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	14201
Folios	15

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

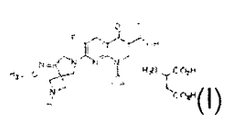
Se acepta la reivindicación de prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

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):



(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[1]	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, divulga (ejemplo 1, pg. 25, ln 26 – pg. 26, ln 13) el compuesto 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, un método para su preparación y composiciones farmacéuticas que la contienen.

[1] 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.

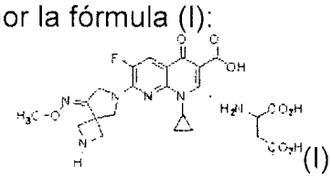
D3
http://www.google.com/patents?id=UVkgAAAAEBAJ&pg=PA1&lpg=PA1&dq=us+4957922&source=bl&ots=fIRPcNg6Xs&sig=t8pD45cy49srZWoO-ZL9OR_icqo&hl=en&sa=X&ei=cdoFUNGZJIyo8QSfIYWACA&ved=0CDYQ6AEwAA#v=onepage&q=us%204957922&f=false, divulga (ejemplo 42, col 13 – 16) la sal de ácido aspártico del ácido-1-ciclopropil-6-fluoro-1,4-dihidro-4-oxo-7-(1-piperazinil)-quinolina-3-carboxílico.

D4
http://www.google.com/patents?id=2VgpAAAAEBAJ&pg=PA1&lpg=PA1&dq=us+5563149&source=bl&ots=vLSIG1jJHz&sig=ENLAjOElrvAVae3FS6ndix2rV4Y&hl=en&sa=X&ei=9NoFUMOUHIi08ASBy8z5Bw&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-azabiciclo[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):


(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-azabiciclo[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)[2]. 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[3] 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

[2] 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).
[3] De acuerdo al documento D2, el compuesto de la invención es un antibiótico de la familia de las floroquinolonas (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-azabiciclo[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-azabiciclo[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 07 AGO. 2012

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

Expediente 09-120908 1er Art 45

Antimicrobial Agents and Chemotherapy, June 2006, p. 2261–2264 Vol. 50, No. 6
0066-4804/06/$08.00+0 doi:10.1128/AAC.01407-05

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

Hee-Soo Park,[1] Hyun-Joo Kim,[1] Min-Jung Seol,[1] Dong-Rack Choi,[2] Eung-Chil Choi,[3] and Jin-Hwan Kwak[1]*

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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, ofloxacin, 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 pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphylococci, penicillin-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-diazaspiro [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 investigated.

This work was presented in part at the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 14 to 17 September 2003 [J. H. Kwak et al., abstr. F-415].

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, Republic 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., Daejon, 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 multipin 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_2. The MIC was considered to be the lowest concentration that completely

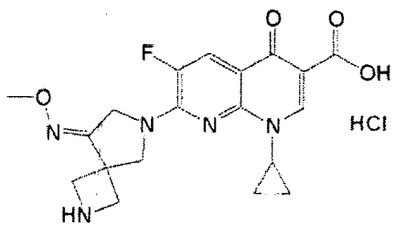

FIG. 1. Chemical structure of DW-224a.

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

Microorganism[a] (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
MSSA (62)	DW-224a	0.008–0.06	0.015	0.03
	Ciprofloxacin	0.06–0.5	0.25	0.5
	Moxifloxacin	0.015–0.25	0.06	0.06
	Gemifloxacin	0.008–0.06	0.015	0.03
MRSA (120)	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
MSCNS (34)	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
MRCNS (28)	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
[illegible]	DW-224a	0.008–[illegible]	0.125	2
	Ciprofloxacin	0.125–>64	2	64
	Moxifloxacin	0.125–64	2	32
	Gemifloxacin	0.008–16	0.125	4
[illegible] (32)	DW-224a	2–32	2	16
	Ciprofloxacin	1–64	8	64
	Moxifloxacin	0.5–64	8	64
	Gemifloxacin	0.5–>64	2	32
VRE (36)	DW-224a	0.125–4	0.5	4
	Ciprofloxacin	0.5–4	4	4
	Moxifloxacin	0.25–4	2	4
	Gemifloxacin	0.015–2	0.5	2
[illegible]	DW-224a	0.008–0.06	0.015	0.03
[illegible] (32)	Ciprofloxacin	1–8	2	8
	Moxifloxacin	0.25–0.5	0.5	0.5
	Gemifloxacin	0.06–0.25	0.125	0.25
[illegible] (35)	DW-224a	0.015–0.125	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
[illegible] (35)	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
Enterobacter cloacae (48)	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
[illegible] (30)	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	0.175
[illegible] (15)	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
[illegible] (25)	DW-224a	0.03–1	0.125	0.25
	Ciprofloxacin	0.015–0.25	0.03	0.06
	Moxifloxacin	0.008–0.125	0.03	0.06
	Gemifloxacin	0.008–0.06	0.015	0.03
Citrobacter freundii (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
Morganella morganii (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
Proteus mirabilis (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
Proteus vulgaris (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
Serratia marcescens (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
Salmonella enterica serovar Typhi (18)	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
Acinetobacter baumannii (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
Acinetobacter calcoaceticus (8)	DW-224a	0.008–1	0.008	—[b]
	Ciprofloxacin	0.015–1	0.25	—
	Moxifloxacin	0.008–1	0.125	—
	Gemifloxacin	0.008–0.5	0.03	—
Stenotrophomonas maltophilia (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
Haemophilus influenzae (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
Moraxella catarrhalis (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
Neisseria gonorrhoeae (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
Pseudomonas aeruginosa (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

MSSA, methicillin-susceptible *S. aureus*; MSCNS, methicillin-susceptible coagulase-negative staphylococci; MRCNS, methicillin-resistant coagulase-negat[illegible] staphylococci; VRE, [illegible]-resistant enterococci.

[illegible] calculated

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

Microorganism inoculum (CFU/mouse)	Mucin (%)	Antimicrobial agent[a]	MIC (μg/ml)	ED_{50} (mg/kg) (95% confidence limits)
S. aureus giorgio (1×10^8)	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 P197 (5×10^7)	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)
S. pneumoniae ATCC 6305 (1×10^8)[b]		DW-224a	0.008	0.53 (0.0029–1.67)
		Ciprofloxacin	0.5	10.46 (3.56–32.26)
		Moxifloxacin	0.06	2.00 (0.41–6.21)
		Gemifloxacin	0.015	0.76 (0.15–2.43)
[illegible]	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)
E. coli 851E (1×10^5)	5	DW-224a	0.03	6.08 (1.64–38.80)
		Ciprofloxacin	0.008	0.78 (0.10–8.20)
		Moxifloxacin	0.06	2.17 (0.61–7.73)
		Gemifloxacin	0.015	1.16 (0.25–4.13)
K. pneumoniae P427 (5×10^5)	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 (0.36–10.68)
		Gemifloxacin	0.03	5.36 (1.42–20.20)
P. vulgaris 1375E (2×10^6)	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)

[a] Antimicrobial agents were orally administered at 1 and 4 h after infection.

[b] Bacterial strains were suspended in 0.9% saline solution containing 5% gastric mucin, except for *S. pneumoniae* ATCC 6305, which was suspended in 0.9% saline solution.

inhibited growth on agar plates, disregarding a single colony or a 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 (Dachan Bio Link Co., Ltd., Eum-sung Gun, Republic of Korea) were used for the systemic infection model. They were maintained in animal rooms kept at 23 ± 2°C with 55 ± 20% relative humidity. Test organisms for infection were cultured in tryptic soy agar medium (Difco) at 37°C for 18 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_{50}) 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_{90}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_{90}, 0.125 μg/ml), *S. pyogenes* (MIC_{90}, 0.06 μg/ml), *Enterococcus faecalis* (MIC_{90}, 2 μg/ml), and *Enterococcus faecium* (MIC_{90}, 16 μg/ml). Against *S. pneumoniae*, the activity of DW-224a (MIC_{90}, 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 2, 1, 0.25, 2, 0.25, and 0.5 μg/ml, respectively. Against *Acinetobacter baumannii* (MIC_{90}, 4 μg/ml), *Acinetobacter calcoaceticus* (MIC_{90}, ≤0.008 μg/ml), and *Stenotrophomonas maltophilia* (MIC_{90}, 1 μg/ml), DW-224a showed an activity comparable to the other compounds. DW-224a was highly active against *H. influenzae* (MIC_{90}, 0.008 μg/ml) and *M. catarrhalis* (MIC_{90}, 0.03 μg/ml). Against *N. gonorrhoeae*, DW-224a (MIC_{90}, 1 μg/ml) was eightfold more active than ciprofloxacin. The activity of DW-224a against *Pseudomonas aeruginosa* (MIC_{90}, 64 μg/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* giorgio the ED_{50}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_{50}, 5.52 mg/kg) was more effective than ciprofloxacin (ED_{50}, >40 mg/kg), moxifloxacin (ED_{50}, 35.35 mg/kg), and gemifloxacin (ED_{50}, 26.58 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_{50}s were 0.53 to 2.65 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_{50}, 6.08 mg/kg) was less active than ciprofloxacin (ED_{50}, 0.78 mg/kg), moxifloxacin (ED_{50}, 2.17 mg/kg), and gemifloxacin (ED_{50}, 1.16 mg/kg). Against *K. pneumoniae* P427, the ED_{50}s of DW-224a, ciprofloxacin, moxifloxacin, and gemifloxacin were 9.77, 0.70, 2.64, and 5.36 mg/kg, respectively. DW-224a (ED_{50}, 4.83 mg/kg) was less active than ciprofloxacin (ED_{50}, 1.41 mg/kg) and gemifloxacin (ED_{50}, 3.41 mg/kg) against *M. morganii* 1375F but more active than moxifloxacin (ED_{50}, 5.28 mg/kg). In general, the ED_{50}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. pyogenes*, 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 fluoroquinolones 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 β-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.

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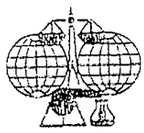

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Handbook of
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Properties, Selection, and Use

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

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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

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.

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_b. 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 $c = c_b$. Beyond $x = h$, the bulk concentration remains uniform at c_b. In the simplest form of the theory, following *Fick*'s First Law, the rate of dissolu-

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. Embonate 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*) *Dagenham 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 experimentally are again entered into the database. The log P value is usually determined concurrently by chromatography using an octanol-loaded HPLC column [10].

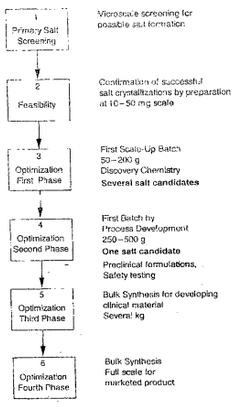

Figure. *Process of salt selection and optimization*

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

lists that are available in the literature [7][11 - 13] (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 then 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*, *Bruker 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*),

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.