous salt, the thermograms of the hydrate in open and hermetically sealed pans (Fig. 4). These exo-

therms were preceded by the liberation of bound water from the salt and, therefore, indicate possible reactivity (or instability) of the salt in an atmosphere of water vapor. The development of color in the sample stored at 35°C/85% relative humidity and not at 35°C alone confirms the instability of the salt in the presence of water. These results also agree with the dissociation of the salt in an aqueous medium. Any liberation of hydrogen chloride, in the presence of moisture, may create a strongly acidic environment on the surface of the solid and possibly be responsible for the reactivity of the salt.

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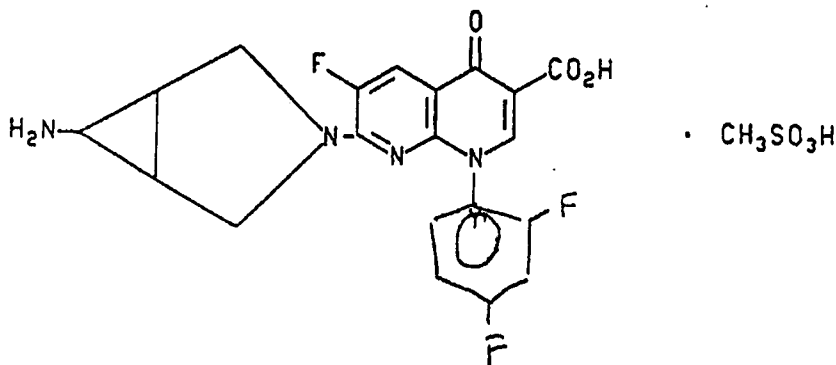


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(54) Title: NOVEL CRYSTAL FORM OF ANHYDROUS 7-([1 α , 5 α , 6 α]-6-AMINO-3-AZABICYCLO[3.1.0]HEX-3-YL)-6-FLUORO-1-(2,4-DIFLUOROPHENYL)-1,4-DIHYDRO-4-OXO-1,8-NAPHTHYRIDINE-3-CARBOXYLIC ACID, METHANESULFONIC ACID SALT



(57) Abstract

The anhydrate of 7-([1 α , 5 α , 6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt has advantageous stability for formulation as an antibacterial agent.

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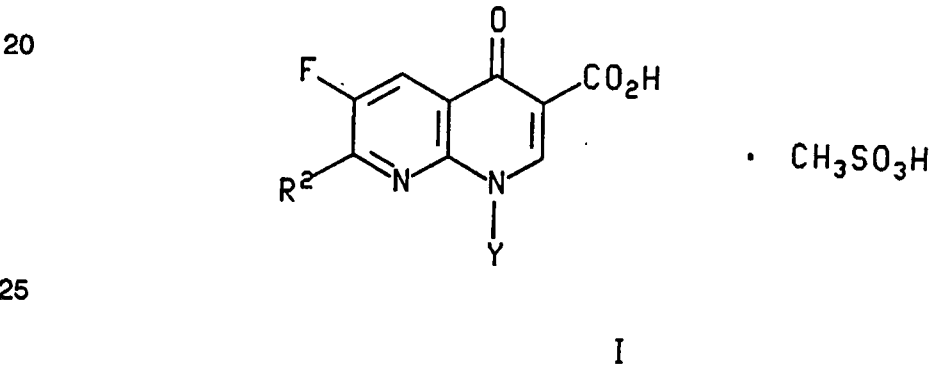
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5 NOVEL CRYSTAL FORM OF ANHYDROUS 7-([1 α ,5 α ,6 α]-6-AMINO-3-
AZABICYCLO[3.1.0]HEX-3-YL)-6-FLUORO-1-(2,4-DIFLUOROPHENYL)-1,4-
DIHYDRO-4-OXO-1,8-NAPHTHYRIDINE-3-CARBOXYLIC ACID,
METHANESULFONIC ACID SALT

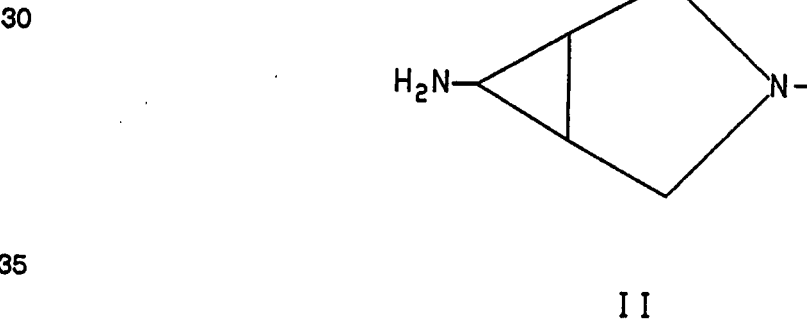
10 Background of the Invention

10 The invention is directed to a novel crystal form of anhydrous 7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt, a method of using said compound in the treatment of a bacterial infection in mammals, especially humans, and to pharmaceutical compositions useful therefor.

15 United States Patent No. 5,229,396, which is incorporated herein by reference, discloses 7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt of the formula



wherein Y is o,p-difluorophenyl and R² is



having antibacterial activity.

30 32

Summary of the Invention

The invention is directed to a novel crystal form of anhydrous 7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt which possesses valuable and nonobvious properties. Since the anhydrate is substantially hydrophobically stable, formulation problems of the active ingredient during tableting or capsulation operations are alleviated.

Detailed Description of the Invention

The 7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt referred to in United States Patent No. 5,229,396 characterized by the major peaks in the following X-ray powder diffraction pattern

Peak no.	1	2	3	4	5	6	7	8	9	10
2 θ ($^{\circ}$) Cu	5.0	9.8	13.0	14.8	19.7	20.9	22.0	23.0	28.1	29.3
d space	17.9	9.0	6.8	6.0	4.5	4.2	4.0	3.9	3.2	3.0

is substantially hygroscopic and can pick up water from the atmosphere to form a monohydrate. The monohydrate is characterized by the major peaks in the following X-ray powder diffraction pattern

Peak no.	1	2	3	4	5	6	7	8
2 θ ($^{\circ}$) Cu	4.7	9.4	12.4	13.1	13.6	14.2	17.0	17.9
d space	18.7	9.4	7.1	6.7	6.5	6.3	5.2	5.0
Peak no.	9	10	11	12	12	14	15	
2 θ ($^{\circ}$) Cu	18.7	21.0	22.0	24.2	24.2	26.6	27.2	
d space	4.7	4.2	4.0	3.7	3.7	3.5	3.3	

The novel crystal form of 7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt (hereinafter "the anhydrate") is hydrophobically stable and characterized by the major peaks in the following X-ray powder diffraction pattern.

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Peak no.	1	2	3	4	5	6	7	8
2 θ (°) Cu	4.5	7.7	9.1	13.6	15.0	18.2	18.6	22.8
d space	19.5	11.5	9.7	6.5	5.9	4.9	4.8	3.9

5 The anhydrate may be prepared by heating 7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt or its derived monohydrate in an organic solvent or a mixture thereof with an aprotic co-solvent, such as isopropanol, dimethylsulfoxide, n-propanol, tetrahydrofuran or n-butanol, preferably
10 n-butanol or tetrahydrofuran/n-butanol, to reflux or to a temperature between about 70°C to about 90°C, preferably about 85°C. Depending on the reaction temperature and other conditions, the reaction time generally ranges from about 1 hour to about 20 hours, preferably about 2 hours to about 16 hours.

15 The crystal slurry formed is cooled to a temperature between about 20°C to about 30°C, preferably about 25°C, for a time period between about 2 hours to about 24 hours, preferably about 2 hours to about 12 hours. The crystalline product is then filtered from the mother liquid and dried under vacuum until all the solvent has been removed.

20 The anhydrate may be administered as an antibacterial agent as described in above-mentioned United States Patent No. 5,229,396. Administration to a subject may be alone, but the anhydrate will generally be administered in admixture with a pharmaceutical carrier selected with regard to the intended route of administration and standard pharmaceutical practice. For example, it can be administered orally or in the form of tablets containing such excipients as starch or lactose, or in capsules either
25 alone or in admixture with excipients, or in the form of elixirs or suspensions containing flavoring or coloring agents. In the case of animals, it is advantageously contained in an animal feed.

30 The invention also provides pharmaceutical compositions comprising an antibacterially effective amount of the anhydrate together with a pharmaceutically acceptable diluent or carrier.

 The anhydrate can be administered to humans for the treatment of bacterial diseases by either the oral or parenteral routes, and may be administered orally at dosage levels of about 0.1 to 500 mg/kg/day, advantageously 0.5-50 mg/kg/day given

in a single dose or up to 3 divided doses. For intramuscular or intravenous administration, dosage levels are about 0.1-200 mg/kg/day, advantageously 0.5-50 mg/kg/day. While intramuscular administration may be a single dose or up to 3 divided doses, intravenous administration can include a continuous drip. Variations will necessarily occur depending on the weight and condition of the subject being treated and the particular route of administration chosen as will be known to those skilled in the art.

The antibacterial activity of the anhydrate is shown by testing according to the Steer's replicator technique which is a standard in vitro bacterial testing method described by E. Steers et al., Antibiotics and Chemotherapy, 9, 307 (1959).

The hydration properties were determined gravimetrically over a range of relative humidities using a VTI microbalance system for moisture sorption studies (Model MB300W).

PREPARATION A

7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt

7-([1 α ,5 α ,6 α]-6-tert-butyloxycarbonylamino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, ethyl ester, (25 g) and methanesulfonic acid (11 g) was added to a mixture of water (250 mL) and tetrahydrofuran (250 mL). The resultant slurry was heated to reflux (about 66°C) temperature and held at this temperature for 20 hours after which time a clear solution was obtained. The solution was cooled to 35-40°C and concentrated under reduced pressure to about half its original volume. The resultant crystal slurry was cooled slowly to room temperature (about 20°C) and then further stirred at 10°C for 2 hours. The crystalline product 7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt was isolated by filtration and washed with a mixture of tetrahydrofuran (12.5 mL) and water (12.5 mL). The crystals were dried under vacuum at 30-35° until the residual water content of the crystals was below 0.2%. Yield 21.2 g, 90%.

The crystals of 7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-

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1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt are characterized by the major peaks in the following X-ray powder diffraction pattern.

5

Peak no.	1	2	3	4	5	6	7	8	9	10
2θ(°) Cu	5.0	9.8	13.0	14.8	19.7	20.9	22.0	23.0	28.1	29.3
d space	17.9	9.0	6.8	6.0	4.5	4.2	4.0	3.9	3.2	3.0

10

The crystals of 7-([1α,5α,6α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt can pick up water from the atmosphere and form a monohydrate. The monohydrate is characterized by the major peaks in the following X-ray powder diffraction pattern.

15

Peak no.	1	2	3	4	5	6	7	8
2θ(°) Cu	4.7	9.4	12.4	13.1	13.6	14.2	17.0	17.9
d space	18.7	9.4	7.1	6.7	6.5	6.3	5.2	5.0
Peak no.	9	10	11	12	12	14	15	
2θ(°) Cu	18.7	21.0	22.0	24.2	24.2	26.6	27.2	
d space	4.7	4.2	4.0	3.7	3.7	3.5	3.3	

20

Example 1

7-([1α, 5α, 6α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt, anhydrous

25

7-([1α,5α,6α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt or its monohydrate (20 g) was stirred with isopropanol (220 ml). The crystal suspension was refluxed for 16 hours or until microscopic examination had shown that the crystal form had changed to a hexagonal form. The crystal slurry was cooled to 20-25°C and stirred at this temperature for about 1 hour. The crystalline product was filtered from the mother liquor, washed with isopropanol (about 50 mL) and dried under vacuum at 40°C until all the solvent had been removed. Yield 98%.

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The product is a new polymorphic form of 7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt, anhydrous, characterized by the following major peaks in its X-ray powder diffraction pattern.

5

Peak no.	1	2	3	4	5	6	7	8
2 θ (°) Cu	4.5	7.7	9.1	13.6	15.0	18.2	18.6	22.8
d space	19.5	11.5	9.7	6.5	5.9	4.9	4.8	3.9

Example 2

10

7-([1 α , 5 α , 6 α]-6-amino-3-azabicyclo[3.1.0]hex-3y)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt, anhydrous

15

7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt or its monohydrate (7 g) was dissolved in dimethylsulfoxide, DMSO (21 mL) by heating to 80-85°C until complete solution was obtained. Isopropanol (150 mL) was added dropwise to the solution at about 85°C to induce crystallization. The crystal suspension was held at reflux temperature about 85°C for 2-16 hours or until microscopic examination had shown that the crystal form had changed to a hexagonal form. The resultant crystal slurry was cooled to 20-25°C. The crystalline product was filtered from the mother liquor, washed with isopropanol (about 50 mL) and dried under vacuum at 50°C until all the solvents had been removed. Yield 77%.

The product is the same as in Example 1.

Example 3

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7-([1 α , 5 α , 6 α]-6-amino-3-azabicyclo[3.1.0]hex-3y)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt, anhydrous

30

7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt or its monohydrate (55.6 g) was dissolved in dimethylsulfoxide, DMSO (159 mL) by heating to 80-85°C until complete solution was obtained. The solution was cooled to 20-25°C and stirred for 2 hours until a crystal slurry formed. Dichloromethane (1200 mL) was

-7-

added dropwise to the solution at about 25°C to fully induce crystallization. The crystal suspension was held at room temperature overnight or until microscopic examination had shown that the crystal form had changed to a hexagonal form. The crystalline product was filtered from the mother liquor, washed with dichloromethane (3 x 119 mL) and dried under vacuum at 50°C until all the solvent had been removed. Yield 91%.

The product is the same as in Example 1.

Example 4

7-([1 α , 5 α , 6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt, anhydrous

7-([1 α , 5 α , 6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt or its monohydrate (1 g) was stirred with n-propanol (44 mL). The crystal suspension was refluxed for 3 hours or until microscopic examination had shown that the crystal form had changed to a hexagonal form. The crystal slurry was cooled at 20-25°C and stirred overnight. The crystalline product was filtered from the mother liquor, washed with n-propanol (about 10 mL) and dried under vacuum at 50-55°C until all the solvent had been removed. Yield 68%.

The product is the same as in Example 1.

Example 5

7-([1 α , 5 α , 6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt, anhydrous

7-([1 α , 5 α , 6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt or its monohydrate (70 g) was stirred with a mixture of tetrahydrofuran (175 mL) and n-butanol (525 mL). The crystal suspension was heated for 16 hours or until microscopic examination had shown that the crystal form had changed to a hexagonal form. The crystal slurry was cooled to 20-25°C and stirred overnight. The crystalline product was filtered from the mother liquor, washed with a mixture of tetrahydrofuran (25 mL) and n-butanol (75 mL) and dried under vacuum at 80°C until all the solvent had been removed. Yield 95%.

The product is the same as in Example 1.

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Example 6

7-([1 α , 5 α , 6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt, anhydrous

- 5 7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-
1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt or its
monohydrate (5 g) was stirred with n-butanol containing up to 1% water (220 mL). The
crystal suspension was heated to reflux for 5 hours or until microscopic examination
had shown that the crystal form had changed to a hexagonal form. The crystal slurry
10 was cooled to 20-25°C and stirred overnight. The crystalline product was filtered from
the mother liquor, washed with n-butanol (about 20 mL) and dried under vacuum at 50-
55°C until all the solvent had been removed. Yield 92%.

The product is the same as in Example 1.

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CLAIMS

1. 7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt characterized by the following major peaks in its X-ray powder diffraction pattern

Peak no.	1	2	3	4	5	6	7	8
2 θ ($^{\circ}$) Cu	4.5	7.7	9.1	13.6	15.0	18.2	18.6	22.8
d space	19.5	11.5	9.7	6.5	5.9	4.9	4.8	3.9

2. A pharmaceutical composition having antibacterial activity comprising the compound according to claim 1 in an amount effective in the treatment of a bacterial infection, and a pharmaceutically acceptable carrier.
3. A method of treating a bacterial infection which comprises administering to a subject in need of treatment an antibacterial amount of the compound according to claim 1.
4. A process for preparing the compound according to claim 1, which comprises heating 7-([1 α ,5 α ,6 α]-6-amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, methanesulfonic acid salt or its derived monohydrate in the presence of an alcohol or mixture thereof with an aprotic co-solvent.

INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 95/07211

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D471/04 A61K31/435 //(C07D471/04,221:00,221:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 413 455 (PFIZER) 20 February 1991 see claim 11; example 13B & US,A,5 229 396 (BRIGHTY) cited in the application -----	1,2

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

7 February 1996

Date of mailing of the international search report

19. 02. 96

Name and mailing address of the ISA

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Authorized officer

Alfaro Faus, I

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 95/07211

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 3 is directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 95/07211

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AT-T- 124040	15-07-95
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		CA-A- 2127561	17-02-91
		CN-A, B 1049501	27-02-91
		DE-D- 69020262	27-07-95
		DE-T- 69020262	26-10-95
		IL-A- 95331	31-07-95
		JP-A- 7149758	13-06-95
		JP-A- 3086875	11-04-91
		JP-B- 7002734	18-01-95
		PL-B- 166381	31-05-95
		US-A- 5266569	30-11-93
		US-A- 5164402	17-11-92
		US-A- 5229396	20-07-93



US008859567B2

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(12) **United States Patent**
Park et al.

(10) **Patent No.:** **US 8,859,567 B2**
(45) **Date of Patent:** **Oct. 14, 2014**

(54) **HYDRATE OF 1-[(2S)-2-AMINO-4-[2,4-BIS(TRIFLUOROMETHYL)-5,8-DI-HYDRO-PYRIDO[3,4-D]PYRIMIDIN-7(6H)-YL]-4-OXOBUTYL]-5,5-DIFLUORO-PIPERIDIN-2-ONE TARTRATE**

(75) **Inventors:** **Ki Sook Park**, Daejeon (KR); **Jung Min Yun**, Daejeon (KR); **Bong Chan Kim**, Daejeon (KR); **Kyu Young Kim**, Daejeon (KR); **Ji Hye Lee**, Daejeon (KR)

(73) **Assignee:** **LG Life Sciences Ltd.**, Seoul (KR)

(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) **Appl. No.:** **13/879,571**

(22) **PCT Filed:** **Oct. 31, 2011**

(86) **PCT No.:** **PCT/KR2011/008186**

§ 371 (c)(1),
(2), (4) **Date:** **Apr. 15, 2013**

(87) **PCT Pub. No.:** **WO2012/060590**

PCT Pub. Date: **May 10, 2012**

(65) **Prior Publication Data**
US 2013/0203787 A1 Aug. 8, 2013

(30) **Foreign Application Priority Data**
Nov. 1, 2010 (KR) 10-2010-0107868

(51) **Int. Cl.**
C07D 471/04 (2006.01)
A61K 31/519 (2006.01)

(52) **U.S. Cl.**
CPC **C07D 471/04** (2013.01)

USPC **514/264.1**; 514/258.1; 544/279;
546/118

(58) **Field of Classification Search**
CPC **C07D 471/04**; **A61K 31/519**
USPC **514/264.1**, **258.1**; **544/279**; **546/118**
See application file for complete search history.

(56) **References Cited**

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Primary Examiner — Sreeni Padmanabhan

Assistant Examiner — Irina Neagu

(74) *Attorney, Agent, or Firm* — Birch, Stewart, Kolasch & Birch, LLP

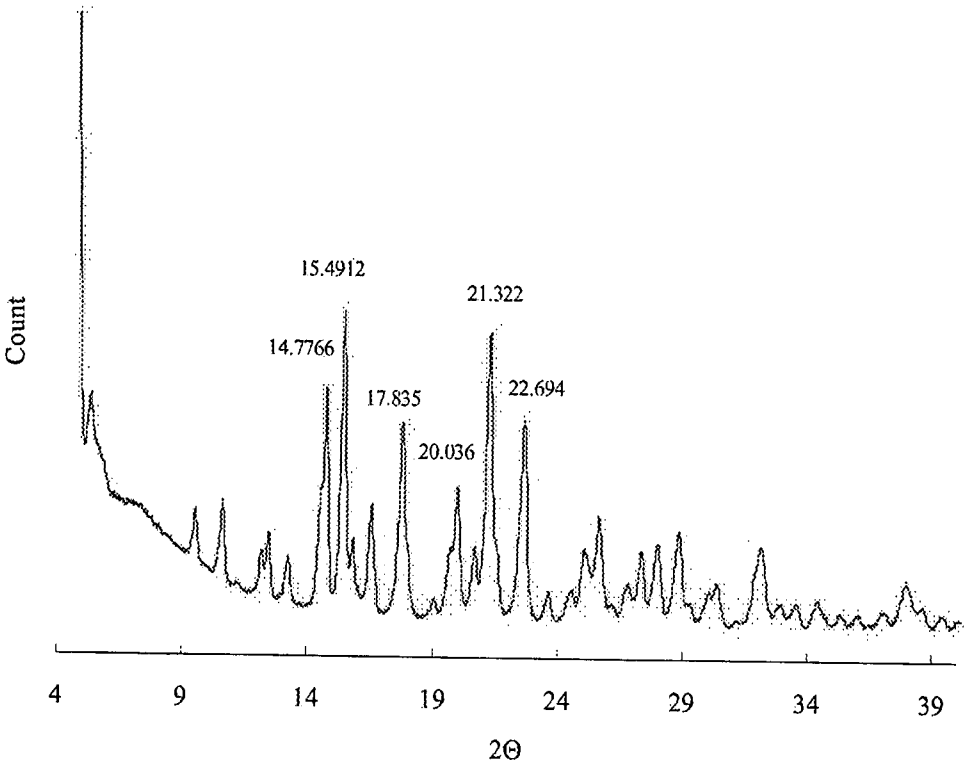
(57) **ABSTRACT**

The present invention relates to 1.5 hydrate of 1-[(2S)-2-amino-4-[2,4-bis(trifluoromethyl)-5,8-dihydropyrido[3,4-d]pyrimidin-7(6H)-yl]-4-oxobutyl]-5,5-difluoropiperidin-2-one tartrate, a process for preparing the same, and a pharmaceutical composition for inhibiting DPP-IV, which comprises said compound as the active component.

6 Claims, 16 Drawing Sheets
(13 of 16 Drawing Sheet(s) Filed in Color)

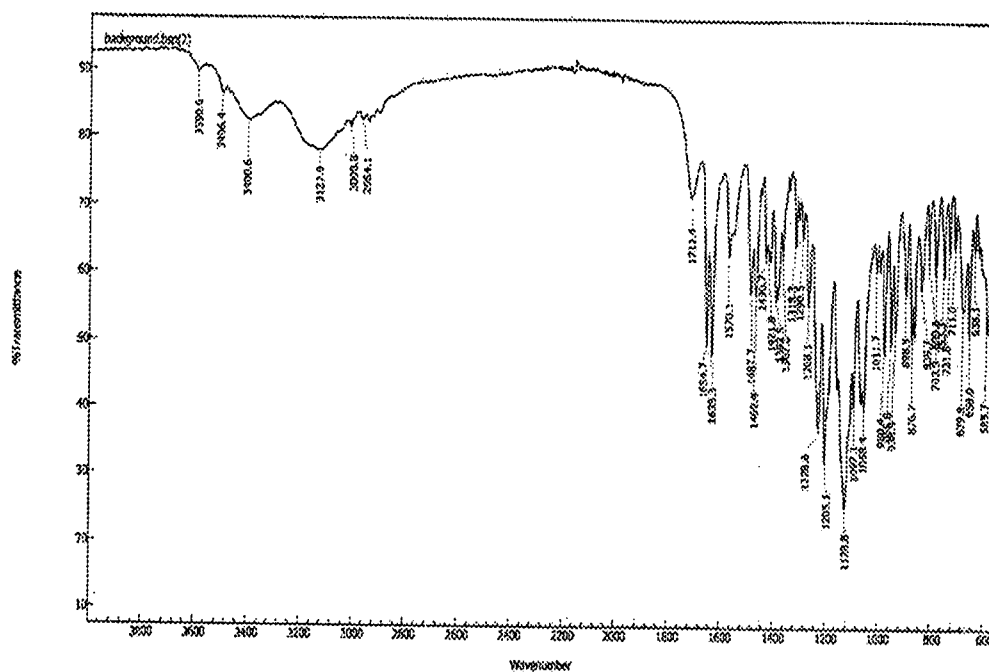
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Figure 1



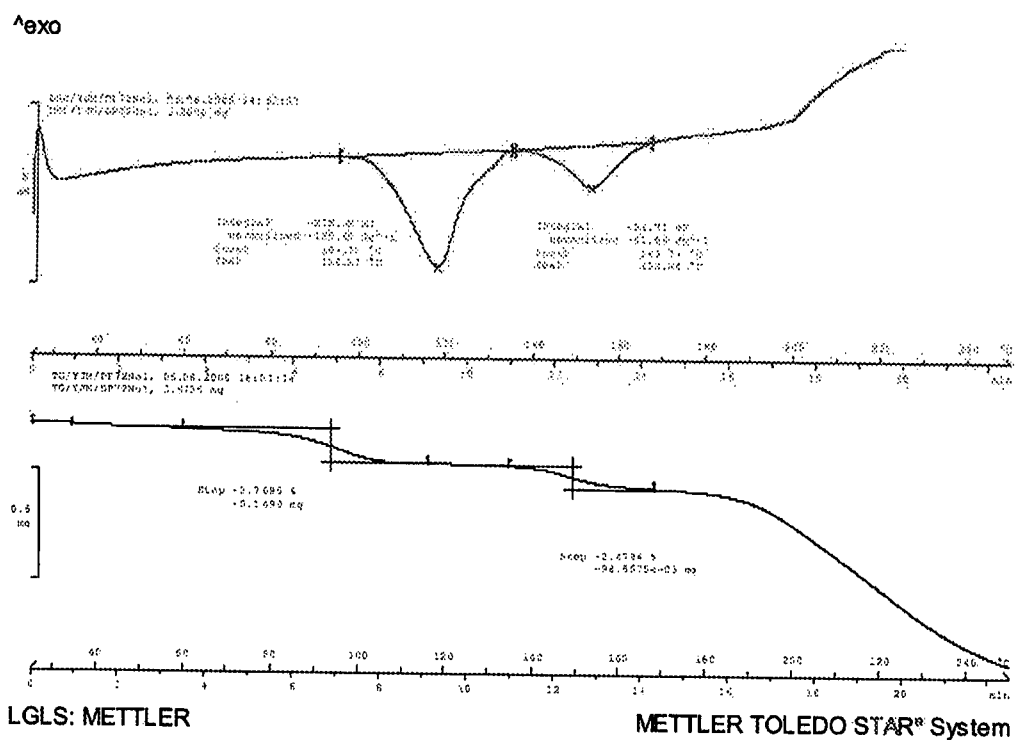
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Figure 2



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Figure 3



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Figure 4

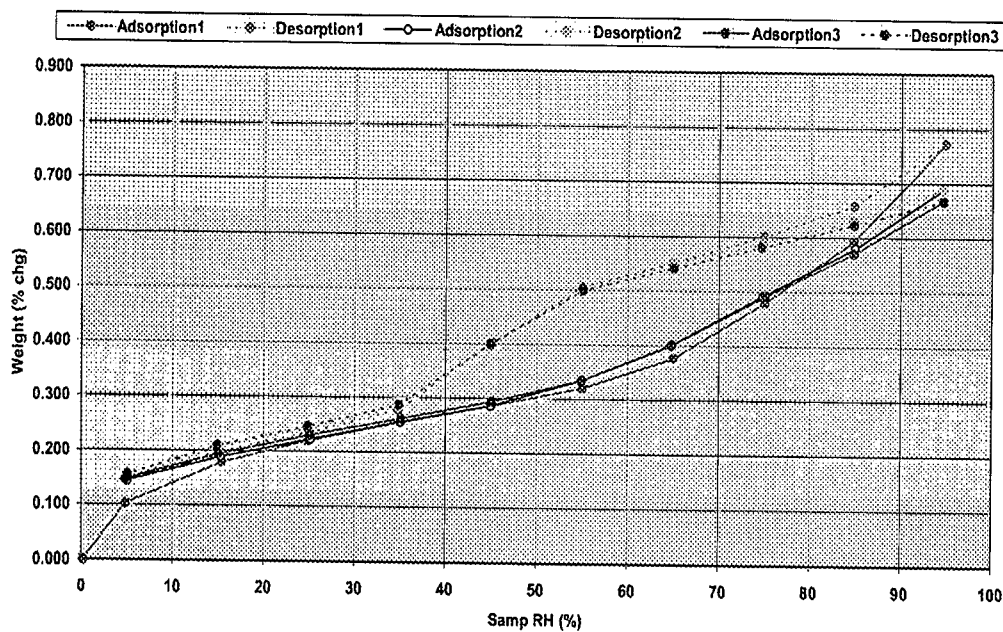


Figure 5

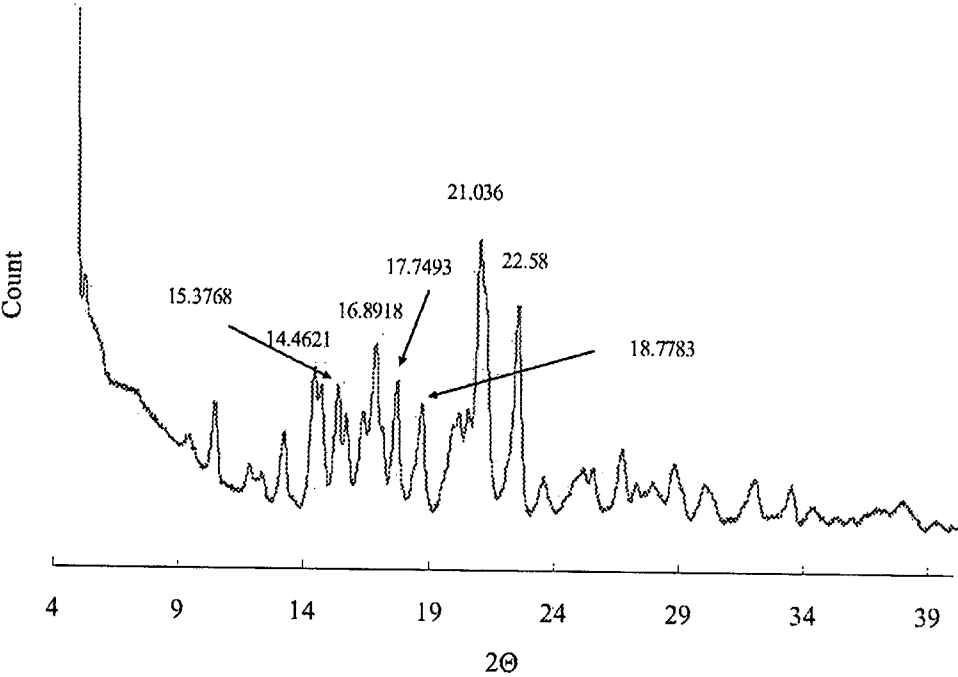
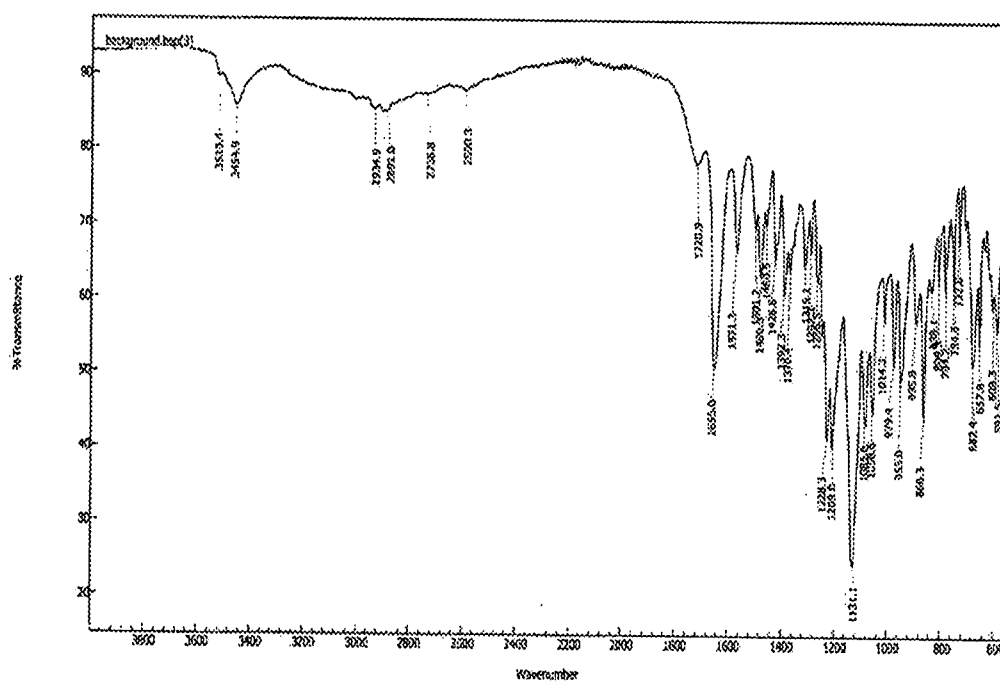
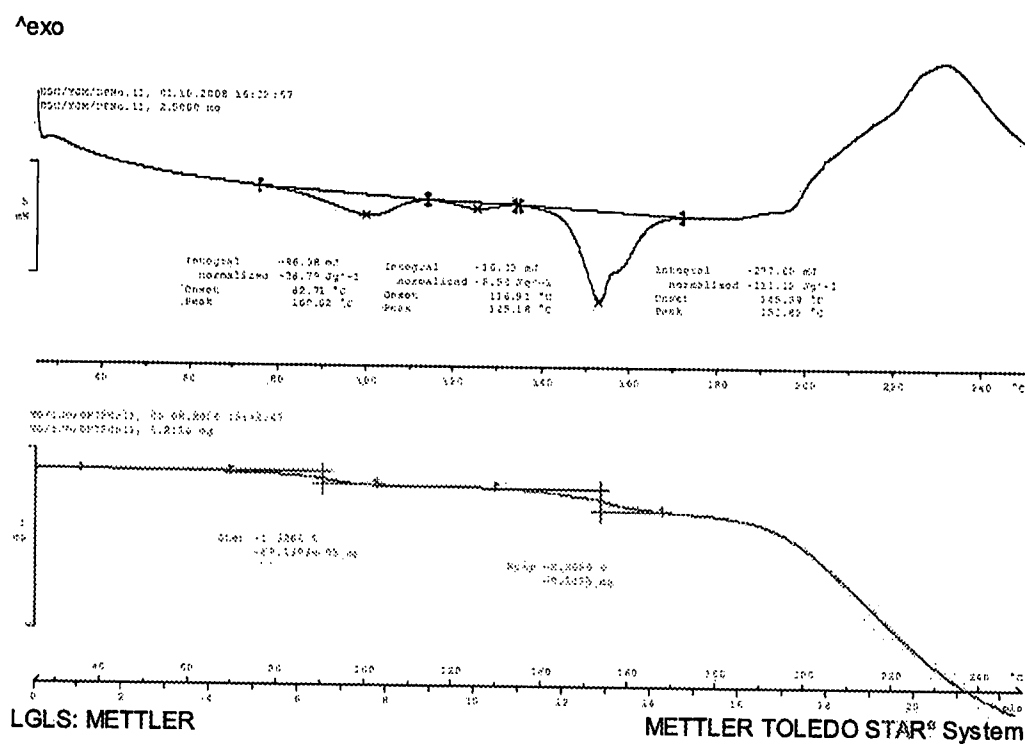


Figure 6



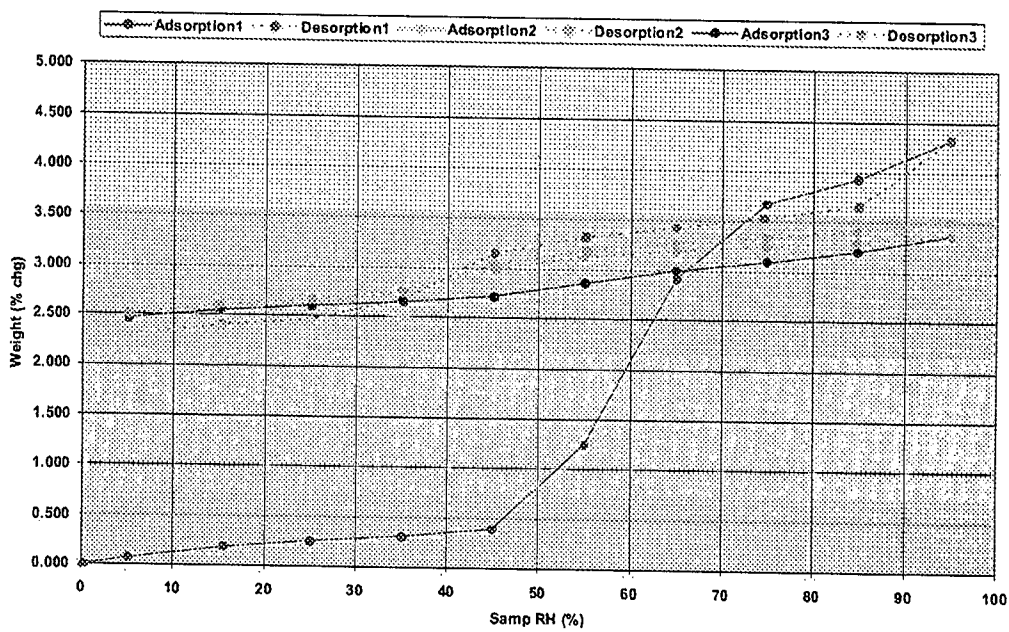
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Figure 7



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Figure 8



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Figure 9

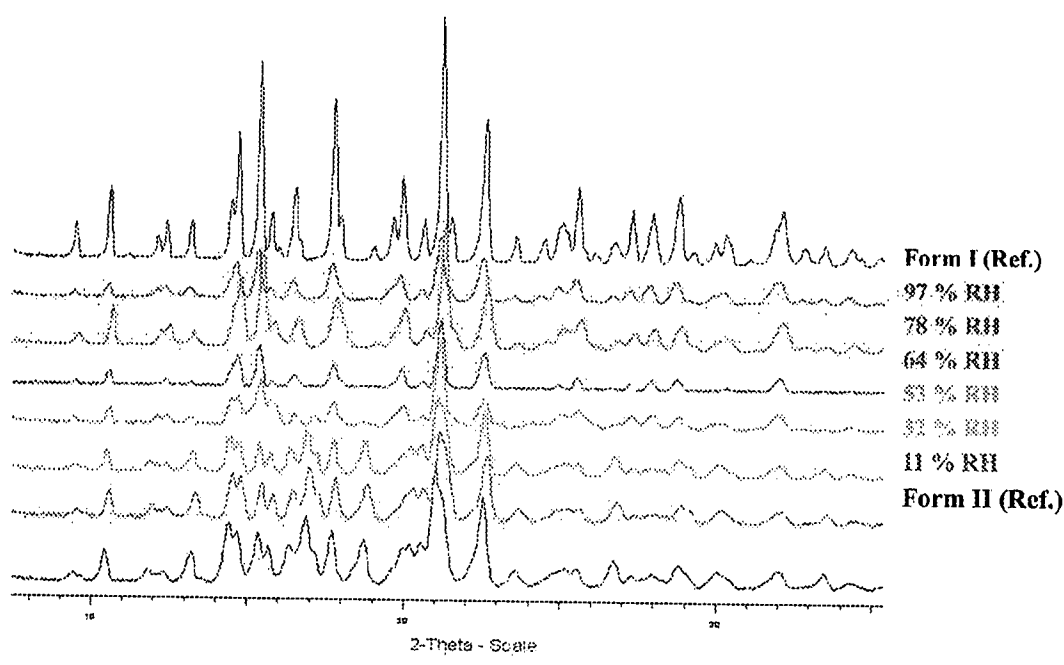
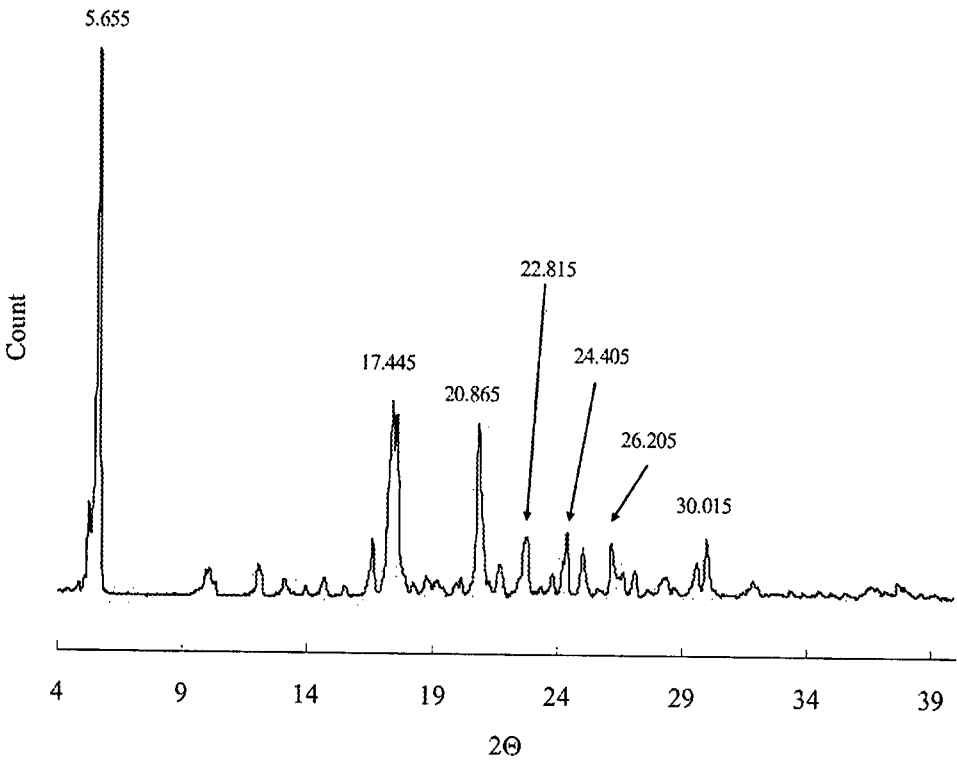


Figure 10



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Figure 11

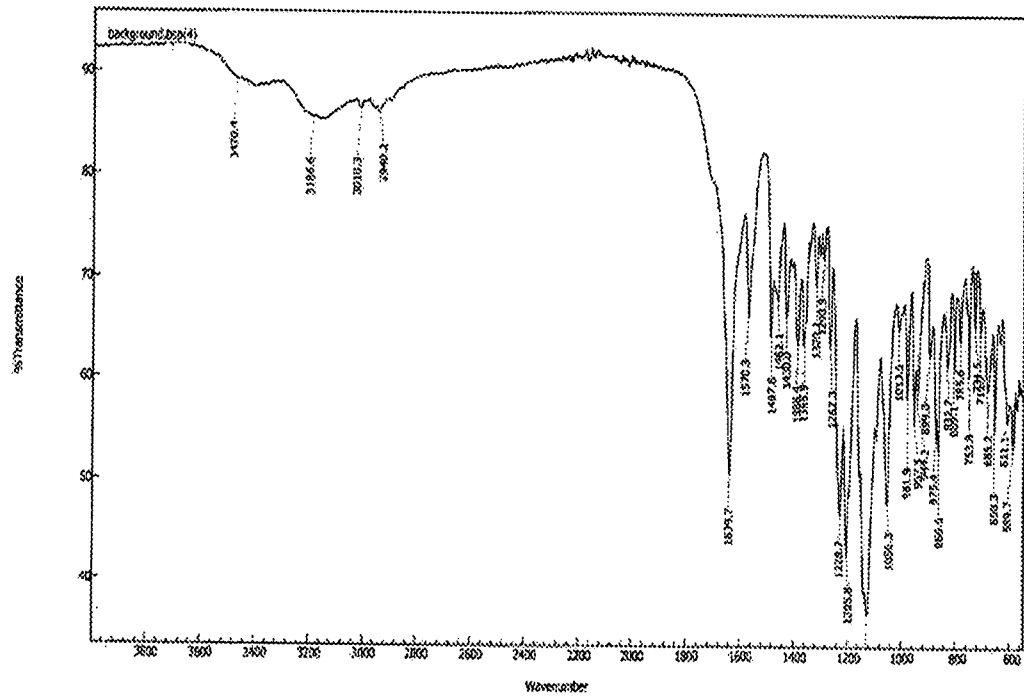
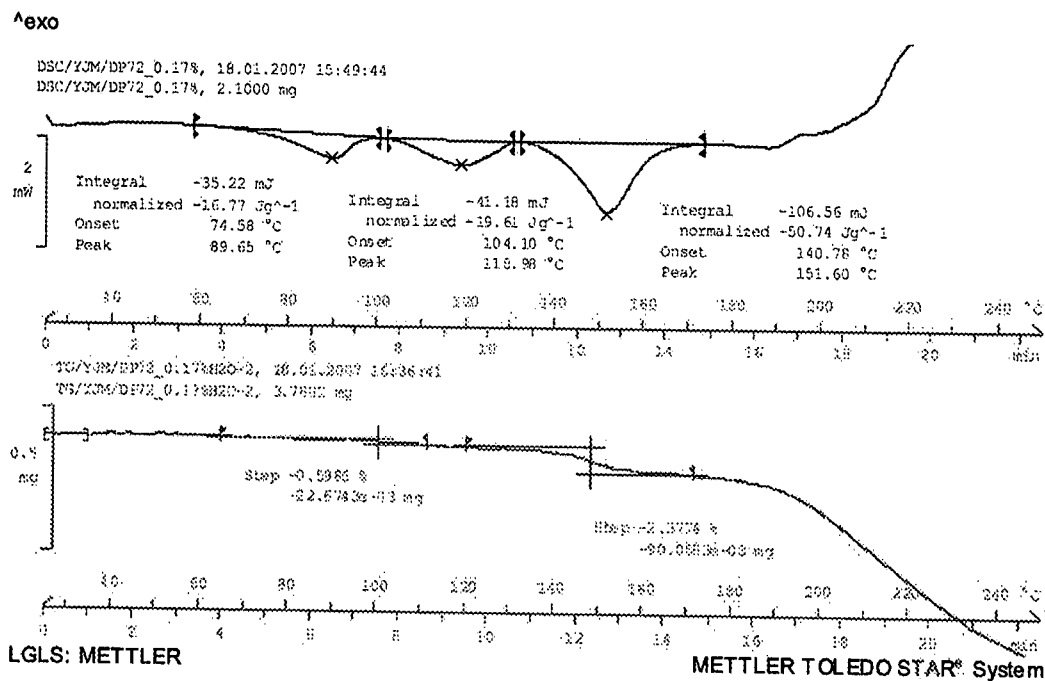
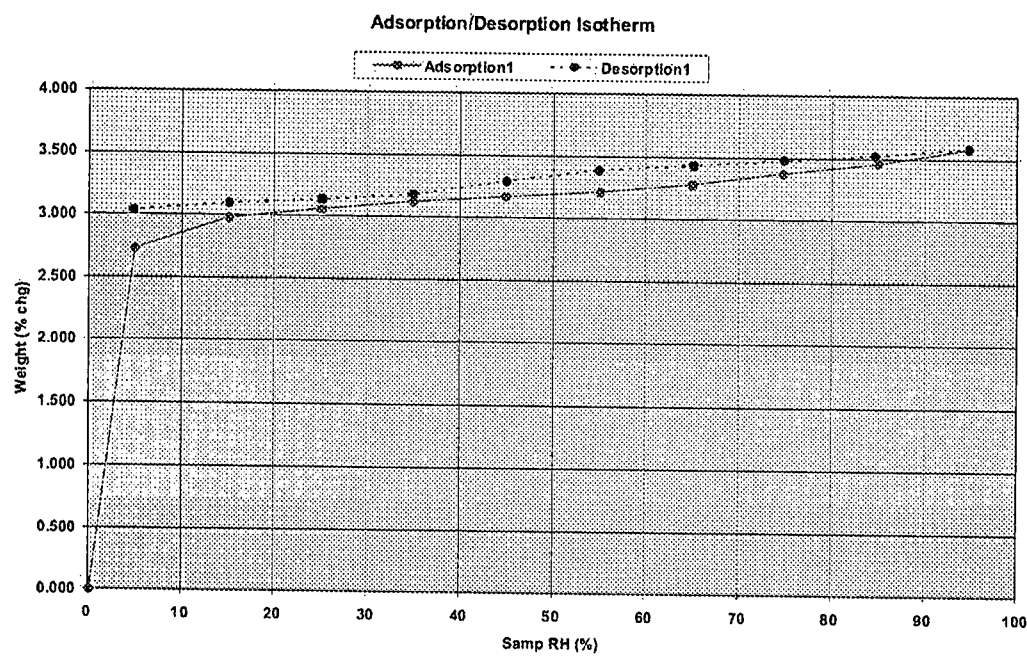


Figure 12



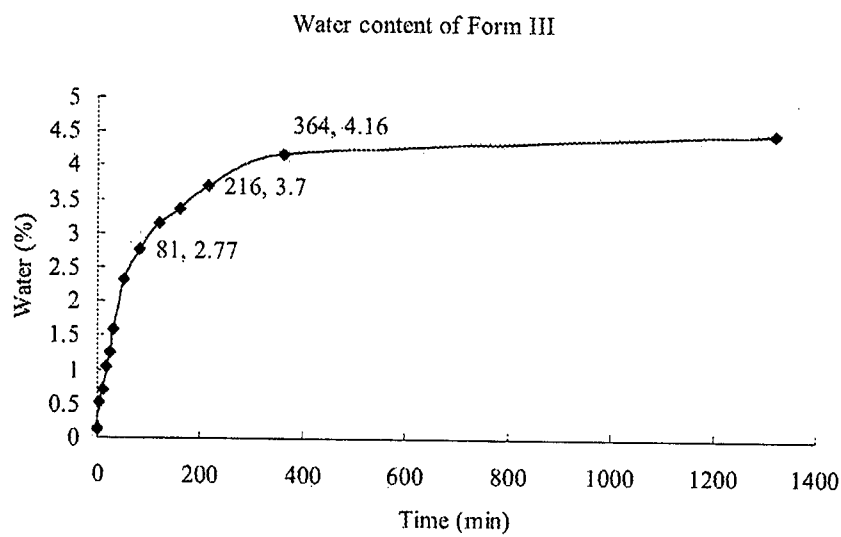
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Figure 13



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87

Figure 14



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Figure 15

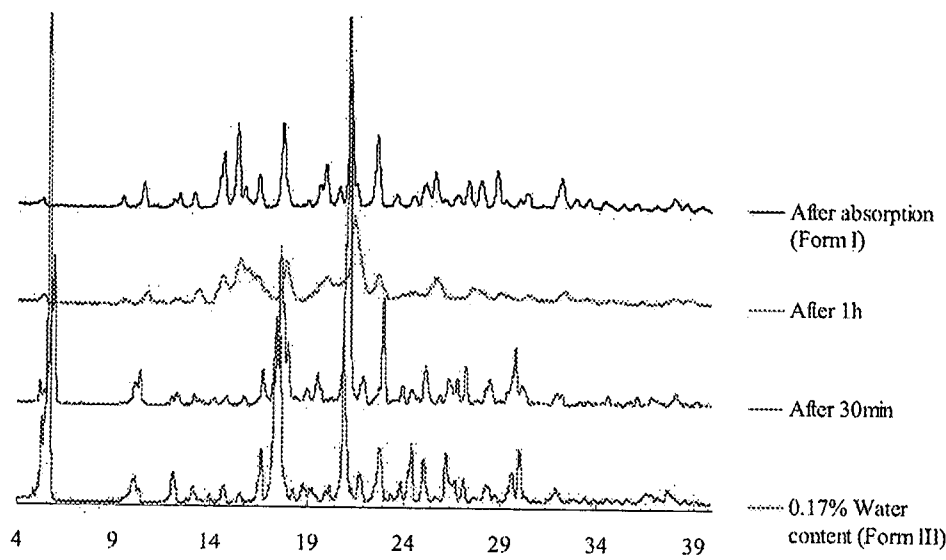
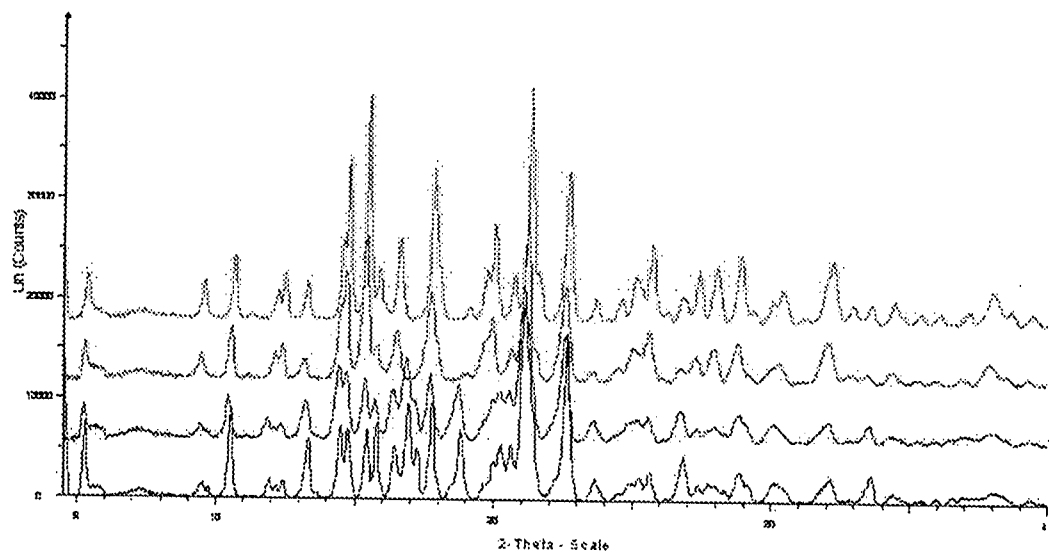


Figure 16

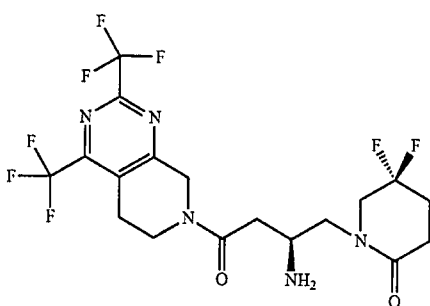


1
HYDRATE OF 1-[(2S)-2-AMINO-4-[2,4-BIS(TRIFLUOROMETHYL)-5,8-DI-HYDRO-PYRIDO[3,4-D]PYRIMIDIN-7(6H)-YL]-4-OXOBUTYL]-5,5-DIFLUORO-PIPERIDIN-2-ONE TARTRATE

This application is a National Stage of International Application No. PCT/KR11/08186, filed on 31 Oct. 2011, which claims the benefit of Korean Patent Application No. 10-2010-0107868, filed on 1 Nov. 2010.

TECHNICAL FIELD

The present invention relates to 1.5 hydrate of 1-[(2S)-2-amino-4-[2,4-bis(trifluoromethyl)-5,8-dihydropyrido[3,4-d]pyrimidin-7(6H)-yl]-4-oxo-butyl]-5,5-difluoropiperidin-2-one tartrate represented by the following formula 1 (hereinafter, referred to as "Compound 1"), and a process for preparing the same.



BACKGROUND ART

Compound 1 is disclosed in Korean Patent Application No. 10-2006-0029138, which is incorporated herein by reference in its entirety, and exhibits superior inhibitory activity against dipeptidyl peptidase-IV (DPP IV) and thus is useful as an agent for treating diabetes. Typical examples of diseases caused by DPP IV can include, but are not limited to, diabetes, obesity, etc. Among diabetes, it is particularly useful for the treatment and prevention of type II diabetes. The term "treatment" as used above means that when the compound is used for individuals manifesting symptoms of a disease, it can interrupt or delay the progress of disease; and the term "prevention" as used above means that when the compound is used for individuals who do not manifest symptoms of a disease but have the risk of onset of disease, it can interrupt or delay the sign of disease.

The investigation of physical and chemical properties of a new drug is necessary for efficient and successful development of the new medicine. Particularly, by studying the presence of polymorphs and pseudopolymorphs of the drug and differences in physical and chemical properties between respective polymorphs the preferable crystal form of the drug can be selected in view of the pharmaceutical aspect (Remington's Pharmaceutics, Chapter 75 Preformulation); (Byrn, S. R., Solid State Chemistry of Drugs, Academic Press, New York, 1982). When the polymorphs are present in the solution, they are chemically identical, but in the solid state they respectively have definitely different X-ray diffraction patterns and exhibit differences in various physical and chemical properties. Particularly, respective polymorphs can have dif-

ferences in bioavailability due to the differences in dissolution rates, and exhibit unexpected properties in the aspect of thermodynamic stability.

When a certain drug is present in the form of polymorphs, the crystal forms having different structures can be obtained depending on the conditions of recrystallization such as recrystallizing solvent, drug concentration, heating and cooling rates, temperature, stirring rate, and the like, during the procedures for preparing the drug. Therefore, in order to obtain the same crystal form a special attention is required for the management of manufacturing procedures.

Hydrates as one of pseudopolymorphs comprise a water molecule within the crystal of drug, and have a crystal form different from anhydrate. Difference in the crystal structure can be distinguished by X-ray diffraction pattern. Since in hydrates only physical properties such as crystallinity, hygroscopic property, melting point, solubility, dissolution rate, etc. are changed without any change of chemical properties providing pharmacological effects, they have a very important significance in the pharmaceutical aspect, like polymorphs (Morris, K. R. et al., Int. J. Pharm., 108, 1994, 195-206).

The knowledge which is understood up to date from various references relating to the technical field to which the present invention belongs is that there is no general tendency, for example, to prefer the hydrate to the anhydrate or vice versa, for the improvement of pharmaceutical properties including drug stability, hygroscopic property, etc. Ultimately, determination of the forms having the optimal pharmaceutical properties for respective compounds must be made by a person skilled in the relevant technical field through continuous study case by case.

Particularly, it can never be anticipated among any conceivable forms of a certain drug, i.e. free compound, salt, anhydrate and hydrate, which one can exhibit a stability with the hygroscopic property that is not changed depending on the surrounding humidity. Furthermore, among the hydrates the most stable hydration number cannot be predicted. And, even though the hydration number is the same, it is also unpredictable which crystal form would be the most stable. This is a phenomenon that is inconsistently revealed since it cannot be anticipated and belongs to the experimental area which can be confirmed only through repeated experiments.

DISCLOSURE OF INVENTION

Technical Problem

Thus, the present inventors conducted intensive study to provide a stable polymorph or pseudopolymorph of Compound 1. As a result, we have surprisingly found that 1.5 hydrate of tartrate salt of Compound 1 exhibits a superior stability against the change of relative humidity as compared to the anhydrate or other hydrates having a similar hydration number, and thus, completed the present invention. Up to date, the crystal form of Compound 1 has never been publicly disclosed.

Solution to Problem

Therefore, the present invention provides 1.5 hydrate of tartrate salt of Compound 1.

In addition, the present invention provides a process for preparing 1.5 hydrate of tartrate salt of Compound 1.

Advantageous Effects of Invention

Since 1.5 hydrate of tartrate salt of Compound 1 obtained as a crystal form that can be developed according to the

3

present invention, is superior to the similar crystal forms in terms of a stability, particularly, a storage stability, it can be very advantageously used in preparing the pharmaceutical composition containing Compound 1 as an active component. That is, 1.5 hydrate according to the present invention neither loses any water molecule in the crystal nor accepts water molecules any more in the broad range of relative humidity to maintain the water content thereof, and therefore, shows substantially no change of weight depending on the change of humidity. In case of unstable crystal forms, the water content thereof can vary with environments or additives during the storage and formulation process. For instance, in quantifying the standard material and the sample for the purpose of quantification, if the experiment is not conducted in a drying room, some experimental error may be caused to incur the problems of quality control. However, since in the 1.5 hydrate according to the present invention the water content does not sensitively vary with environments in the broad range of relative humidity, the product with a uniform standard can always be obtained during the storage and formulation process, and further, an error in the quality control is very small. As above, the 1.5 hydrate according to the present invention shows a great advantage in terms of handling and quality control.

Further, 1.5 hydrate of tartrate salt of Compound 1 according to the present invention does not show any change in the crystal form depending on the change of humidity. On the contrary, the present inventors have identified that 0.5 hydrate and anhydrate of tartrate salt of Compound 1 absorb much water as the relative humidity is raised, to be converted into a more stable 1.5 hydrate according to the present invention. It was also identified that even in the experiment for an accelerating stability (40° C./75% RH) the 0.5 hydrate is converted into the 1.5 hydrate after a lapse of some hours.

In addition, it is possible to control the preparation of 1.5 hydrate of tartrate salt of Compound 1 according to various methods as provided in the present invention.

BRIEF DESCRIPTION OF DRAWINGS

The patent or application file contains at least one color drawing. Copies of this patent or patent application publication with color drawing will be provided by the USPTO upon request and payment of the necessary fee.

FIG. 1 represents the X-ray powder diffraction (XRD) spectrum of the crystal form I as the 1.5 hydrate of tartrate salt of Compound 1.

FIG. 2 represents the Infrared spectroscopy (FT-IR) spectrum of the crystal form I as the 1.5 hydrate of tartrate salt of Compound 1.

FIG. 3 represents the result of Differential scanning calorimetric (DSC) or Thermogravimetric (TG) analysis of the crystal form I as the 1.5 hydrate of tartrate salt of Compound 1.

FIG. 4 represents the dynamic vapor adsorption/desorption isotherm of the crystal form I as the 1.5 hydrate of tartrate salt of Compound 1.

FIG. 5 represents the X-ray powder diffraction (XRD) spectrum of the crystal form II as the 0.5 hydrate of tartrate salt of Compound 1.

FIG. 6 represents the Infrared spectroscopy (FT-IR) spectrum of the crystal form II as the 0.5 hydrate of tartrate salt of Compound 1.

FIG. 7 represents the result of Differential scanning calorimetric (DSC) or Thermogravimetric (TG) analysis of the crystal form II as the 0.5 hydrate of tartrate salt of Compound 1.

4

FIG. 8 represents the dynamic vapor adsorption/desorption isotherm of the crystal form II as the 0.5 hydrate of tartrate salt of Compound 1.

FIG. 9 represents the X-ray powder diffraction (XRD) spectrum of the crystal form II as the 0.5 hydrate of tartrate salt of Compound 1, during the course of moisture adsorption study.

FIG. 10 represents the X-ray powder diffraction (XRD) spectrum of the crystal form III as the anhydrate of tartrate salt of Compound 1.

FIG. 11 represents the Infrared spectroscopy (FT-IR) spectrum of the crystal form III as the anhydrate of tartrate salt of Compound 1.

FIG. 12 represents the result of Differential scanning calorimetric (DSC) or Thermogravimetric (TG) analysis of the crystal form III as the anhydrate of tartrate salt of Compound 1.

FIG. 13 represents the dynamic vapor adsorption/desorption isotherm of the crystal form III as the anhydrate of tartrate salt of Compound 1.

FIG. 14 represents the moisture adsorption curve of the crystal form III as the anhydrate of tartrate salt of Compound 1, with time lapse at normal temperature and normal humidity.

FIG. 15 represents the X-ray powder diffraction (XRD) spectrum of the crystal form III as the anhydrate of tartrate salt of Compound 1, during the moisture adsorption study.

FIG. 16 represents the X-ray powder diffraction (XRD) spectrum obtained after conducting the experiment of Test Example 8, indicating XRD of the crystal form II, XRD of the sample of the crystal form II after 8-week storage at 60° C./5% RH, XRD of the sample of the crystal form II after 8-week storage at 40° C./75% RH, and XRD of the crystal form I, in the order from the bottom.

MODE FOR THE INVENTION

As identified to respectively have the characteristic crystal forms according to the present invention, in the present specification the 1.5 hydrate of tartrate salt of Compound 1 is designated as "the crystal form I"; the 0.5 hydrate is designated as "the crystal form II"; and the anhydrate is designated as "the crystal form III". Herein, the water content of 1.5 hydrate is 3.0~5.5%, that of 0.5 hydrate is 1.0~2.5%, and that of anhydrate is 0~1.0%.

The crystallinities of the crystal forms I, II and III are different from each other as can be identified from X-ray diffraction diagrams shown in FIGS. 1, 5 and 10.

As a result from the analysis by Differential Scanning calorimetry for the crystal form I, the endothermic peak occurring with releasing water contained in the crystal lattice at 90~130° C. has a broad section in which the melting point is included, and the endothermic peak occurs one more time in the section of 130~160° C. with undergoing the chemical dehydration of tartrate salt of Compound 1. In addition, the thermogravimetric analysis shows the weight loss of about 4.0% that is equivalent to the water contained in the lattice in the first endothermic section of differential scanning calorimetry and the weight loss of about 2.5% that is equivalent to the water removed by dehydration from the structure of tartrate salt of Compound 1 in the second endothermic section (FIG. 3). The analysis for the crystal forms II and III by Differential Scanning calorimetry shows three endothermic points. Specifically, the first endothermic point occurs with releasing water contained in the lattice, the second endothermic point occurs with the melting point, and the third endothermic point occurs via chemical dehydration reaction. Further, according

5

to the thermogravimetric analysis the first endothermic section shows the weight loss that is equivalent to the water contained in the lattice (about 1.3% in the crystal form II, and about 0.6% in the crystal form III), and the third endothermic section shows the weight loss of about 2.4% that is equivalent to the water removed by dehydration (FIGS. 7 and 12). Since the water content in the lattice of tartrate salt of Compound 1 as above was consistent with the water content quantified by Karl-Fischer method (Mettler Toledo DL37 KF Coulometer), it was proved that the endothermic peaks were caused by evaporation of water molecules.

Hereinafter, we intend to explain that the crystal form I according to the present invention has unexpected superior characteristics from any aspect in comparison with other crystal form II or III by more specifically comparing the characteristics of respective crystal forms.

Crystal Form I

The present invention relates to the crystal form of 1.5 hydrate of tartrate salt of Compound 1. As a result of the analysis of the properties thereof the crystal form I exhibits the following characteristics:

(a) The water content of the crystal form I is in the range of 3.0~5.5%.

(b) The characteristic peak values (2 θ) of XRD spectrum measured at CuK α , 40 kV, 30 mA are 15, 18, 20, 21 and 23°.

(c) Infrared (IR) spectrum shows a characteristic absorbance at about 3591, 3401, 3128, 1712, 1655, 1636, 1229, 1205, 1129 and 1058 cm⁻¹.

(d) Differential scanning calorimetry spectrum shows the endo-thermal peaks in two broad temperature ranges of about 90~130° C. and 130~160° C.

(e) The water content measured by Karl-Fisher method is about 4.0%.

(f) When the temperature is raised from 25° C. to 250° C., the weight loss of about 4.0% and about 2.5% occur in the range of 70~110° C. and 140~170° C., respectively (This is the result obtained from TG of FIG. 3. Since energy change occurs slightly later than the weight change, there is generally a difference between the results of DSC and TG.).

(g) Tartrate salt of Compound 1 can be crystallized from water, acetonitrile/water, ethanol/water, ethanol/hexane or ethyl acetate/hexane solvent, and preferably crystallized from water.

(h) Crystal form I can be prepared by subjecting the crystal form II or III to moisture absorption.

(i) The weight change is absent or, if any, 0.8% or less depending on the change of external humidity in the range of 5~95% RH, and the crystal form is not changed with the change of humidity.

Crystal Form II

In order to find out other crystal forms than the crystal form I the present inventors prepared 0.5 hydrate of tartrate salt of Compound 1 (crystal form II). Although the 0.5 hydrate is the same tartrate salt of the same compound and has a similar hydration number, it shows unstable storage stability as follows, in comparison with the 1.5 hydrate (crystal form I).

(a) The water content of the crystal form II is in the range of 1.0~2.5%.

(b) The characteristic peak values (2 θ) of XRD spectrum measured at CuK α , 40 kV, 30 mA are 14, 15, 17, 18, 19, 21 and 23°.

6

(c) Infrared (IR) spectrum shows a characteristic absorbance at about 3455, 2891, 1721, 1655, 1571, 1228, 1209, 1131, 1086 and 1059 cm⁻¹.

(d) Differential scanning calorimetry spectrum shows the endo-thermal peaks in three broad temperature ranges of about 80~115° C., 115~135° C. and 135~173° C., and the melting point at about 117° C.

(e) The water content measured by Karl-Fisher method is about 2.0%.

(f) When the temperature is raised from 25° C. to 250° C., the weight loss of about 1.3% and about 2.4% occur in the range of 70~104° C. and 137~168° C., respectively.

(g) The weight change of 4.0% or more occurs over the change of external humidity in the range of 5~95% RH and moisture is rapidly absorbed from 45% RH, giving a weight increase of 3.7% at 75% RH. If a total of 4.0% or more moisture is contained, the crystal form II is converted into the crystal form I. It has been shown that the crystal form II is converted into the crystal form I from about 60% RH (see FIGS. 8 and 9). That is, the crystal form varies with the change of humidity to reach the more stable crystal form I. In addition, it is converted into the crystal form I within 8 weeks in the accelerating (40° C./75% RH) stability test.

Crystal Form III

In order to find out other crystal forms than the crystal form I the present inventors prepared anhydrate of tartrate salt of Compound 1 (crystal form III). The anhydrate shows unstable storage stability as follows, in comparison with the 1.5 hydrate (crystal form I).

(a) The water content of the crystal form III is in the range of 0~1.0%.

(b) The characteristic peak values (2 θ) of XRD spectrum measured at CuK α , 40 kV, 30 mA are 6, 17, 21, 23, 24, 26 and 30°.

(c) Infrared (IR) spectrum shows a characteristic absorbance at about 3470, 3187, 2940, 1640, 1570, 1229, 1206, 1130 and 1056 cm⁻¹.

(d) Differential scanning calorimetry spectrum shows the endo-thermal peaks in three broad temperature ranges of about 65~100° C., 100~130° C. and 132~170° C., and the melting point at about 104° C.

(e) The water content measured by Karl-Fisher method is about 0.1%.

(f) When the temperature is raised from 25° C. to 250° C., the weight loss of about 0.6% and about 2.4% occur in the range of 62~110° C. and 120~173° C., respectively.

(g) The weight change of 3.5% or more occurs over the change of external humidity in the range of 5~95% RH and moisture is rapidly absorbed from 5% RH, so that the crystal form III is converted into the crystal form I from about 15% RH (see FIGS. 13, 14 and 15). That is, the crystal form varies with the change of humidity to arrive at the more stable crystal form I.

The above results suggest that in the range of relative humidity under which the formulation is conventionally practiced the crystal forms II and III are unstable and thus automatically converted into the crystal form I. Such tendency is shown more remarkably in case of the crystal form III.

1.5 Hydrate of tartrate salt of Compound 1 according to the present invention exhibits a strong DPP-IV inhibitory activity in the same way as the corresponding free base disclosed in Korean Patent Application No. 10-2006-0029138. In addition, the 1.5 hydrate of the present invention shows improved physical and chemical properties as compared to the crystal forms having other hydration state. Therefore, the 1.5 hydrate

7

according to the present invention is considerably easy to handle, quality control and formulate as compared to the crystal forms having other hydration state.

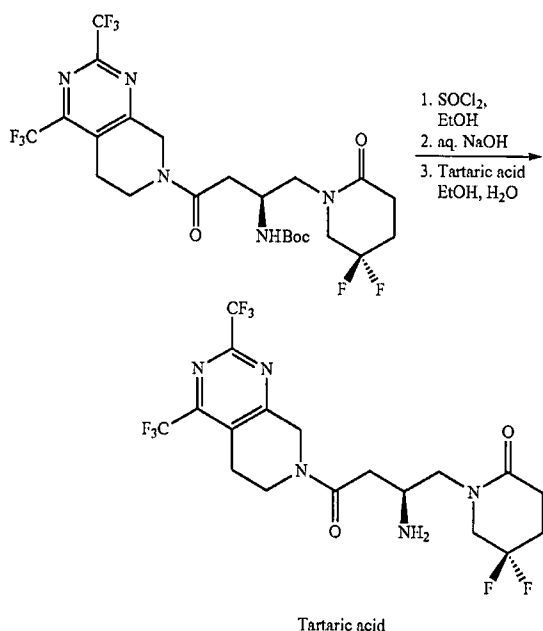
The 1.5 hydrate according to the present invention has the DPP-IV inhibitory activity as mentioned above, and therefore, can be formulated for convenient administration in the pharmaceutical and veterinary field. Formulation can be conducted according to the techniques and methods known in the art in relation to other formulations having DPP-IV inhibitory activity, particularly with reference to the disclosure of Korean Patent Application No. 10-2006-0029138 which is incorporated herein by reference in its entirety.

Therefore, a pharmaceutical composition for inhibiting DPP-IV, comprising the 1.5 hydrate according to the present invention as the active component together with a pharmaceutically acceptable carrier, is covered by the scope of the present invention. The composition according to the present invention is characterized in that it is used particularly for the treatment and prevention of diabetes or obesity.

The present invention is illustrated more in detail by means of the following Examples and Test Examples. However, the following Examples and Test Examples are provided only to assist the understanding of the present invention but it is not intended that the scope of the present invention is limited in any manner by these Examples and Test Examples.

Example 1

Preparation of 1.5 Hydrate of Tartrate Salt of Compound 1 (Crystal Form I)



1.87 kg of the compound 2 was dissolved in about 9 L of ethanol. 0.94 kg of SOCl_2 was added at 0–10° C. and then stirred while maintaining low temperature. After concentrating under reduced pressure, the concentrate was dissolved in 11.2 L of MTBE (methyl t-butyl ether), and the resulting mixture was adjusted with 10 N NaOH solution to pH 7–8. After separating the layers, the aqueous layer was extracted with about 3.7 L of MTBE and twice with 3.7 L of MTBE, and then concentrated under reduced pressure. The resulting

8

brown turbid solution was dissolved in 12 L of ethanol, 0.47 kg of L-tartaric acid dissolved in about 1.5 L of water was added thereto, and then stirred for 1 hour. The resulting crystalline slurry was filtered, washed with water and ethanol (1:8), and then dried to obtain 1.13 kg (yield 97.5%) of the title compound.

$^1\text{H NMR}$ (500 MHz, CD_3OD) δ : 3.38 (m, 2H), 2.59 (m, 2H), 2.82–2.99 (m, 2H), 3.11 (bt, 1H), 3.21 (bt, 1H), 3.50–3.55 (m, 1H), 3.72–3.91 (m, 5H), 3.98 (t, $J=5.2$ Hz, 1H), 4.38 (s, 2H), 4.97–5.00 (m, 2H).

Example 2

Recrystallization of 1.5 Hydrate of Tartrate Salt of Compound 1 (Crystal Form I) from Water

50 g of tartrate salt of Compound 1 obtained from Example 1 was added to 250–500 ml of water, and dissolved in water while adjusting the solution with 10 N NaOH to pH 6–7. 11.7 g of L-tartaric acid dissolved in 23.5 ml of water was added, and crystals were obtained with varying the temperature, stirring rate and stirring time as shown in the following Table 1. Then, the crystals were filtered and dried to obtain the crystal form I. The stirring rate was varied in the range of 50–400 rpm, and the temperature was varied in the range of 5–32° C. The volume of water used for recrystallization, the stirring rate, temperature and stirring time are represented in the following Table 1.

TABLE 1

Entry	Volume (mL)	RPM	Temperature	Time (h)	Yield (%)
1	250	50	25.0	0.50	96.0 (48.0 g)
2	250	100	25.0	1.00	92.8 (46.4 g)
3	250	400	25.0	5.00	93.6 (46.8 g)
4	375	400	25.0	1.00	92.4 (46.2 g)
5	500	50	25.0	5.00	46.6 (23.3 g)
6	500	400	25.0	3.00	88.2 (44.1 g)
7	375	225	31.8	2.75	94.0 (47.0 g)
8	375	225	15.0	2.75	91.0 (45.5 g)
9	250	50	5.0	0.50	81.4 (40.7 g)
10	250	400	5.0	5.00	101.8 (50.9 g)

Example 3

Recrystallization of 1.5 Hydrate of Tartrate Salt of Compound 1 (Crystal Form I) from the Mixed Solvent

5 g of tartrate salt of Compound 1 was dissolved in 25–60 ml of solvent mixtures comprised of water and acetonitrile in different ratios. Crystals were precipitated with varying the temperature in the presence or absence of stirring, filtered and dried to recrystallize the tartrate salt of Compound 1. The conditions used for recrystallization are listed in the following Table 2.

TABLE 2

Entry	Acetonitrile/water	Stirring	Temperature (° C.)
1	4/1	N	25.0
3	6/1	N	25.0
4	6/1	N	0.0
5	8/1	N	25.0
6	8/1	Y	25.0
8	1/1	N	25.0
9	1/1	Y	20.0

Test Example 1

Powder X-ray Diffractometry

About 20 mg of the sample was charged in the sample holder and mounted on Powder X-ray diffractometer to obtain the diffraction pattern in the range of 3–40°/2θ. The diffraction patterns as obtained are attached to the present specification as FIGS. 1, 5 and 10, respectively. Specific conditions for analysis are as follows.

Instrument: Bruker 4D Endeavor

Time per step: 0.3 s
Stepsize: 0.03°
Scan Mode: step
Voltage/Current: 40 kV/30 mA
Cu-target (Ni-filter)
Divergence slit: 0.3
Detector: PSD: LynxEye

Instrument: Philips X-ray Generator (PW1710)

Time per step: 0.5 s
Stepsize: 0.03°
Scan Mode: step
Voltage/Current: 40 kV/30 mA
Cu-target (Ni-filter)
Source Slit: 1.0 mm
Detector Slits: 0.15 mm, 1.0 mm

Test Example 2

Infrared Spectroscopy

The infrared spectra for respective crystal forms according to the present invention were obtained using ZASCO FT-IR 4200 provided with DTGS detector. The resolution of respective spectra was 4 cm⁻¹, and the number of scan was 16. In the present Test Example 1–2 mg of the sample was placed on the accessory of ATR (Attenuated Total Reflectance) and the equipment was operated to obtain the spectrum. Background data was obtained by operating the equipment without any material in ATR. The spectra thus obtained are attached to the present specification as FIGS. 2, 6 and 11, respectively.

Test Example 3

Differential Scanning Calorimetry (DSC)

Differential Scanning calorimetry (DSC) was conducted using Mettler Toledo's DSC821°. About 2–3 mg of the sample was charged into the aluminum pan, and the weight thereof was accurately recorded. The pan was covered with a lid through which a hole was punched. The pan was mounted on the equipment and heated from 25 to 250° C. in the rate of 10° C./min under nitrogen purge. Indium metal was used as the standard for calibration. The spectra thus obtained are attached to the present specification as FIGS. 3, 7 and 12, respectively.

Test Example 4

Thermogravimetry (TG)

Thermogravimetry (TG) was conducted using Mettler Toledo TGA850. About 4–5 mg of the sample was charged into the aluminum pan. The pan was mounted on the equipment and then heated from 25 to 250° C. in the rate of 10° C./min under nitrogen purge. Nickel and Aluminum™ were used as the standard for calibration. The results of TG analysis as obtained are attached to the present specification as FIGS.

3, 7 and 12, respectively, together with the results of DSC analysis according to said Test Example 3.

Test Example 5

Dynamic Vapor Adsorption/Desorption Analysis

The dynamic vapor adsorption/desorption data were collected on VTI-SA Vapor Sorption Analyzer. While maintaining 25° C., the vapor adsorption and desorption were repeated three times at intervals of 5% RH in the range of relative humidity (RH) 5–95%. The samples were not dried prior to the analysis. The equilibrium standard used for analysis was that the weight change within 2 minutes is less than 0.01%.

As the result of analysis, FIG. 4 shows a result of the vapor adsorption/desorption isotherm of the crystal form I, from which it can be seen that the crystal form I shows the weight change of 0.8% or less according to the change of external humidity in the range of 5–95% RH. That is, the crystal form I according to the present invention is very stable against the change of relative humidity. FIG. 8 shows a result of the vapor adsorption/desorption isotherm of the crystal form II, from which it can be seen that in the crystal form II the weight increases by 4.3% when the relative humidity is raised up to 95% in the initial moisture adsorption test. After moisture desorption, the crystal form II gives the same graph of moisture behavior as the crystal form I. From such result, it could be confirmed that the crystal form II was converted into the crystal form I during the course of the initial moisture adsorption. FIG. 13 shows a result of the vapor adsorption/desorption isotherm of the crystal form III, from which it can be seen that in the crystal form III the weight increases by 3.6% when the relative humidity is raised up to 95%. Furthermore, it can be seen that decreasing the humidity causes the moisture desorption of 0.6% or less so that the crystal form III becomes to contain the same moisture content as the crystal form I.

Test Example 6

Powder X-ray Diffraction Test of the Crystal Form II During Moisture Adsorption

About 50 mg of the crystal form II was placed in a glass vial, put under the relative humidity of 11%, 32%, 53%, 64%, 78% and 97%, respectively, for two or more days to induce the moisture adsorption, and then subjected to the powder X-ray diffraction test according to the conditions as presented in said Test Example 1 to identify any change of the crystal form at the time of moisture adsorption (see FIG. 9).

Respective relative humidity was provided by making the saturated aqueous solution of salt as shown in the following Table 3 and placing the solution in a desiccator, which was then sealed.

TABLE 3

Relative humidity 11%	Saturated aqueous solution of LiCl
Relative humidity 32%	Saturated aqueous solution of MgCl ₂
Relative humidity 53%	Saturated aqueous solution of Mg(NO ₃) ₂ •6H ₂ O
Relative humidity 64%	Saturated aqueous solution of NaNO ₂
Relative humidity 78%	Saturated aqueous solution of NaCl
Relative humidity 97%	Saturated aqueous solution of KNO ₃

Test Example 7

Powder X-ray Diffraction Test of the Crystal Form III During Moisture Adsorption

The crystal form III was placed on a XRD holder and subjected to XRD test over the time (after 30 minutes, 1 hour

and 5 hours) while allowing the moisture adsorption at room temperature under atmosphere (see FIG. 15). In addition, the weight change at room temperature under atmosphere was recorded by times to obtain a graph (see FIG. 14). It could be known that the crystal form III rapidly absorbs water so that it is converted into the crystal form I within about one hour.

Test Example 8

Thermal Stability of the Crystal Form I and the Crystal Form II

About 50 mg of each of the crystal form I and the crystal form II was placed in Duma bottle, and then kept at 40±2° C., 75±5% RH or 60±2° C., 5±5% RH. After 2 weeks, 4 weeks and 8 weeks, each sample was removed from Duma bottle and analyzed by XRD for identifying any change of the crystal form and HPLC for identifying the stability. For HPLC analysis the sample was dissolved in a mixture of acetonitrile/water/trifluoroacetic acid=30/70/0.1 (v/v/v) and then subjected to the analysis. The conditions for HPLC analysis are as follows:

Conditions for HPLC Analysis

Column: Atlantis dC18 (4.6 mm I.D×250 mm L, Particle Size 5, µm, Waters)
Column Temperature: 10° C.
Mobile phase:
Mobile phase A: MeCN/TFA=100/0.1 (v/v)
Mobile phase B: H₂O/TFA=100/0.1 (v/v)
Gradient condition:

Time (min.)	A (%)	B (%)
Initial	38	62
25	38	62
35	80	20
40	38	62
55	38	62

Flow rate: 0.7 ml/min.
Detection: 256 nm, UV
Injection volume: 10 µl
Total analysis time: 55 min.
The results of the stability for the crystal form I and the crystal form II are shown in the following Table 4.

TABLE 4

Time (week)	40° C./75% RH		60° C./5% RH	
	Crystal form I	Crystal form II	Crystal form I	Crystal form II
0	99.4	98.4	99.4	98.4
2	99.3	98.4	98.8	98.1
4	99.2	98.3	98.6	97.8
8	99.2	98.2	98.4	97.5

As shown in Table 4, it could be confirmed that upon keeping the crystal form I and the crystal form II at 40±2° C., 75±5% RH or 60±2° C., 5±5% RH they exhibit a superior stability up to 8 weeks. However, according to the result of XRD analysis the crystal form I did not show any change up

to 8 weeks, but the crystal form II was converted into the crystal form I at 8 week under the condition of 40° C./75% RH (see FIG. 16).

Test Example 9

Determination of Density of the Crystal Form I and the Crystal Form II

About 20~30 ml of each of the crystal form I and the crystal form II was introduced into a 50 ml measuring cylinder. Here, the samples were slowly introduced so that they are not closely contacted with each other. After reading a scale on the measuring cylinder, the weight thereof was measured to calculate the bulk-density. After measuring the bulk-density, the measuring cylinder containing the sample was put on ERWEKA densitometer and then subjected to impulses 250 times to measure the tapdensity (Table 5). The result of measurement shows that both the bulk-density and the tap-density of the crystal form I were higher than those of the crystal form II. The Carr index was calculated from the bulk-density and the tap-density. As a result, it was lower in the crystal form I than in the crystal form II. Since it is generally understood that the lower the Carr index, the better the flowability, it could be identified by comparing the Carr indexes that the crystal form I can be easily handled in processing procedures including tableting as compared to the crystal form II.

TABLE 5

Determination of density and particle size of the crystal form I and the crystal form II			
Form	Density		
	Bulk-density	Tap-density	Carr index
I	0.45	0.54	16.22
II	0.34	0.44	23.47

- The invention claimed is:
1. 1.5 Hydrate of 1-[(2S)-2-amino-4-[2,4-bis(trifluoromethyl)-5,8-dihydropyrido[3,4-d]pyrimidin-7(6H)-yl]-4-oxobutyl]-5,5-difluoropiperidin-2-one tartrate salt having a water content in the range of 3.5-5.5%.
 2. The 1.5 hydrate according to claim 1, wherein characteristic peak values (2θ) in XRD diffractogram are 15, 18, 20, 21 and 23°.
 3. A process for preparing the 1.5 hydrate according to claim 1, characterized in that 1-[(2S)-2-amino-4-[2,4-bis(trifluoromethyl)-5,8-dihydropyrido[3,4-d]pyrimidin-7(6H)-yl]-4-oxobutyl]-5,5-difluoropiperidin-2-one tartrate salt is recrystallized from water, acetonitrile/water, ethanol/water, ethanol/hexane or ethyl acetate/hexane solvent.
 4. The process according to claim 3, wherein the recrystallizing solvent is water.
 5. A pharmaceutical composition for inhibiting DPP-IV, which comprises the 1.5 hydrate according to claim 1 as the active component together with a pharmaceutically acceptable carrier.
 6. The composition according to claim 5, wherein it is used for the treatment of diabetes or obesity.

* * * * *



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Application No. 11 838 195.3 - 1462	Ref. REG/P43776EP	Date 24.02.2015
Applicant LG Life Sciences Ltd		

Communication under Rule 71(3) EPC

1. Intention to grant

You are informed that the Examining Division intends to grant a European patent on the basis of the above application with the text and drawings and the related bibliographic data as indicated below.

A copy of the relevant documents is enclosed.

1.1 In the text for the Contracting States:

AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT
RO RS SE SI SK SM TR

Description, Pages

1-17 as published

Claims, Numbers

1-6 as published

Drawings, Sheets

1/8-8/8 as published

With the following amendments to the above-mentioned documents proposed by the division

Description, Pages 1

Claims, Numbers 5

SA 67

1.2 Bibliographic data

The title of the invention in the three official languages of the European Patent Office, the international patent classification, the designated Contracting States, the registered name(s) of the applicant(s) and the other bibliographic data are shown on the **enclosed EPO Form 2056**.

2. Invitation

You are invited, **within a non-extendable period of four months** of notification of this communication,

2.1 to EITHER approve the text communicated above and verify the bibliographic data (R. 71(5) EPC)

(1) by filing a translation of the claim(s) in the other two official languages of the EPO

Fee code EUR

(2a) by paying the fee for grant including the fee for publication:
minus any amount already paid (R. 71a(5) EPC):

007 915.00
0.00

Total amount: 915.00

(3) by paying additional claims fees under Rule 71(4) EPC;
number of claims fees payable: 0
minus any amount already paid (R. 71a(5) EPC):

016 0.00
0.00

Total amount: 0.00

Important: If the translations of the claims and fees have already been filed and paid respectively in reply to a previous communication under Rule 71(3) EPC, e.g. in the case of resumption of examination after approval (see Guidelines C-V, 6), **agreement as to the text to be granted** (R. 71a(1) EPC) must be expressed within the same time limit (e.g. by approving the text and verifying the bibliographic data, by confirming that grant proceedings can go ahead with the documents on file and/or by stating which translations of the claims already on file are to be used).

Note 1: See "Important notes concerning fee payments" below.

Note 2: Any overpaid "minus" amounts will be refunded when the decision to grant (EPO Form 2006A) has been issued.

Note 3: For the calculation of the grant fee under Art. 2(2), No. 7, RFees (old fee structure), the number of pages is determined on the basis of a clean copy of the application documents, in which text deleted as a result of any amendments by the Examining Division is not shown. Such clean copy is made available via on-line file inspection only.

2.2 OR, in the case of disapproval, to request reasoned amendments or corrections to the text communicated above or keep to the latest text submitted by you (R. 71(6) EPC).

In this case the translations of the claims and fee payments mentioned under point 2.1 above are NOT due.

The terms "amendment(s)" and "correction(s)" refer only to amendments or corrections of the application documents and not of other documents (e.g. bibliographic data, the designation of the inventor, etc.).

If filing amendments, you must identify them and indicate the basis for them in the application as filed. Failure to meet either requirement may lead to a communication from the Examining Division requesting that you correct this deficiency (R. 137(4) EPC).

2.3 Bibliographic data

Where you request a change or correction of bibliographic data in response to the Rule 71(3) communication, this will **not** cause the sending of a second communication under Rule 71(3) EPC. You will still have to pay the fees and file translations in reply to the Rule 71(3) communication in the case of 2.1 above, unless you also file a reasoned request for amendments or corrections in response to the Rule 71(3) communication (see case 2.2 above).

3. Loss of rights

If neither of the two possible actions above (see points 2.1 or 2.2) is performed in due time, the European patent application will be deemed to be withdrawn (R. 71(7) EPC).

4. Further procedure

4.1 In the case of point 2.1 above

- 4.1.1 The decision to grant the European patent will be issued, and the **mention of the grant** of the patent will be published in the European Patent Bulletin, if the requirements concerning the translation of the claims and the payment of all fees are fulfilled and there is agreement as to the text to be granted (R. 71a(1) EPC).

Note on payment of the renewal fee:

If a renewal fee becomes due before the next possible date for publication of the mention of the grant of the European patent, publication will be effected only after the renewal fee and any additional fee have been paid (R. 71a(4) EPC).

Under Article 86(2) EPC, the obligation to pay renewal fees to the European Patent Office terminates with the payment of the renewal fee due in respect of the year in which the mention of the grant of the European patent is published.

Note on payment of the designation fee(s):

If the designation fee(s) become(s) due after the communication under Rule 71(3) EPC, the mention of the grant of the European patent will not be published until the designation fee(s) has/have been paid (R. 71a(3) EPC).

- 4.1.2 After publication, the **European patent specification** can be downloaded free of charge from the EPO publication server <https://data.epo.org/publication-server> or ordered from the Vienna sub-office upon payment of a fee (OJ EPO 2005, 126).

Until 31 December 2013, each proprietor may request in writing to receive the certificate for the European patent together with one copy of the new patent specification only if the request is filed within three-month or the additional two-month period (R. 95(3) and (4) EPC). If such request has been previously filed, it has to be confirmed within the same period. The requested copy is free of charge. Any request or confirmation of a request filed after 31 December 2013 or after expiry of these periods will not be dealt with and the certificate will be delivered without a copy of the new European patent specification (cf. Notice from the European Patent Office dated 16 July 2013 concerning communication of the certificate for a European patent, OJ 2013, 418).

4.1.3 Filing of translations in the Contracting States

As regards translation requirements prescribed by the Contracting States under Article 65(1) EPC, please consult the website of the European Patent Office

www.epo.org → Law & practice → Legal texts, National law relating to the EPC

www.epo.org → Law & practice → All Legal texts → London Agreement

In the case of a valid extension or validation

As regards translation requirements prescribed by the Extension or or Validation States, please consult the website of the European Patent Office

www.epo.org → Law & practice → Legal texts, National law relating to the EPC

Failure to supply a prescribed translation in a Contracting State, or in an Extension or Validation State may result in the patent being deemed to be void *ab initio* in the State concerned (Art. 65(3) EPC).

4.2 In case of 2.2 above

If the present communication under Rule 71(3) EPC is based on an auxiliary request and, within the time limit, you maintain the main request or a higher ranking request which is not allowable, the application will be refused (Art. 97(2) EPC).

If the Examining Division gives its consent to the requested amendments or corrections, it will issue a new communication under Rule 71(3) EPC; otherwise, it shall resume the examination proceedings (R. 71(6) EPC).

5. Filing of a divisional application

Any divisional application relating to this European patent application must be filed directly with the European Patent Office in Munich, The Hague or Berlin and will be in the language of the proceedings for the present application, or if the latter was not in an official language of the EPO, the divisional application may be filed in the language of the present application as filed (see Article 76(1) and Rule 36(2) EPC). Any such divisional application must be filed while the present application is still pending and the time limit for filing divisional applications must be observed (R. 36(1) EPC; Guidelines A-IV, 1.1.1).

6. Important notes concerning fee payments

6.1 For all payments, please refer to the relevant **fee code(s)**.

6.2 Automatic debiting procedure

The fee for grant, including the fee for publication, and any additional claims fees due under Rule 71(4) EPC will be debited automatically on the date of filing of the translations of the claims, or on the last day of the period of this communication. However, if the designation fee(s) become(s) due as set out in Rule 71a(3) EPC and/or a renewal fee becomes due as set out in Rule 71a(4) EPC, these should be paid separately by another permitted way of payment in order not to delay the publication of the mention of the grant. The same applies in these circumstances to the payment of extension fees. For further details see the Arrangements for the automatic debiting procedure (AAD) and accompanying information from the EPO concerning the automatic debiting procedure (Annexes A.1 and A.2 to the Arrangements for deposit accounts (ADA) Supplementary publication 4 - OJ EPO 2014).

6.3 Important information relating to fee amounts

Following any amendment to the Rules relating to Fees, the amount(s) mentioned in this communication may be different from the amount(s) **actually due on the date of payment**. The latest version of the Schedule of fees and expenses, published as a Supplement to the Official Journal of the EPO, is also available on the EPO website (www.epo.org) and can be found under www.epo.org/schedule-of-fees, which allows the viewing, downloading and searching for individual fee amounts, both current and previous. Please note that procedural fees are usually adjusted every two years, on even years, with effect from 1 April.

42 70

Date 24.02.2015

Sheet 5

Application No.: 11 838 195.3

Examining Division:

Chairman:	Bissmire, Stewart
2nd Examiner:	Miniejew, Catherine
1st Examiner:	Baston, Eckhard



Hutterer, Georg
For the Examining Division
Tel. No.: +49 89 2399 - 8066

Enclosure(s): Text intended for grant

EPO Form 2056

63 71

WO 2012/060590

18

PCT/KR2011/008186

Claims

- [Claim 1] 1.5 Hydrate of
1-[(2S)-2-amino-4-[2,4-bis(trifluoromethyl)-5,8-dihydro-pyrido[3,4-*d*]pyrimidin-7(6H)-yl]-4-oxobutyl]-5,5-difluoropiperidin-2-one tartrate salt having a water content in the range of 3.5-5.5%.
- [Claim 2] The 1.5 hydrate according to Claim 1, wherein characteristic peak values (2 θ) in XRD diffractogram are 15, 18, 20, 21 and 23°.
- [Claim 3] A process for preparing the 1.5 hydrate according to Claim 1, characterized in that
1-[(2S)-2-amino-4-[2,4-bis(trifluoromethyl)-5,8-dihydropyrido[3,4-*d*]pyrimidin-7(6H)-yl]-4-oxobutyl]-5,5-difluoropiperidin-2-one tartrate salt is recrystallized from water, acetonitrile/water, ethanol/water, ethanol/hexane or ethyl acetate/hexane solvent.
- [Claim 4] The process according to Claim 3, wherein the recrystallizing solvent is water.
- [Claim 5] A pharmaceutical composition for use in
inhibiting DPP-IV, which comprises
the 1.5 hydrate according to Claim 1 as the active component together with a pharmaceutically acceptable carrier.
- [Claim 6] The composition according to Claim 5, wherein it is used for the treatment or prevention of diabetes or obesity.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800.176.089-2

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RECIBIDO DE : CAVELIER ABOGADOS

NI 860.041.367

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*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR PAGO
CONSIGNACION	SIC ** RECIBO **	999999999	38708	09/04/2015	530.000.00
CONSIGNACION	SIC ** RECIBO **	999999999	40711	14/04/2015	1.000.00
CONSIGNACION	SIC ** RECIBO **	999999999	41723	16/04/2015	478.000.00

*** Conceptos Pagados ***

CANT. RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1 50005-01-03 CAMBIO DE MODALIDAD Y MODIFICACIONES EN CREACION CORRECCION A SOLICITUD EN TRAMITE / MODIFICA		132.000.00	132.000.00
			\$132.000.00

SON: **CIENTO TREINTA Y DOS MIL PESOS MONEDA CORRIENTE***

Responsable:

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 13-108961- -00009-0000

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Abril 20 de 2015 - 11:53:22