

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

2020  
 Bogotá, D.C.,

Abogada  
 LUZ CLEMENCIA DE PAEZ

|        |            |            |
|--------|------------|------------|
| ASUNTO | Radicación | 09 120 908 |
|        | Trámite    | 11         |
|        | Evento     | 378        |
|        | Actuación  | 519        |
|        | Oficio     |            |
|        | Folios     | 15         |

**9 1 4 2 0 1**

Patente de Invención



Patente de Modelo de Utilidad

Via Nacional

Via PCT (Fase Nacional)



Capítulo I



Capítulo II

No. Solicitud Internacional

PCT/KR2008/002106

Fecha de Presentación Internacional

14/Abril/2008

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

### 1. Documentos estudiados

|                   |                    |                 |
|-------------------|--------------------|-----------------|
| Descripción:      | Folios 45 a 101    | 27/Octubre/2009 |
| Reivindicaciones: | Folios 102 a 104   | 27/Octubre/2009 |
| Prioridad:        | KR 10-2007-0036574 | 13/Abril/2007   |

### 2. Análisis de la Prioridad

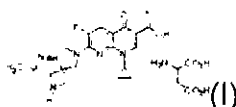
Se acepta la reivindicación de prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

### 3. Objeto de la invención

Título de la solicitud: "Aspartato de ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico, método para preparar el mismo, y composición farmacéutica antimicrobiana que comprende la misma".

El problema planteado en esta solicitud consiste en la necesidad de suministrar agentes antimicrobianos derivados de quinolona mejorados contra *Staphylococcus aureus* resistentes a meticilina (MRSA).

Para resolver este problema técnico la solicitud en estudio proporciona una sal de ácido aspártico del ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico representada por la fórmula (I):



El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07D471/04//487/10, A61K31/47, A61P31/00.

4. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de las reivindicaciones 10 no es patentable de acuerdo con el Art citado.

5. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicación 3 no es clara, concisa, ni está soportada en la descripción cuando emplea el término “disolvente” sin especificar a cual hace referencia, lo cual es impreciso, no permite distinguir la invención del estado de la técnica, tampoco permite que la persona del oficio normalmente versada en la materia distinga cual es el disolvente en el cual es posible llevar a cabo la reacción. Se sugiere eliminar el término o definir los disolventes, de acuerdo a lo soportado en la descripción, de manera precisa de tal manera que pueda definirse claramente el objeto de la invención.

La reivindicación 3 no es clara porque hace referencia a un procedimiento pero este no se define mediante características como alguna condición específica en que debiera realizarse. Se sugiere, entonces, definir la invención por sus características esenciales.

La reivindicación 9 es idéntica a la reivindicación 1. Se sugiere eliminar esta reivindicación del pliego reivindicatorio.

6. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad 13 de abril de 2007 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

| Item | Documento             | Título                                                                                                        | Fecha de Publicación* |
|------|-----------------------|---------------------------------------------------------------------------------------------------------------|-----------------------|
| D1   | WO99/00393            | Quinolone carboxylic acid derivatives                                                                         | 07/Enero/1999         |
| D2   | Artículo <sup>1</sup> | In vitro and in vivo antibacterial activities of DW-224a, a new fluoronaphthyridone                           | Junio/2006            |
| D3   | US 5.563.149          | Infusion solutions of 1-cyclopropyl-6-fluoro-1,4-di-hydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic acid | 18/Septiembre/1990    |
| D4   | US 4.957.922          | Aqueous solutions of pyridone carboxylic acids                                                                | 08/Octubre/1996       |

D1  
[http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=9900393A1&KC=A1&FT=D&ND=&date=19990107&DB=&locale=en\\_EP](http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=9900393A1&KC=A1&FT=D&ND=&date=19990107&DB=&locale=en_EP), divulga (ejemplo 1, pg. 25, In 26 – pg. 26, In 13) el compuesto metano-sulfonato de ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-díaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico, un método para su preparación y composiciones farmacéuticas que la contienen.

<sup>1</sup> Park H, et al; In vitro and in vivo antibacterial activities of DW-224a, a new fluoronaphthyridone; Antimicrobial agents and chemotherapy; Vol. 50, No. 6; Junio 2006; pg. 2261-2264, fls. 158 - 161.

D2 (fls 158 - 161) divulga (pg. 2261, fl 158) la sal clorhidrato de ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico.

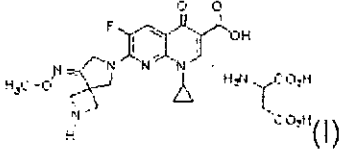
D3  
[http://www.google.com/patents?id=UVkgAAAAEBAJ&pg=PA1&lpg=PA1&dq=us+4957922&source=bl&ots=fIRPcNg6Xs&sig=t8pD45cy49srZW0O-ZL9OR\\_icqo&hl=en&sa=X&ei=cdoFUNGZJlyo8QSfIYWACA&ved=0CDYQ6AEwAA#v=onepage&q=us%204957922&f=false](http://www.google.com/patents?id=UVkgAAAAEBAJ&pg=PA1&lpg=PA1&dq=us+4957922&source=bl&ots=fIRPcNg6Xs&sig=t8pD45cy49srZW0O-ZL9OR_icqo&hl=en&sa=X&ei=cdoFUNGZJlyo8QSfIYWACA&ved=0CDYQ6AEwAA#v=onepage&q=us%204957922&f=false), divulga (ejemplo 42, col 13 – 16) la sal de ácido aspártico del ácido-1-ciclopropil-6-fluoro-1,4-dihidro-4-oxo-7-(1-piperazinil)-quinolina-3-carboxílico.

D4  
<http://www.google.com/patents?id=2VgpAAAAEBAJ&pg=PA1&lpg=PA1&dq=us+5563149&source=bl&ots=vLSIG1jJHz&sig=ENLAjOEIrvAVae3FS6ndix2rV4Y&hl=en&sa=X&ei=9NoFUMOUHli08ASBy8z5Bw&ved=0CDYQ6AEwAA#v=onepage&q=us%205563149&f=false>, divulga (col. 4, ejemplo 8, col. 5, tabla 1) la sal de ácido aspártico del ácido-1-ciclopropil-6-fluoro-4-oxo-7-([1α,5α,6β]-6-amino-1-metil-3-azabicyclo[3.2.0]heptan-3-il)- 1,8-naftidrina-3-carboxílico.

7. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La reivindicación 1 consiste en una sal de ácido aspártico del ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico representada por la fórmula (I):



Comparación de las características técnicas esenciales, con las del estado de la técnica:

| Características esenciales                                                                                                 | D1                                                                                                                         | D2                                                                                                                         | D3                                                                                                        | D4                                                                                                                             |
|----------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| Sal de ácido aspártico de:                                                                                                 | Sal de ácido metano-sulfónico (ejemplo 1, pg. 25, ln 26 -- pg. 26, ln 13)                                                  | Sal de ácido clorhídrico de (pg. 2261, fl 158):                                                                            | Sal de ácido aspártico de (ejemplo 42, col 13 – 16):                                                      | Sal de ácido aspártico de (col. 4, ejemplo 8, col. 5, tabla 1):                                                                |
| ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico | ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico | ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico | Ácido-1-ciclopropil-6-fluoro-1,4-dihidro-4-oxo-7-(1-piperazinil)-quinolina-3-carboxílico (Ciprofloxacina) | Ácido-1-ciclopropil-6-fluoro-4-oxo-7-([1α,5α,6β]-6-amino-1-metil-3-azabicyclo[3.2.0]heptan-3-il)- 1,8-naftidrina-3-carboxílico |

Los documentos D1 y D2 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulgan la sal metano-sulfonato y la sal clorhidrato de ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico respectivamente.

La diferencia entre la invención, definida en la reivindicación 1 y los compuesto de D1

y D2 consiste en que divulga de manera específica la sal aspartato del ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico.

El efecto que logra el incluir esta característica es que la sal tiene mayor solubilidad y menor toxicidad.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular como la necesidad de sales del ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico con mayor solubilidad y menor toxicidad a los ya conocidos en el estado del arte.

Sin embargo, es de conocimiento común para la persona del oficio normalmente versada en la materia que el mejoramiento de la disolución de fármacos que son ácido o bases débiles poco solubles es uno de los principales motivos para la formación de sales (pg. 19 y 28, fl 163). Además, que la selección y la preparación de una sal adecuada dan la oportunidad de modificar características fisicoquímicas y mecánicas de los fármacos que permiten el desarrollo de formas farmacéuticas con mejor biodisponibilidad, estabilidad, entre otros (pg. 163, fl 164). Asimismo, que la elección de una sal en particular está determinada por la acidez o basicidad del grupo ionizable, la seguridad del grupo potencial para la formación de la sal (contra-ión), indicaciones del medicamento, ruta de administración y la dosis, entre otros (pg. 167, fl 166)<sup>2</sup>. Además, la formación de sales con ácido aspártico ya esta divulgada en los documentos D3 y D4, en donde diferentes agentes antimicrobianos del tipo fluoroquinolona<sup>3</sup> forman sales con este ácido para mejorar la solubilidad de los compuestos (D3: ejemplo 42, col 13 - 16; D4: col. 4, ejemplo 8, col. 5, tabla 1).

Por otra parte, aunque en la descripción se encuentra soporte de la mayor solubilidad de la sal aspartato del ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico en comparación con el clorhidrato, no se encuentra soporte respecto del metano-sulfonato, además, en D4 esta divulgado que la formación de la sal aspartato con un agente antimicrobiano del tipo fluoroquinolona incrementa de manera sorprendente su solubilidad (col. 4, ejemplo 8, col. 5, tabla 1). Asimismo, aunque en la descripción se encuentra soporte de la toxicidad de la sal de la invención respecto de la sal metano-sulfonato y del clorhidrato, esto sólo ocurre para el D-aspartato, no se encuentran soportes correspondientes al L-aspartato. Por lo anterior, no se puede concluir que la sal aspartato del ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico reclamada en esta solicitud tenga un efecto técnico inesperado o una ventaja superior en comparación con las sales clorhidrato y metano-sulfonato del estado de la técnica cercano.

En consecuencia, la persona del oficio normalmente versada en la materia, se vería suficientemente motivada de acuerdo a sus conocimientos comunes y lo divulgado en los documentos D3 o D4 para formar sales de ácido aspártico con el ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico, en lugar del ácido metano sulfónico o el ácido clorhídrico de D1 o D2, respectivamente, para llegar así al objeto de la reivindicación 1 de la

<sup>2</sup> Handbook of pharmaceutical salts. Properties, selection and use; Wiley-VCH; 2002; Chapter 7 "A procedure for salt selection and optimization"; pgs. 162-167; pgs 19 y 28; (fls 162 - 166).

<sup>3</sup> De acuerdo al documento D2, el compuesto de la invención es un antibiótico de la familia de las fluoroquinolonas (pg. 2261, fl 158, col. 1)

solicitud. Por lo cual se considera obvia.

Conclusión: La reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18) según lo divulgado en los documentos D3 con D1, D3 con D2, D4 con D1 o D4 con D2.

Puesto que las reivindicaciones dependientes 2 y 5 - 9 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

La reivindicación 3 consiste en un método para preparar la sal de ácido aspártico del ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico de la reivindicación 1, que comprende la etapa de hacer reaccionar ácido 1-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico con ácido aspártico en un disolvente.

Comparación de las características técnicas esenciales, con las del estado de la técnica:

| Características esenciales                                                                                                                                                                    | D1                                                                                                                                                                                                                                              | D3                                                                                                                                                                    | D4                                                                                                                                                                                                                     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Método para preparar la sal de ácido aspártico del ácido-ciclopropil-6-fluoro-7-(8 metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico, que comprende: | Método para preparar la sal de ácido metano sulfónico del ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico, que comprende: (ejemplo 1, pg. 25, ln 26 – pg. 26, ln 13) | Método para preparar la sal de ácido aspártico de ácido-1-ciclopropil-6-fluoro-1,4-dihidro-4-oxo-7-(1-piperazinil)-quinolina-3-carboxílico (ejemplo 42, col 13 – 16): | Método para preparar la sal de ácido aspártico de ácido-1-ciclopropil-6-fluoro-4-oxo-7-([1α,5α,6β]-6-amino-1-metil-3-azabicyclo[3.2.0]heptan-3-il)- 1,8-naftidrina-3-carboxílico (col. 4, ejemplo 8, col. 5, tabla 1): |
| Hacer reaccionar ácido 1-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico                                                 | Hacer reaccionar ácido 1-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico                                                                                                   | Hacer reaccionar ácido-1-ciclopropil-6-fluoro-1,4-dihidro-4-oxo-7-(1-piperazinil)-quinolina-3-carboxílico (Ciprofloxacina)                                            | Hacer reaccionar ácido-1-ciclopropil-6-fluoro-4-oxo-7-([1α,5α,6β]-6-amino-1-metil-3-azabicyclo[3.2.0]heptan-3-il)- 1,8-naftidrina-3-carboxílico                                                                        |
| Con ácido aspártico                                                                                                                                                                           | Con ácido metano-sulfónico                                                                                                                                                                                                                      | Con ácido aspártico                                                                                                                                                   | Con ácido aspártico                                                                                                                                                                                                    |
| En un disolvente                                                                                                                                                                              | En un disolvente                                                                                                                                                                                                                                | En un disolvente                                                                                                                                                      | En un disolvente                                                                                                                                                                                                       |

El documento D1 se considera el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulga el método para preparar la sal metano-sulfonato de ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico que comprende hacer reaccionar ácido 1-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico con ácido metano sulfónico en un disolvente como etanol.

La diferencia entre la invención, definida en la reivindicación 1 y el proceso de D1 consiste en que divulga de manera específica que la reacción del ácido 1-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico se hace con ácido aspártico.

Esta diferencia no parece conceder un efecto técnico inesperado al proceso reclamado en esta solicitud ya que no parece presentar ventajas superiores en comparación con los procesos del estado de la técnica cercano.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular como la necesidad de un procedimiento para la preparación de sales del ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico alternativo a los ya conocidos en el estado del arte.

Sin embargo, el proceso para la formación de sales con ácido aspártico ya esta divulgada en los documentos D3 y D4, en donde diferentes agentes antimicrobianos del tipo fluoroquinolona reaccionan con ácido aspártico en un disolvente como agua (D3: ejemplo 42, col 13 - 16; D4: col. 4, ejemplo 8).

En consecuencia, la persona del oficio normalmente versada en la materia, se vería suficientemente motivada para utilizar el ácido aspártico de acuerdo a sus conocimientos comunes y lo divulgado en los documentos D3 o D4 en el proceso de preparación de sales de ácido-ciclopropil-6-fluoro-7-(8-metoxiimino-2,6-diaza-espiro[3.4]oct-6-il)-4-oxo-1,4-dihidro-[1,8]naftidrina-3-carboxílico de D1, para llegar así al objeto de la reivindicación 3 de la solicitud. Por lo cual se considera obvia.

Conclusión: La reivindicación 3 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18) según lo divulgado en los documentos D3 con D1, o D4 con D1.

Puesto que la reivindicación dependiente 4 no menciona características inventivas adicionales tampoco se pueden considerar inventivas.

8. Conclusión

Los requisitos de patentabilidad de la presente Solicitud están afectados así:

| Item | Documento    | Reivindicaciones afectadas | Requisito que afecta |
|------|--------------|----------------------------|----------------------|
| D1   | WO99/00393   | 1 - 9                      | Nivel Inventivo      |
| D2   | Artículo     | 1 - 9                      | Nivel Inventivo      |
| D3   | US 5.563.149 | 1 - 9                      | Nivel Inventivo      |
| D4   | US 4.957.922 | 1 - 9                      | Nivel Inventivo      |

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



**JOSÉ LUIS SALAZAR LÓPEZ**  
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha 17/4 AGO. 2012

Elaborado por: Rubiela Pacanchique Vargas *OKU*  
Revisado por: Jacqueline Alfonso Rozo

## In Vitro and In Vivo Antibacterial Activities of DW-224a, a New Fluoronaphthyridone

Hee-Soo Park,<sup>1</sup> Hyun-Joo Kim,<sup>1</sup> Min-Jung Seol,<sup>1</sup> Dong-Rack Choi,<sup>2</sup> Eung-Chil Choi,<sup>3</sup>  
 and Jin-Hwan Kwak<sup>1\*</sup>

<sup>1</sup>School of Life and Food Sciences, Hanyang Global University, Kyungbuk, Republic of Korea<sup>1</sup>; Dong Wha Pharmaceutical Industry Ltd., Anyang City, Republic of Korea<sup>2</sup>; and College of Pharmacy, Seoul National University, Seoul, Republic of Korea<sup>3</sup>

Received 31 October 2005; Returned for modification 27 December 2005; Accepted 21 March 2006

**DW-224a showed the most potent in vitro activity among the quinolone compounds tested against clinical isolates of gram-positive bacteria. Against gram-negative bacteria, DW-224a was slightly less active than the other fluoroquinolones. The in vivo activities of DW-224a against gram-positive bacteria were more potent than those of other quinolones.**

The classic fluoroquinolones such as ciprofloxacin, norfloxacin, fleroxacin, and ofloxacin have shown strong activity against gram-negative bacteria, but the effectiveness of these compounds against gram-positive bacteria has been debated (1, 4). Recently, the emergence of multidrug-resistant gram-positive organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphylococci, trimethoprim-resistant *Streptococcus pneumoniae*, and vancomycin-resistant enterococci, has generated worldwide concern in the medical community (2, 5, 6, 11). Therefore, recent efforts have been directed toward the synthesis of novel quinolone compounds that can provide improved activities against gram-positive organisms yet retain the broad-spectrum activity of ciprofloxacin (1, 4, 7).

DW-224a is a new group of fluoroquinolone antibiotics with the formula {1-cyclopropyl-6-fluoro-7-[8-(methoxyimino)-2,6-dihydrospiro[3,4]oct-6-yl]-4-oxo-1,4-dihydro[1,8]naphthyridine-3-carboxylic acid hydrochloride} (Fig. 1), synthesized by the research laboratory of Dong Wha Pharmaceutical Industry, Ltd., Anyang City, Republic of Korea.

In this study, we compared the in vitro activities of DW-224a with those of ciprofloxacin, moxifloxacin, and gemifloxacin against clinical isolates. The comparative in vivo efficacy of DW-224a against systemic bacterial infections in mice was also evaluated.

This work was presented in part at the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., 14 to 18 September 2003 [J. H. Kwak et al., abstr. P-1515].

The studied compounds were obtained as follows. DW-224a, ciprofloxacin, moxifloxacin, and gemifloxacin were synthesized at the R & D Center, Dong Wha Pharmaceutical Industry, Ltd., Anyang City, Republic of Korea. Oxacillin was purchased from Sigma Aldrich Korea. Test organisms used in this study were originally isolated from human clinical specimens. Fecal samples were obtained from general hospitals in Seoul, Re-

public of Korea, between 2001 and 2004. The challenge organisms used in the mouse systemic infections were obtained as follows: *Streptococcus pneumoniae* ATCC 6305 was obtained from the Korean Culture Center of Microorganisms, Seoul, Republic of Korea; *Staphylococcus aureus* Giorgio, *Escherichia coli* 851E, and *Morganella morganii* 1375E were kindly provided by LG Life Sciences Ltd., Daejeon, Republic of Korea (10); and *Klebsiella pneumoniae* P427, *Streptococcus pyogenes* B144, and MRSA strain P197 were selected through the screening of clinical isolates.

In vitro MICs were determined by an agar dilution method as described by the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) (8). Mueller-Hinton agar medium was used for testing aerobic and facultative organisms. *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Moraxella catarrhalis* were grown on Mueller-Hinton agar supplemented with 5% defibrinated sheep blood (Hankuk Media Ltd., Gunpo City, Republic of Korea). Mueller-Hinton agar supplemented with 3% Fildes enrichment (Oxoid Ltd., Basingstoke, Hampshire, England) was used for *Haemophilus influenzae*. GCII agar (Difco) supplemented with 1% IsoVitaleX (Becton Dickinson) was used for *Neisseria gonorrhoeae*. Test strains were grown for 18 h and diluted with the same fresh medium to a density of approximately  $10^7$  CFU/ml. These test strains were applied with a multipip inoculator to agar plates containing serial dilutions of the antimicrobial agents, yielding  $10^4$  CFU per spot. Plates were incubated at 37°C for 18 h and examined for growth. *Neisseria gonorrhoeae* was incubated under 5% CO<sub>2</sub>. The MIC was considered to be the lowest concentration that completely

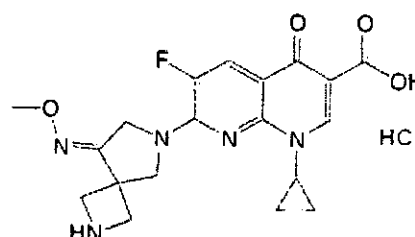


FIG. 1. Chemical structure of DW-224a.

\*Corresponding author. Mailing address: School of Life and Food Sciences, Hanyang Global University, Pok-ku, Pohang City, Kyungbuk 712-708, Republic of Korea. Phone: 82-54-260-1353. Fax: 82-54-260-2135. E-mail: jhkwak@hanyang.edu

TABLE 1. In vitro antibacterial activities of DW-224a and quinolones against clinical isolates

| Microorganism <sup>a</sup><br>(no. of isolates) | Antimicrobial<br>agent | MIC (µg/ml)  |        |        |
|-------------------------------------------------|------------------------|--------------|--------|--------|
|                                                 |                        | Range        | 50%    | 90%    |
| <i>Staphylococcus aureus</i> (20)               | DW-224a                | 0.005–0.06   | 0.015  | 0.03   |
|                                                 | Ciprofloxacin          | 0.06–6       | 0.25   | 0.5    |
|                                                 | Moxifloxacin           | 0.015–0.25   | 0.06   | 0.06   |
|                                                 | Gemifloxacin           | 0.005–0.06   | 0.015  | 0.03   |
| <i>Staphylococcus epidermidis</i> (20)          | DW-224a                | 0.008–32     | 2      | 4      |
|                                                 | Ciprofloxacin          | 0.125–>64    | 32     | >64    |
|                                                 | Moxifloxacin           | 0.03–>64     | 4      | 16     |
|                                                 | Gemifloxacin           | 0.008–64     | 2      | 4      |
| <i>Staphylococcus saprophyticus</i> (14)        | DW-224a                | 0.008–0.25   | 0.015  | 0.125  |
|                                                 | Ciprofloxacin          | 0.125–2      | 0.125  | 2      |
|                                                 | Moxifloxacin           | 0.03–2       | 0.125  | 0.25   |
|                                                 | Gemifloxacin           | 0.008–0.25   | 0.015  | 0.25   |
| <i>Staphylococcus sciuri</i> (18)               | DW-224a                | 0.008–4      | 0.25   | 2      |
|                                                 | Ciprofloxacin          | 0.06–64      | 8      | 32     |
|                                                 | Moxifloxacin           | 0.06–16      | 2      | 8      |
|                                                 | Gemifloxacin           | 0.008–8      | 0.25   | 1      |
| <i>Enterococcus faecalis</i> (10)               | DW-224a                | 0.008–4      | 0.125  | 2      |
|                                                 | Ciprofloxacin          | 0.125–64     | 2      | 64     |
|                                                 | Moxifloxacin           | 0.08–64      | 2      | 32     |
|                                                 | Gemifloxacin           | 0.008–16     | 0.125  | 4      |
| <i>Enterococcus faecium</i> (22)                | DW-224a                | 2–32         | 2      | 16     |
|                                                 | Ciprofloxacin          | 1–64         | 8      | 64     |
|                                                 | Moxifloxacin           | 0.5–64       | 8      | 64     |
|                                                 | Gemifloxacin           | 0.5–>64      | 2      | 32     |
| <i>Enterobacter cloacae</i> (16)                | DW-224a                | 0.125–4      | 0.5    | 4      |
|                                                 | Ciprofloxacin          | 0.5–4        | 4      | 4      |
|                                                 | Moxifloxacin           | 0.125–4      | 2      | 4      |
|                                                 | Gemifloxacin           | 0.015–2      | 0.7    | 2      |
| <i>Enterobacter aerogenes</i> (12)              | DW-224a                | 0.015–0.06   | 0.015  | 0.03   |
|                                                 | Ciprofloxacin          | 1–8          | 2      | 8      |
|                                                 | Moxifloxacin           | 0.015–0.5    | 0.5    | 0.5    |
|                                                 | Gemifloxacin           | 0.06–0.25    | 0.125  | 0.25   |
| <i>Enterobacter coli</i> (15)                   | DW-224a                | 0.015–0.75   | 0.03   | 0.06   |
|                                                 | Ciprofloxacin          | 0.5–4        | 1      | 2      |
|                                                 | Moxifloxacin           | 0.125–0.5    | 0.25   | 0.25   |
|                                                 | Gemifloxacin           | 0.015–0.125  | 0.03   | 0.125  |
| <i>Enterobacter cloacae</i> (15)                | DW-224a                | 0.015–64     | 0.06   | 1      |
|                                                 | Ciprofloxacin          | 0.008–64     | 0.015  | 0.5    |
|                                                 | Moxifloxacin           | 0.03–32      | 0.06   | 0.5    |
|                                                 | Gemifloxacin           | 0.008–16     | 0.015  | 0.25   |
| <i>Enterobacter cloacae</i> (148)               | DW-224a                | 0.008–8      | 0.06   | 2      |
|                                                 | Ciprofloxacin          | 0.008–2      | 0.015  | 0.5    |
|                                                 | Moxifloxacin           | 0.008–4      | 0.06   | 1      |
|                                                 | Gemifloxacin           | 0.008–2      | 0.015  | 0.5    |
| <i>Enterobacter cloacae</i> (136)               | DW-224a                | 0.03–1       | 0.125  | 0.25   |
|                                                 | Ciprofloxacin          | 0.008–0.25   | 0.015  | 0.06   |
|                                                 | Moxifloxacin           | 0.008–1      | 0.06   | 0.125  |
|                                                 | Gemifloxacin           | 0.008–0.5    | 0.06   | 0.125  |
| <i>Enterobacter cloacae</i> (22)                | DW-224a                | 0.06–8       | 0.125  | 1      |
|                                                 | Ciprofloxacin          | 0.015–2      | 0.06   | 0.5    |
|                                                 | Moxifloxacin           | 0.03–4       | 0.25   | 1      |
|                                                 | Gemifloxacin           | 0.015–2      | 0.06   | 1      |
| <i>Acinetobacter baumannii</i> (2)              | DW-224a                | 0.03–1       | 0.125  | 0.25   |
|                                                 | Ciprofloxacin          | 0.015–0.25   | 0.03   | 0.06   |
|                                                 | Moxifloxacin           | 0.015–0.25   | 0.03   | 0.06   |
|                                                 | Gemifloxacin           | 0.015–0.25   | 0.03   | 0.06   |
| <i>Citrobacter freundii</i> (13)                | DW-224a                | 0.008–16     | 0.06   | 1      |
|                                                 | Ciprofloxacin          | 0.008–2      | 0.015  | 0.25   |
|                                                 | Moxifloxacin           | 0.015–8      | 0.125  | 2      |
|                                                 | Gemifloxacin           | 0.008–4      | 0.03   | 0.5    |
| <i>Morganella morganii</i> (38)                 | DW-224a                | 0.008–4      | 0.25   | 0.5    |
|                                                 | Ciprofloxacin          | 0.008–0.5    | 0.015  | 0.03   |
|                                                 | Moxifloxacin           | 0.008–4      | 0.25   | 0.25   |
|                                                 | Gemifloxacin           | 0.008–2      | 0.03   | 0.125  |
| <i>Proteus mirabilis</i> (21)                   | DW-224a                | 0.125–64     | 0.25   | 2      |
|                                                 | Ciprofloxacin          | 0.015–2      | 0.015  | 0.06   |
|                                                 | Moxifloxacin           | 0.125–16     | 0.25   | 0.5    |
|                                                 | Gemifloxacin           | 0.125–32     | 0.125  | 0.25   |
| <i>Proteus vulgaris</i> (18)                    | DW-224a                | 0.125–1      | 0.25   | 0.5    |
|                                                 | Ciprofloxacin          | 0.008–0.5    | 0.015  | 0.125  |
|                                                 | Moxifloxacin           | 0.125–1      | 0.25   | 0.5    |
|                                                 | Gemifloxacin           | 0.03–0.25    | 0.06   | 0.125  |
| <i>Serratia marcescens</i> (39)                 | DW-224a                | 0.25–64      | 2      | 32     |
|                                                 | Ciprofloxacin          | 0.06–8       | 1      | 4      |
|                                                 | Moxifloxacin           | 0.008–16     | 1      | 4      |
|                                                 | Gemifloxacin           | 0.125–16     | 2      | 8      |
| <i>Salmonella enterica</i> serovar Typhi (13)   | DW-224a                | 0.06–0.06    | 0.06   | 0.06   |
|                                                 | Ciprofloxacin          | 0.03–0.125   | 0.125  | 0.125  |
|                                                 | Moxifloxacin           | 0.015–0.06   | 0.03   | 0.03   |
|                                                 | Gemifloxacin           | 0.06–0.125   | 0.06   | 0.125  |
| <i>Acinetobacter baumannii</i> (37)             | DW-224a                | 0.008–8      | 0.25   | 4      |
|                                                 | Ciprofloxacin          | 0.008–64     | 0.125  | 16     |
|                                                 | Moxifloxacin           | 0.015–8      | 0.03   | 4      |
|                                                 | Gemifloxacin           | 0.008–8      | 0.125  | 4      |
| <i>Acinetobacter calcoaceticus</i> (8)          | DW-224a                | 0.008–1      | 0.008  | 0.06   |
|                                                 | Ciprofloxacin          | 0.015–1      | 0.25   | —      |
|                                                 | Moxifloxacin           | 0.008–1      | 0.125  | —      |
|                                                 | Gemifloxacin           | 0.008–0.5    | 0.03   | —      |
| <i>Stenotrophomonas maltophilia</i> (27)        | DW-224a                | 0.06–64      | 0.5    | 1      |
|                                                 | Ciprofloxacin          | 0.015–16     | 0.5    | 2      |
|                                                 | Moxifloxacin           | 0.03–64      | 0.25   | 1      |
|                                                 | Gemifloxacin           | 0.015–32     | 0.25   | 1      |
| <i>Haemophilus influenzae</i> (15)              | DW-224a                | <0.008–0.008 | <0.008 | 0.008  |
|                                                 | Ciprofloxacin          | <0.008       | <0.008 | <0.008 |
|                                                 | Moxifloxacin           | 0.008–0.015  | 0.008  | 0.015  |
|                                                 | Gemifloxacin           | <0.008       | <0.008 | <0.008 |
| <i>Moraxella catarrhalis</i> (20)               | DW-224a                | <0.008–0.03  | 0.015  | 0.03   |
|                                                 | Ciprofloxacin          | <0.008–0.06  | 0.03   | 0.06   |
|                                                 | Moxifloxacin           | 0.015–0.06   | 0.06   | 0.06   |
|                                                 | Gemifloxacin           | <0.008–0.03  | <0.008 | 0.015  |
| <i>Neisseria gonorrhoeae</i> (27)               | DW-224a                | 0.015–2      | 1      | 1      |
|                                                 | Ciprofloxacin          | 0.03–16      | 8      | 8      |
|                                                 | Moxifloxacin           | 0.03–4       | 2      | 2      |
|                                                 | Gemifloxacin           | 0.008–2      | 1      | 1      |
| <i>Pseudomonas aeruginosa</i> (22)              | DW-224a                | <0.008–64    | 8      | >64    |
|                                                 | Ciprofloxacin          | 0.03–64      | 2      | 64     |
|                                                 | Moxifloxacin           | 0.03–>64     | 64     | >64    |
|                                                 | Gemifloxacin           | <0.008–64    | 2      | 64     |

<sup>a</sup> MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; MSSSE, methicillin-susceptible enterococci; MRSE, methicillin-resistant enterococci; MSSNS, methicillin-susceptible coagulase-negative staphylococci; MRNS, methicillin-resistant coagulase-negative staphylococci; VRE, vancomycin-resistant enterococci; MSSGN, methicillin-susceptible gram-negative bacilli; MRGN, methicillin-resistant gram-negative bacilli.

TABLE 2. Comparative in vivo activities of DW-224a against systemic infections in mice

| Microorganism inoculum (CFU/mouse)                    | Mucin (%) | Antimicrobial agent <sup>a</sup> | MIC (µg/ml) | ED <sub>50</sub> (mg/kg) (95% confidence limits) |
|-------------------------------------------------------|-----------|----------------------------------|-------------|--------------------------------------------------|
| <i>S. aureus</i> Giorgio (1 × 10 <sup>6</sup> )       | 5         | DW-224a                          | <0.008      | 1.11 (0.17–4.64)                                 |
|                                                       |           | Ciprofloxacin                    | 0.125       | 16.52 (1.27–18.99)                               |
|                                                       |           | Moxifloxacin                     | 0.03        | 1.78 (0.33–7.66)                                 |
|                                                       |           | Gemifloxacin                     | <0.008      | 1.37 (0.27–7.67)                                 |
| MRSA P107 (5 × 10 <sup>6</sup> )                      | 5         | DW-224a                          | 0.25        | 15.52 (9.02–26.58)                               |
|                                                       |           | Ciprofloxacin                    | 8           | >40                                              |
|                                                       |           | Moxifloxacin                     | 1           | 35.35 (20.38–61.33)                              |
|                                                       |           | Gemifloxacin                     | 0.25        | 26.58 (15.03–47.28)                              |
| <i>S. pneumoniae</i> ATCC 6305 (1 × 10 <sup>5</sup> ) |           | DW-224a                          | 0.008       | 0.53 (0.0029–1.67)                               |
|                                                       |           | Ciprofloxacin                    | 0.5         | 10.46 (3.56–32.26)                               |
|                                                       |           | Moxifloxacin                     | 0.06        | 2.00 (0.41–8.21)                                 |
|                                                       |           | Gemifloxacin                     | 0.015       | 0.76 (0.15–2.43)                                 |
| <i>S. pneumoniae</i> 1375F (2 × 10 <sup>5</sup> )     | 5         | DW-224a                          | 0.06        | 2.65 (0.65–13.73)                                |
|                                                       |           | Ciprofloxacin                    | 4           | >40                                              |
|                                                       |           | Moxifloxacin                     | 0.25        | 9.04 (2.16–73.6)                                 |
|                                                       |           | Gemifloxacin                     | 0.06        | 2.83 (0.37–15.24)                                |
| <i>S. pneumoniae</i> 1375F (1 × 10 <sup>4</sup> )     | 5         | DW-224a                          | 0.03        | 6.08 (1.64–38.80)                                |
|                                                       |           | Ciprofloxacin                    | 0.008       | 0.78 (0.10–3.20)                                 |
|                                                       |           | Moxifloxacin                     | 0.06        | 2.17 (0.61–7.73)                                 |
|                                                       |           | Gemifloxacin                     | 0.015       | 1.16 (0.25–4.13)                                 |
| <i>K. pneumoniae</i> P427 (5 × 10 <sup>5</sup> )      | 5         | DW-224a                          | 0.06        | 9.77 (2.77–36.20)                                |
|                                                       |           | Ciprofloxacin                    | 0.06        | 0.70 (0.00–2.59)                                 |
|                                                       |           | Moxifloxacin                     | 0.25        | 2.64 (0.36–18.68)                                |
|                                                       |           | Gemifloxacin                     | 0.03        | 5.36 (1.42–20.20)                                |
| <i>E. coli</i> 1375F (2 × 10 <sup>6</sup> )           | 5         | DW-224a                          | 0.125       | 4.83 (0.49–26.77)                                |
|                                                       |           | Ciprofloxacin                    | 0.008       | 1.41 (0.17–5.91)                                 |
|                                                       |           | Moxifloxacin                     | 0.125       | 5.28 (1.50–18.45)                                |
|                                                       |           | Gemifloxacin                     | 0.06        | 3.41 (0.77–12.48)                                |

<sup>a</sup> Antibiotics were orally administered at 1 and 4 h after infection.

<sup>b</sup> Bacterial suspensions were prepared in 0.9% saline solution containing 5% gastric mucin, except for *S. pneumoniae* ATCC 6305, which was suspended in 0.9% saline solution.

deficient growth on agar plates, disregarding a single colony or faint haze caused by the inoculum.

The in vivo activity of DW-224a against systemic infections in mice was determined. Four-week-old male ICR mice weighing 18 to 22 g (Daehan Bio Link Co., Ltd., Eum-sung Gun, Republic of Korea) were used for the systemic infection model. Mice were maintained in animal rooms kept at  $23 \pm 2^\circ\text{C}$  with 50 to 70% relative humidity. Test organisms for infection were cultured in tryptic soy agar medium (Difco) at  $37^\circ\text{C}$  for 24 h. For *S. pneumoniae* and *S. pyogenes*, tryptic soy agar medium was supplemented with 5% defibrinated sheep blood. For use as inocula, bacterial strains were suspended in 0.9% saline solution containing 5% gastric mucin (Difco), except for *S. pneumoniae* ATCC 6305, which was suspended in 0.9% saline solution. Mice were used in groups of six for each inoculum and were challenged intraperitoneally with a single 0.5-ml portion of the bacterial suspension, corresponding to an inoculum range of 10 to 100 times the minimal lethal dose of bacteria. Four dose levels were used for each antibiotic, depending on the in vitro antimicrobial activity of the compound. Antibiotics at various dose regimens were orally administered to mice twice, at 1 and 4 h postinfection. Mortality was recorded for 7 days, and the median effective dose needed to protect 50% of the mice (ED<sub>50</sub>) was calculated by the Probit method (3). The challenge inoculum

was sufficient to kill 100% of the untreated control mice, which died within 48 h postinfection.

The in vitro efficacy of DW-224a against the clinical isolates is presented in Table 1. DW-224a was slightly superior to gemifloxacin and much more active than moxifloxacin and ciprofloxacin against clinical isolates of gram-positive bacteria. Against methicillin-sensitive *S. aureus* and MRSA, the antibacterial activity of DW-224a was comparable to that of gemifloxacin but 2- to 16-fold more potent than those of moxifloxacin and ciprofloxacin. The MIC<sub>50</sub>s of DW-224a against methicillin-sensitive *S. aureus* and MRSA were 0.03 µg/ml and 4 µg/ml, respectively. DW-224a was also more active than ciprofloxacin and moxifloxacin and was slightly superior to gemifloxacin against *Staphylococcus epidermidis* (MIC<sub>50</sub>, 0.125 µg/ml), *S. pyogenes* (MIC<sub>50</sub>, 0.06 µg/ml), *Enterococcus faecalis* (MIC<sub>50</sub>, 2 µg/ml), and *Enterococcus faecium* (MIC<sub>50</sub>, 16 µg/ml). Against *S. pneumoniae*, the activity of DW-224a (MIC<sub>50</sub>, 0.03 µg/ml) was 8-fold higher than that of gemifloxacin and at least 16-fold higher than those of moxifloxacin and ciprofloxacin. Among the compounds tested, DW-224a showed the most potent antibacterial activities against gram-positive bacteria. Against most members of the family *Enterobacteriaceae*, the activity of DW-224a was twofold or fourfold lower than those of ciprofloxacin and gemifloxacin, respectively, but it was com-

parable to that of moxifloxacin. DW-224a inhibited 90% of the *E. coli*, *K. pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Enterobacter aerogenes*, and *Morganella morganii* isolates at concentrations of 1, 1.0, 25, 2.0, 25, and 0.5  $\mu\text{g/ml}$ , respectively. Against *Acinetobacter baumannii* (MIC<sub>90</sub>, 4  $\mu\text{g/ml}$ ), *Acinetobacter baumannii* (MIC<sub>90</sub>, 0.008  $\mu\text{g/ml}$ ), and *Stenotrophomonas maltophilia* (MIC<sub>90</sub>, 1  $\mu\text{g/ml}$ ), DW-224a showed an activity comparable to the other compounds. DW-224a was 1/250 active against *H. influenzae* (MIC<sub>90</sub>, 0.008  $\mu\text{g/ml}$ ) and *M. catarrhalis* (MIC<sub>90</sub>, 0.03  $\mu\text{g/ml}$ ). Against *V. gonorrhoeae*, DW-224a (MIC<sub>90</sub>, 1  $\mu\text{g/ml}$ ) was eightfold more active than ciprofloxacin. The activity of DW-224a against *Pseudomonas aeruginosa* (MIC<sub>90</sub>, 64  $\mu\text{g/ml}$ ) was inferior to those of ciprofloxacin and gemifloxacin, but it was comparable to that of moxifloxacin.

The protective efficacy of DW-224a against systemic infections in mice was compared with those of ciprofloxacin, moxifloxacin, and gemifloxacin (Table 2). DW-224a exhibited the most potent protective effects against systemic infections caused by gram-positive bacteria. Against infection caused by *S. aureus* 3090 the ED<sub>50</sub>s of DW-224a, ciprofloxacin, moxifloxacin, and gemifloxacin were 1.11, 16.52, 1.78, and 1.37 mg/kg of body weight, respectively. Especially against quinolone-resistant *S. aureus* P197 (an MRSA), DW-224a (ED<sub>50</sub>, 0.52 mg/kg) was more effective than ciprofloxacin (ED<sub>50</sub>, 4.0 mg/kg), moxifloxacin (ED<sub>50</sub>, 35.35 mg/kg), and gemifloxacin (ED<sub>50</sub>, 16.58 mg/kg). DW-224a was also the most effective drug against infections caused by streptococci, including *S. pneumoniae* and *S. pyogenes* strains, for which the ED<sub>50</sub>s were 0.53 and 0.63 mg/kg, respectively. But, DW-224a was shown to be less effective than the test compounds against infections caused by gram-negative organisms. Against *E. coli* 851E, DW-224a (ED<sub>50</sub>, 6.08 mg/kg) was less active than ciprofloxacin (ED<sub>50</sub>, 0.78 mg/kg), moxifloxacin (ED<sub>50</sub>, 2.17 mg/kg), and gemifloxacin (ED<sub>50</sub>, 1.16 mg/kg). Against *K. pneumoniae* P427, the ED<sub>50</sub>s of DW-224a, ciprofloxacin, moxifloxacin, and gemifloxacin were 9.77, 0.70, 2.64, and 5.36 mg/kg, respectively. DW-224a (ED<sub>50</sub>, 4.83 mg/kg) was less active than ciprofloxacin (ED<sub>50</sub>, 0.41 mg/kg) and gemifloxacin (ED<sub>50</sub>, 3.41 mg/kg) against *M. morganii* 1375E but more active than moxifloxacin (ED<sub>50</sub>, 5.28 mg/kg). In general, the ED<sub>50</sub>s of DW-224a were well correlated with in vitro MICs.

DW-224a demonstrated potent in vitro and in vivo antibacterial activities against both gram-positive and gram-negative bacteria. The overall antibacterial activities of DW-224a against gram-positive pathogens, such as MRSA, methicillin-resistant coagulase-negative staphylococci, *S. pneumoniae*, *S. aureus*, and *E. faecalis*, were more potent than those of the reference compounds, such as ciprofloxacin, moxifloxacin, and gemifloxacin. DW-224a especially showed increased potency against *S. pneumoniae*, which is the bacterial strain most frequently isolated from patients with community-acquired pneumonia and continues to be a significant cause of mortality. However, DW-224a was slightly less active than other fluoro-

quinolones against members of the family *Enterobacteriaceae*. It is a general trend that the improving antibacterial activities of the fluoroquinolones against gram-positive bacteria is likely associated with a relative decrease in activity against gram-negative bacteria, but DW-224a was very active against gram-negative respiratory pathogens such as strains of *H. influenzae* and *M. catarrhalis*, known as clinically significant pathogens. These findings suggest that DW-224a, with its expanded antipneumococcal activity, may be very useful for the treatment of community-acquired respiratory tract infections, because  $\beta$ -lactam resistance in *H. influenzae* and *M. catarrhalis* is a documented problem in the community (9).

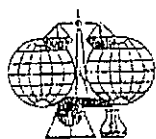
DW-224a at two times the MIC had very rapid bactericidal activity against *S. pneumoniae* and *S. aureus*, regrowth after 24 h of incubation was not detected, and the minimal bactericidal concentrations of DW-224a were twofold higher than the MICs for all test strains (J. Kwak, M. J. Seol, H. Kim, H. Park, D. Choi, and Y. Jung, Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-1947, 2004).

In view of its improved antibacterial activities against gram-positive bacteria and good pharmacokinetic profiles in animals, the clinical usefulness of DW-224a should be established by further studies.

This study was supported by a grant from the Korea Health 21 R & D Project, Ministry of Health and Welfare, Republic of Korea (01-PJ1-PG4-01PT01-0013).

## REFERENCES

- Ball, P. 2003. Adverse drug reactions: implications for the development of fluoroquinolones. *J. Antimicrob. Chemother.* 51(Suppl. S1):21-27.
- Bell, J. M., J. D. Turnidge, and SENTRY Antimicrobial Surveillance Participants. 2002. High prevalence of oxacillin-resistant *Staphylococcus aureus* isolates from hospitalized patients in Asia-Pacific and South Africa: results from SENTRY Antimicrobial Surveillance Program, 1998-1999. *Antimicrob. Agents Chemother.* 46:879-881.
- Bliss, C. I. 1985. *Statistics in bioassay*. Academic Press, Inc., New York, N.Y.
- Emmerson, A. M., and A. M. Jones. 2003. The quinolones: decades of development and use. *J. Antimicrob. Chemother.* 51(Suppl. S1):13-20.
- Endtz, H. P., N. van den Braak, H. A. Verbrugg, and A. van Belkum. 1999. Vancomycin resistance: status quo and quo vadis. *Eur. J. Clin. Microbiol. Infect. Dis.* 18:683-690.
- Levy, S. B., and B. Marshall. 2004. Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.* 10:s122-s129.
- Lode, H., and M. Allewell. 2002. Role of newer fluoroquinolones in lower respiratory tract infections. *J. Antimicrob. Chemother.* 49:709-712.
- National Committee for Clinical Laboratory Standards. 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6, 6th ed., vol. 23. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- Nilfus, A. M., L. L. Shen, D. Hensley-Rudloff, L. S. Ahmer, J. M. Beyer, D. J. Balli, Y. Cai, and R. K. Flamm. 2003. In vitro antibacterial potency and spectrum of ABT-492, a new fluoroquinolone. *Antimicrob. Agents Chemother.* 47:3260-3269.
- Oh, J.-I., K.-S. Paek, M.-J. Ahn, M.-Y. Kim, C. Y. Hong, I.-C. Kim, and J.-H. Kwak. 1996. In vitro and in vivo evaluations of LB20304, a new fluoronaphthyridone. *Antimicrob. Agents Chemother.* 40:1564-1568.
- Song, J.-H., S.-I. Jung, K. S. Ko, N. Y. Kim, J. S. Son, H.-H. Chang, H. K. Ki, W. S. Oh, J. Y. Suh, K. R. Peck, N. Y. Lee, Y. Yang, Q. Lu, A. Chongthaleong, C.-H. Chiu, M. K. Lalitha, J. Perera, T. T. Yee, G. Kumarasinghe, F. Jamal, A. Kamarulzaman, N. Parasakthi, P. H. Vnn, C. Carlos, T. So, T. K. Ng, and A. Shibli. 2004. High prevalence of antimicrobial resistance among clinical *Staphylococcus pneumoniae* isolates in Asia (an ANSORP study). *Antimicrob. Agents Chemother.* 48:2101-2107.



International Union  
of Pure and Applied Chemistry (IUPAC)

Handbook of  
Pharmaceutical Salts  
Properties, Selection, and Use

P. Heinrich Stahl, Camille G. Wermuth (Eds.)



Verlag Helvetica Chimica Acta · Zürich



WILEY-VCH

Weinheim · New York · Chichester  
Brisbane · Singapore · Toronto

mation. Further confirmation of the nature of the excess solid phase may be obtained by powder X-ray diffractometry or elemental analysis. If the excess solid phase is entirely in the free form, the experiment must be repeated with a higher solid to solvent ( $H_2O$ ) ratio until the excess solid contains the salt. In some cases, complete conversion of the salt to the free form may occur at all solid to solution ratios, often leading to formation of a 'gel'.

As a result of the above phenomenon, in some cases, an attempt to make a solution of a 1:1 salt at a concentration well below the solubility of the salt (as estimated from the  $K_{sp}$  value) will be unsuccessful due to precipitation of the free unionized form. To further develop this point Anderson and Flora [2] defined the term 'stoichiometric solubility'. The stoichiometric solubility of a 1:1 salt is the concentration of salt which can be dissolved in pure  $H_2O$  with no formation of a precipitate of the unionized free form. The authors pointed out that the difficulties of low 'apparent solubility' of a salt due to free form precipitation may be encountered in the preclinical stages of development when concentrated solutions in  $H_2O$  are required for toxicology testing. However, appropriate pH adjustment should overcome this problem.

### 3. Dissolution Behavior of Acids, Bases, and Their Salts

The term *dissolution* refers to the overall process by which a solid compound dissolves in a liquid medium, while *dissolution rate* is the kinetic descriptor giving the rate at which the dissolution takes place. The concept of solubility, on the other hand, implies that the process of dissolution has reached equilibrium, and the solution is saturated. Dissolution rate of solids is of paramount importance in the development of pharmaceutical products and quality control. Salt formation is one of the most commonly employed techniques to improve dissolution of weakly acidic or basic drugs.

A number of theories of the dissolution of solids have been proposed [17] [29] [30]. However, the simple diffusion model may be adequate to describe the dissolution behavior of most pharmaceutical solids in aqueous and non-aqueous media. The diffusion layer model (Fig. 7) assumes that a thin film of saturated solution of concentration  $c_s$  exists at the interface of the dissolving solid and the dissolution medium. The dissolution rate is controlled by the diffusion rate of solute molecules from this thin saturated film into the bulk solution. The concentration of the solute in the bulk is denoted by  $c_0$ . As the distance  $x$  increases from  $x=0$  (at the surface of the solid) to  $x=h$  (at the beginning of the bulk solution), the concentration decreases from  $c=c_s$  to  $c=c_0$ . Beyond  $x=h$ , the bulk concentration remains uniform at  $c_0$ . In the simplest form of the theory, following Fick's First Law, the rate of dissolu-

## Chapter 2

### Solubility and Dissolution of Weak Acids, Bases, and Salts

by Madhu Pudipeddi\*, Abu T. M. Serajuddin, David J. W. Grant,  
and P. Heinrich Stahl

#### Contents

1. Introduction
2. Solubility Behavior of Acids, Bases, and Their Salts
  - 2.1. Measurement of Aqueous Solubility
  - 2.2. General Features of pH-Solubility Profiles
  - 2.3.  $pH_{max}$
  - 2.4. Difficulties in Determination of Salt Solubility
3. Dissolution Behavior of Acids, Bases, and Their Salts
  - 3.1. Measurement of Dissolution Rate
  - 3.2. Dissolution into Reactive Media
4. Relevance of pH Relations of Solubility and Dissolution to Salt Selection and Formulation
  - 4.1. Solution Formulations
  - 4.2. Solid Formulations
5. Non-Ideal Solubility Behavior

#### REFERENCES

#### 1. Introduction

Improvement of dissolution rate of weakly acidic or weakly basic drugs that are poorly soluble is one of the primary reasons for preparation of pharmaceutical salt forms [1-7]. Salt forms have also been used to control drug dissolution [8] [9]. This chapter examines the pH-dependencies of the solubility and dissolution rate of pharmaceutical acids, bases, and salts, and the relevance of these dependencies to the selection of the final form.

- 4.3.5. Basic Physicochemical Properties of the Hydromorphone RPR30763F  
4.3.6. Discussion of Results

5. Optimization - Second Phase: Drug Substance Form for Practical Development  
6. Optimization - Third Phase: Drug-Substance Form for Clinical Development  
7. Optimization - Fourth Phase: Drug-Substance Form for Marketing  
REFERENCES

## 1. Introduction

Until ca. 15 years ago the drug discovery processes adopted by pharmaceutical companies involved the screening of large numbers of compounds that had been synthesized by the company's research laboratories over many years. Alternative sources of compounds were also available to some companies from university laboratories, or as extracts from a multitude of natural sources. In the last 15 years, there has been a rapid change towards the use of combinatorial-chemistry techniques which are now being used routinely to prepare many thousands of compounds per year. As described in *Chapt. 6*, these compounds are generally dissolved in dimethyl sulfoxide (DMSO) to enable screening for activity in an enzyme- or receptor-based assay system. Initial screening usually involves a highly automated 'high throughput screen' (HTS). If the number of 'hits' found is large, the numbers are usually refined by a more involved and specific 'low throughput screen' (LTS) and other selection processes, until a manageable number of 'leads' is available. Often, several of these 'leads' show only moderate activity, and further structural refinement and optimization is invariably necessary, using a mixture of computational techniques and intuition by the chemistry team, until a smaller number (usually 1-5) of highly active potential 'candidates' remain.

These 'candidates' are usually relatively impure free bases, free acids, or neutral molecules, and are often amorphous. Because they are dissolved and stored in DMSO prior to being used in screening procedures, there is no need to ensure that they are crystalline. Also, the preparation of any salts of these compounds is rarely necessary as the neutral/covalent molecule usually exhibits better solubility in both DMSO and the assay system to be used by the pharmacology group. As can be seen readily, these new procedures favor lipophilic molecules, whereas the majority of marketed drug substances are hydrophilic. The need for water-soluble candidates for use in drug development and the vast majority of dosage forms has been recognized [1-4] for many years before the advent of 'combinatorial chemistry'.

Because drug substances are designed to bind with a receptor, enzyme, protein etc., they normally possess several functional groups capable of hydrogen bonding. Some of these functional groups may give potential for salt

formation and thus increased hydrophilicity. This salt formation also gives the pharmaceutical chemist and formulation scientist the opportunity to modify the physicochemical (e.g., melting point, hygroscopicity, chemical stability, dissolution rate, solution pH, crystal form etc.) and mechanical (e.g., hardness, elasticity etc.) characteristics of the potential drug substance and to develop dosage forms with good bioavailability, stability, manufacturability, and patient compliance.

For weakly basic drug substances, salts of an inorganic acid (e.g., hydrochloride, sulfate or phosphate), a sulfonic acid (mesylate, esylate, or isethionate), a carboxylic acid (acetate, maleate, or fumarate), a hydroxy acid (citrate or tartrate), or possibly an amino acid (arginine or lysine) could be considered. In the past, hydrochloride salts have often been the first choice for weakly basic drugs, since, as a consequence of the low counter-ion  $pK_a$ , salts can nearly always be formed, and recrystallization from organic solvents is normally straightforward. However, the potential disadvantages of hydrochloride salts include unacceptably high acidity in formulations (e.g., parenteral products), the risk of corrosion of manufacturing plant and equipment, less than optimal solubility due to the risk of salting out, and the potential for poor stability, if the drug is acid labile and hygroscopic [2].

Historically, prior to the severe regulatory constraints that abound today, it had been possible to develop the neutral species (free acid or free base) together with one or more different salts. These different chemical forms were often used for specific purposes. An excellent example of this concerns the phenothiazine drug substance, prochlorperazine. The free base was used in suppositories, as it was the most lipophilic form, the mesylate salt was used in injections and in sachets for the preparation of an oral solution, whilst the less soluble maleate was used for the manufacture of tablets.

Occasionally, salts were also prepared in former times to decrease drug substance solubility for use in suspension formulations where very low solubility is necessary to prevent 'Ostwald ripening', for taste masking (e.g., dextropropoxyphene napsylate suspension), or to prepare an extended release product. Emmonate salts have been used in suspension formulations to increase the duration of action (e.g., chlorpromazine embonate). On some occasions, the selection of a salt with only modest aqueous solubility may be more suitable for use in tablet products prepared by wet granulation, since the use of highly soluble salts can be detrimental to the granulation process. Depending on the dose required, aqueous solubilities in the range of 0.1-1.0 mg/ml will normally be sufficient to satisfy the dissolution requirements for standard, solid, oral dosage forms of drugs with good-to-moderate potency. However, for parenteral solution products, higher solubilities, perhaps 10 mg/ml or greater, depending on the required dose and dose volume, may be required. For parenteral formulations, the pH of solution (normally

within an acceptable range of 3–10 for intravenous solutions) should be monitored to help ensure that the formulation will be well tolerated.

Manipulation of drug substance solubility by selection of salts may also be employed to modify the pharmacokinetic profile of the drug (e.g., benzathine penicillin and insulin zinc complexes used in parenteral formulations). Salt formation may be also advantageous where the melting point of the active moiety is low, and it is necessary to mill or micronize the active ingredient to achieve adequate homogeneity. A suitably stable salt may have a melting point that is 50–100 °C higher than that of the free acid or free base. Also, being more ionic, the crystals are likely to be less plastic and more easily deformed by brittle fracture.

*Chapt. 6* describes some of the theoretical aspects behind salt formation. This chapter describes some of the procedures adopted by the *Preformulation* team at the *Aventis Pharma* (previously *Rhône-Poulenc Rorer*) *Drug Research Centre* (DRC). These procedures are similar to a systematic approach proposed by *Morris et al.* [3] but were devised empirically with one aim of how to obtain the maximum amount of information from the minimum amount of precious candidate batch. The outline of the process is shown in the *Figure*.

Where possible, we prepare a range of salts for each new candidate and compare their properties in our preformulation program, which is designed to give us the relative suitabilities of each one. Only one salt form is developed, and its properties should be appropriate to the primary route of administration and the intended dosage form proposed for initial marketing. The properties of the drug substance required for one dosage form may be quite different from those required for another. An understanding of the influence of drug substance, or its salt, properties on the finished product is essential to ensure selection of the optimum salt form. Some of these aspects are dealt with in more detail in *Chapt. 4*. The procedures demonstrated here are also described in outline form in [5].

## 2. Salt Formation – Is It Necessary, Is It Feasible?

Each new molecule synthesized within the *Discovery Chemistry* group has an entry prepared in the compound database. Among the first information added as an entry into this database for each compound is the calculated  $pK_a$  value of each ionizable group in the molecule [6–9]. Another early entry is the calculated  $\log P$  value. Immediately, a small amount of material is made available, the values are determined experimentally on 1–2 mg of sample by potentiometric titration (e.g., *Sirius Model GLpKa* apparatus, *Sirius Analytical Instruments Ltd.*), and the values determined experimental-

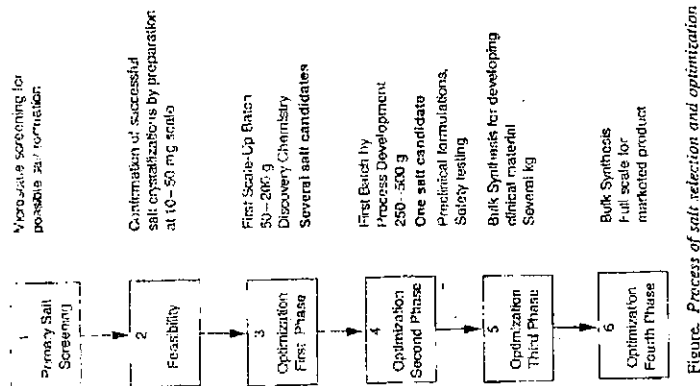


Figure. Process of salt selection and optimization

ly are again entered into the database. The  $\log P$  value is usually determined concurrently by chromatography using an octanol-loaded HPLC column [10].

Synthesis of a working quantity of each new candidate is rarely undertaken at a scale that exceeds 5 g; the primary synthetic aim is to provide sufficient compound for activity screening in both cell and animal models. Once activity is confirmed in these screens, the material remaining from this first batch is usually further sub-divided across a small number of Departments. The amount of drug substance made available to *Physical Chemistry* and the *Preformulation* teams together rarely exceeds 1 g and is often less than 0.5 g.

With the  $pK_a$  value of each ionizable group in the molecule known, either by experimental determination or by calculation, a list of potential salt forming agents (counter-ions) can be selected, for each candidate, based on

ists that are available in the literature [21,22,23] (see also the tables in the Appendix). Based upon studies on numerous candidates over more than 10 years, it is our considered opinion that for the formation of a stable salt, there should be a minimum difference of ca. three units between the  $pK_a$  value of the ionizable group and a possible counter-ion. This is especially so when the drug substance is a particularly weak acid or base where disproportionation back to the component parts often occurs in solution. This results in precipitation of the free acid or free base. Occasionally, an exception may be found where a salt has an acceptable stability, despite there being a smaller difference in the  $pK_a$  values.

To maximize the amount of data obtained from small quantities of candidate drug substances, micro and semi-micro techniques have been developed and are used regularly within our groups. A microplate technique has been devised by the *Aventis Physical Chemistry* group for rapidly and qualitatively screening whether solid salts can be prepared. This involves dissolving ca. 50 mg of sample in a suitable, volatile solvent and adding a fixed volume of this solution, containing ca. 0.5 mg of sample, into each glass microplate well. Concentrated solutions of each potential counter-ion in equimolar proportion, or (if the molecule could form double or triple salts) other appropriate stoichiometric ratios, are prepared, and a few microlitres of each is added sequentially to each well. Thus, all the wells in line 1 ('x'-direction) will contain the same combination of sample and counter-ion 1; all the wells in line 2 contain the same combination of sample and counter-ion 2, etc. Different, potential crystallizing solvents can be investigated systematically in the 'y'-direction. The wells are inspected using an inverted microscope (*Leica*, model *DMIRB*) at regular intervals for the appearance of crystals or solid. Occasionally, crystallization can be promoted by evaporation of any excess solvent in some wells using a slow stream of dry  $N_2$  gas.

Once the possible combinations of counter-ion and solvent(s) are identified, studies at a slightly larger scale (usually 10–50 mg) can be initiated to confirm the formation of a crystalline salt or an amorphous solid and initiate a study of any polymorphism. These studies give a lead to the chemist and can help in the identification of future problems. Melting points are determined by hot-stage microscopy, and samples showing evidence of hygroscopicity can be evaluated using a dynamic vapor sorption (DVS) analyzer (Model *DVS-1*, *Surface Measurement Systems Ltd.*). There is usually sufficient material for a preliminary powder X-ray study using an environmental chamber (model *D8 Advance*, *Brüker Instruments*) to qualitatively determine the presence of crystalline or amorphous material. Frequently, these studies can also give information on the existence of solvates and hydrates, especially if differential scanning calorimetry (DSC; *Mettler Toledo DSC*, model 820), thermal gravimetric analysis (TGA; *Mettler Toledo TGA*, model 850),

Leica DMIRB  
Inverted Microscope

and hot-stage microscopy are also used in the evaluation process. Most of these studies can be accommodated, if care is taken, on 20–25 mg of sample.

In parallel with these studies, a preliminary HPLC method is developed within ca. one week, and, after completion of minimal validation, it is used to give an estimate of the purity of the original sample, whilst IR and other spectroscopic techniques may be used to define the salt and the stoichiometry. Knowledge of the approximate purity is important at this stage, as the presence of high levels of some impurities can often hinder crystallization by 'poisoning' crystal growth or promote the formation of a specific polymorphic form. Immediately after the development of this HPLC method for use in the assessment of the main manufacturing impurities, the analytical chemists turn their attention to the development of the corresponding stability-indicating method. The development and partial validation of this method involves the use of degraded solutions of the drug substance produced in accelerated stress studies. This method takes ca. three weeks to complete and can then be used in the early stability evaluation of salts, their solutions and, ultimately, simple formulations.

Thus, by carefully planned studies using a small quantity of drug substance, it is possible to identify quickly the possibilities for the chemist to investigate, once larger quantities of drug substance are made. These studies may point to a small group of potential salt formers for initial evaluation; they also give the chemist ideas on possible recrystallization solvents that can be tried.

### 3. The Salt-Optimization Process – First Phase

#### 3.1. Choice of the Salt Former (Counter-Ion)

The choice of salt former (counter-ion) depends on several factors. Obviously the primary factor is that it should yield a crystalline salt. Crystallinity in a salt affords a means of purification and removal of unwanted impurities. Lack of crystallinity (i.e., the salt is amorphous) normally would be expected to lead to severe problems and uncertainties, if the product intended to be developed is a solid, oral dosage form. The prospect of unexpected and uncontrolled crystallization at some stage with just one batch, creating a product recall, is something that the *Quality*, *Regulatory*, and *Marketing* groups could not live with. Absence of crystallinity is often less of a problem if the dosage form intended is a liquid.

The choice of salt is also governed largely by the acidity or basicity of the ionizable group, the safety of the counter-ion and the drug indications.

Yodanis Universalist  
Eligible Member