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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

E. _____ S. _____ D. _____

Re: PI.-ARENA PHARMACEUTICALS, INC.-Solicitud de Patente de Invención en Fase Nacional PCT para "DERIVADOS DE 3-FENIL-PIRAZOL COMO MODULADORES DEL RECEPTOR 5-HT_{2A} DE LA SEROTONINA ÚTILES PARA EL TRATAMIENTO DE TRASTORNOS RELACIONADOS CON ÉSTE".-Clase C07D 231/16.-Expediente número 07-052.699.-

1. Me refiero al auto dictado por ese Despacho notificado por fijación en lista de 28 de Octubre de 2011.

2. Con el fin de responder las objeciones expuesta por el señor Examinador en su Concepto Técnico correspondiente al Oficio No. **17087** con respecto a la Claridad, Concisión y Nivel Inventivo de la presente solicitud, me permito dirigir su atención a las siguientes modificaciones y argumentos técnicos por medio de los cuales se superan completamente dichas objeciones y se demuestra la patentabilidad de la solicitud.

3. **CLARIDAD Y CONCISION DEL CAPÍTULO REIVINDICATORIO**

En relación al capítulo reivindicatorio actualmente presentado, el señor Examinador afirma que este carece de claridad y concisión basado en los siguientes argumentos:

(i) Respecto a la reivindicación 1 actualmente presentada, el señor Examinador afirma que esta reivindicación *"no es clara ya que existen expresiones generales en R4, R6, R7 y R8 como heterobiciclo, heteroarilo y heteroaril que deben ser definidas en términos de las especies químicas."* (sic).

En respuesta a este señalamiento, en primer lugar me permito manifestar a ese Despacho que en dicha objeción se hace referencia dos veces a un mismo sustituyente nombrándolo de manera similar ("heteroaril" y "heteroarilo"). No obstante, se ha interpretado que la intención de dicha objeción es hacer también referencia a un tercer sustituyente que se menciona en las definiciones de R₄ y R₈ de la reivindicación en cuestión, que es el sustituyente "heterocíclico".

Teniendo en cuenta lo anterior y acatando el señalamiento realizado por el señor Examinador, me permito manifestar que la reivindicación 1 actualmente presentada ha sido modificada para definir de manera clara el significado de cada uno de los sustituyentes "heterobicíclico", "heterocíclico" y "heteroaril" de conformidad con las definiciones dadas para los términos heterobiciclo, heterociclo y heteroaril que se revelan en las páginas 13 y 14 del texto de la descripción actualmente presentado.

Así las cosas, dentro de la reivindicación 1 ahora modificada claramente se definen los sustituyentes "heterobicíclico", "heterocíclico" y "heteroaril" en términos de sus especies químicas, tal como se lee continuación:

"heteroaril" indica un sistema de anillos aromáticos que puede ser un solo anillo, dos anillos fusionados o tres anillos fusionados en donde por lo menos un carbono del anillo está remplazado por un heteroátomo seleccionado de: O, S y N; en donde N está opcionalmente sustituido con H, acilo C₁₋₄, o alquilo C₁₋₄;

"heterobicíclico" indica un anillo bicíclico no aromático, en donde 1, 2 o 3 carbonos del anillo están remplazados por un heteroátomo seleccionado de: O, S, S(=O), S(=O)₂ y NH; en donde el nitrógeno está opcionalmente sustituido con acilo C₁₋₄ o alquilo C₁₋₄; y 1 o 2 carbonos de anillo están opcionalmente sustituidos con oxo o tiooxo; y

"heterocíclico" indica un anillo de carbonos, no aromático en donde uno, dos o tres carbonos de anillo están remplazados por un heteroátomo seleccionado de: O, S, S(=O), S(=O)₂, NH; en donde N está opcionalmente sustituido con acilo C₁₋₄ o alquilo C₁₋₄; y los átomos de carbono del anillo están opcionalmente sustituidos con oxo o tiooxo."

Por otro lado, dentro de la reivindicación 1 se ha eliminado la referencia a R₁₀ en la expresión "R₉, R₁₀ y R₁₁ son cada uno independientemente H o alquil C₁₋₈", con lo cual en la reivindicación 1 ahora modificada se lee "R₉ y R₁₁ son cada uno independientemente H o alquil C₁₋₈". Esta modificación ha sido realizada con el único propósito de dar mayor claridad al capítulo reivindicatorio, toda vez que dicho sustituyente R₁₀ no se encuentra dentro del alcance de la reivindicación 1 actualmente presentada.

En vista de las anteriores modificaciones queda superada por completo la objeción de claridad que presentó el señor Examinador en su Concepto Técnico antes mencionado.

En conexión con lo anterior, me permito resaltar que las mencionadas modificaciones no constituyen de manera alguna una ampliación al alcance de la invención, tal como fue originalmente presentada, toda vez que se está delimitando

de forma más precisa el objeto de la invención según lo revelado en la descripción de la solicitud.

(ii) Adicionalmente, el señor Examinador afirma que *"la reivindicación 1 no es concisa dado que los términos "sal", "hidratos" y "solvatos" no especifican con exactitud los compuestos a los cuales se refiere."* Por lo tanto, el señor Examinador sugiere aclarar cuáles son los compuestos en forma de sales, hidratos y solvatos que desean reivindicar.

A este respecto, me permito señalar que para la persona medianamente versada en la materia los términos "sales", "hidratos" y "solvatos" sí resultan concisos y permiten identificar con exactitud los compuestos a los cuales se refieren, toda vez que dichos términos se encuentran con frecuencia en el estado de la técnica y además son claramente definidos en el texto de la descripción de la solicitud en trámite. En este mismo sentido, me permito manifestar que la preparación de sales, hidratos y solvatos es bien conocida en el campo de la química, y la persona medianamente versada en la materia, con base en las enseñanzas de la descripción de la solicitud y sus propios conocimientos, puede fácilmente producir y cerciorarse de los efectos de tales derivados de los compuestos de la invención.

En conexión con lo anterior, el señor Examinador reconocerá que, por ejemplo, el término "sal" está definido en la página 63 de la descripción actualmente presentada, en donde se enseña que:

*"Los compuestos según la invención opcionalmente pueden existir en forma de **sales** farmacéuticamente aceptables, incluidas las sales de adición ácida farmacéuticamente aceptables preparadas a partir de ácidos no tóxicos y farmacéuticamente aceptables que incluyen ácidos inorgánicos y orgánicos. Los ácidos representativos incluyen, entre otros, los siguientes: acético, bencensulfónico, benzoico, alcanforsulfónico, cítrico, etensulfónico, dicloroacético, fórmico, fumárico, glucónico, glutámico, hipúrico, bromhídrico, clorhídrico, isetiónico, láctico, maleico, málico, mandélico, metansulfónico, mícico, nítrico, oxálico, pamoico, pantoténico, fosfórico, succínico, sulfúrico, tartárico, p-toluensulfónico y semejantes, tal como aquellas sales farmacéuticamente aceptables mencionadas en el Journal of Pharmaceutical Science, 66, 2 (1977); que por referencia se incorporan en su totalidad a este documento."*(Resaltado fuera de texto)

Adicionalmente, el señor Examinador podrá apreciar que un número de sales de los compuestos de la invención son específicamente descritas en los Ejemplos de la solicitud, junto con los métodos para su preparación y su información fisicoquímica.

Veanse por ejemplo, el Ejemplo 1.59 (sal clorhidrato del compuesto 794) y el Ejemplo 1.66 (sal trifluoroacetato del compuesto 459).

Así las cosas, el término "sales" del capítulo reivindicatorio resulta claro y conciso para la persona medianamente versada en la materia, toda vez que este término es común y frecuentemente usado en los campos de la química y las patentes, y además ha sido debidamente definido en la memoria descriptiva de la solicitud. En consecuencia, la persona versada en la materia no tendrá ninguna duda acerca del significado de dicho término y podrá identificar con exactitud los compuestos en forma de sal a los cuales hace referencia.

Por otro lado, en relación a los hidratos y solvatos de los compuestos de la invención, en la página 63 de la solicitud se describe que *"Los compuestos de esta invención pueden formar solvatos con disolventes estándares de bajo peso molecular, mediante métodos conocidos por los químico farmacéuticos experimentados."*

En este sentido, me permito señalar que el término "solvato" es un término bien conocido en el estado del arte y se usa en la solicitud de una manera consistente con su significado usual, el cual puede ser encontrado en la literatura, véase por ejemplo el documento editado por R. Hilfiker, *"Polymorphism in the Pharmaceutical Industry"*, Wiley-VCH, 2006, Chapter 8 "The importance of Solvates", el cual se adjunta al presente memorial.

Asimismo, la frase "disolventes estándares de bajo peso molecular" no deja ninguna duda para la persona medianamente versada en la materia de cuáles son los disolventes que son usados para preparar dichos solvatos de los compuestos de la invención.

De hecho, la elección del disolvente a ser usado para la preparación de un solvato de un compuesto dado es una tarea rutinaria en el campo de la química, como por ejemplo se evidencia en el documento editado por Harry G. Brittain, *"Polymorphism in Pharmaceutical Solids"*, Vol. 95, Marcel Dekker, Inc. New York, 1999; páginas 202 a 209", el cual se adjunta al presente memorial. En dicho documento, en la página 206, la Tabla 2 lista la distribución de los 15 disolventes más abundantes en el Cambridge Crystallographic Database en función de los solvatos formados, en donde los hidratos se encuentran en la parte superior de la lista.

Además, la persona versada en la materia reconocerá que la preparación de hidratos y solvatos es rutinaria en el campo de la química y uno de los procedimientos más simples que la química puede tener.

Más aún, como se resume a continuación, el estado de la técnica y el conocimiento de una persona medianamente versada en química contienen toda la información necesaria para hacer y caracterizar hidratos y solvatos de compuestos orgánicos.

En este sentido, algunos procedimientos típicos para preparar e identificar hidratos y solvatos de compuestos son descritos en el documento de Brittain. Por ejemplo, dicho documento enseña en su página 204 que *"la simple exposición de un polvo anhidro a una humedad relativa alta puede con frecuencia permitir la formación de un hidrato"*; y en la página 205 enseña que *"con frecuencia, cuando los disolventes son empleados en la purificación de una sustancia farmacéutica nueva por recristalización, se observa que los cristales aislados incluyen moléculas del disolvente."*

Adicionalmente, los métodos para la preparación de hidratos y solvatos usan técnicas que son muy bien conocidas en el estado del arte y que son consideradas muy sencillas, como por ejemplo la exposición de compuestos a agua o a disolventes orgánicos, dejando las muestras expuestas al aire o al vapor de dichos disolventes por cierto tiempo, así como suspendiendo y/o cristalizando las muestras del agua o cualquier otro disolvente. De hecho, existen métodos de exploración sistemática que son usados rutinariamente para la identificación de solvatos e hidratos. (véase el documento de Hilfiker, páginas 222 y 223). En la página 222, Hilfiker discute como *"han sido desarrollados sistemas de cristalización automáticos y estrategias..."* para ayudar a la industria farmacéutica a establecer la presencia de formas cristalinas (incluyendo hidratos y solvatos) de compuestos farmacéuticos en un "corto tiempo". Hilfiker continúa diciendo que *"es improbable que un hidrato estable sea pasado por alto por cualquiera de los programas de exploración"* y prosigue afirmando que *"a menudo, los solvatos pueden ser generados simple y rápidamente por suspensión de una sustancia en un solvente o por mojado y molienda..."*.

Sumado a lo anterior, vale la pena resaltar que hay varias compañías que rutinariamente prestan servicios de exploración de hidratos y solvatos y anuncian como de manera rápida y eficiente ellos pueden identificar las diversas formas. Ejemplos de compañías que ofrecen estos servicios incluyen a Wilmington Pharmatech y Aptuit en los Estados Unidos, y Avantium Technologies en los Países Bajos.

Una vez los hidratos y solvatos son formados, pueden ser fácilmente caracterizados por métodos rutinarios tales como análisis termogravimétrico (TGA), calorimetría diferencial de barrido (DSC), titulación de Karl Fischer, difracción de Rayos X (en polvo o de mono-cristal), espectroscopía infrarroja (IR), microscopía de

luz polarizada, microscopía de platina caliente (ver la página 18, columna derecha, del documento de Vippagunta y col., *Advanced Drug Delivery Reviews* 48 (2001) 3-26, el cual se adjunta al presente memorial) u otras técnicas rutinarias para detectar y cuantificar la presencia de moléculas de hidrato o solvato en la muestra.

Además, me permito señalar que una o más de las descripciones experimentales encontradas en la solicitud en trámite pueden permitir la obtención de hidratos o solvatos de los compuestos de la invención, en donde la persona medianamente versada en la materia reconocerá fácilmente hasta que punto del procedimiento descrito debe seguir para obtener dichos compuestos.

De hecho, ejemplos de hidratos y solvatos de compuestos de la presente invención son conocidos y son el objeto el objeto de la publicación PCT WO2011/075596, la cual se adjunta al presente memorial. Por ejemplo, en el ejemplo 2 de la página 35 de dicho documento se revela el hidrato del compuesto 733 de la presente solicitud. Igualmente, en el Ejemplo 3 de la página 36 del documento WO2011/075596 se revela el solvato de etanol de la sal de oxalato del compuesto 660 de la presente solicitud.

Por ultimo, me permito manifestar a ese Despacho que una solicitud de patente no necesita enseñar, y preferiblemente omite, aquello que es bien conocido en el estado del arte. Como se discutió en detalle anteriormente, la preparación de hidratos y solvatos de compuestos orgánicos es una tarea rutinaria, bien conocida y abundantemente descrita en el estado de la técnica. Por tal razón, tal información no necesita ser incluida en la solicitud.

En consecuencia, cualquier otra revelación relativa a la preparación de las sales, hidratos y solvatos de los compuestos de la invención no necesita ser incluida en la presente solicitud, porque tal revelación únicamente recitaría lo que una persona medianamente versada en la materia ya conoce y considera un trabajo rutinario.

En vista de lo anterior, queda claramente establecido que los términos "hidratos" y "solvatos" contenidos en el capítulo reivindicatorio son claros y concisos para la persona medianamente versada en la materia, toda vez que la selección de los disolventes (que para la presente solicitud se ha establecido que son disolventes estándares de bajo peso molecular) y el método de preparación de dichos compuestos, además de encontrarse ampliamente descritos en la literatura como se mostró anteriormente, son una tarea rutinaria para la persona versada en la materia, razón por la cual dicha persona podrá establecer con exactitud los compuestos en forma de hidratos y solvatos a los cuales hacen referencia dichos términos.

En conclusión, los términos "sales", "hidratos" y "solvatos" contenidos en el capítulo reivindicatorio son totalmente claros y concisos para la persona medianamente versada en la materia y se encuentran completamente sustentados en la memoria descriptiva de la solicitud como se demostró anteriormente.

4. **NIVEL INVENTIVO**

Por otra parte, el señor Examinador considera que el capítulo reivindicatorio en estudio carece de altura inventiva a la luz de las enseñanzas del documento **WO 2004058722 (D1)**.

En relación con este documento, el señor Examinador señala que D1 *"divulga (Abstract) derivados de arilamina y pirazol heteroarilamina, útiles como moduladores de la actividad de receptor 5HT2A de la serotonina, demostrando su actividad en términos de IC50 (Pág. 71/72, ejemplo 11)."*

Adicionalmente, el señor Examinador señala que *"La diferencia entre la invención, definida en la reivindicación 1, y los compuestos de D1 consiste en la inclusión de los radicales V, W unido a R4 y el grupo R8."* (sic).

En conexión con lo anterior, el señor Examinador afirma que *"No hay evidencia de un efecto técnico inesperado derivado de incluir los radicales V,W;R4 y R8, ya que aunque presenta el protocolo para realizar la prueba de acumulación de fosfato de inositol, no presenta ninguna clase de resultados con los compuestos de la invención. Además aunque D1 presenta diferencias estructurales tanto a la molécula de D1 como a la molécula de la invención se les atribuye la misma actividad por lo que no hay evidencia de un efecto técnico inesperado con las diferencias estructurales que se pretenden reivindicar."* (sic).

En consecuencia, el señor Examinador concluye que la persona del oficio normalmente versada en la materia realizaría modificaciones sobre las posiciones 5 y 2 del anillo de los compuestos de D1 obteniendo estructuras con actividad similar como aquellas reclamadas por la reivindicación 1, por lo que dicha reivindicación carece de nivel inventivo.

Por otro lado, respecto a la reivindicación 36 actualmente presentada el señor Examinador señala que el documento D1 *"divulga (Resumen) una composición farmacéutica que contiene derivados de arilamina y pirazol con un portador farmacéuticamente aceptable (reivindicación 5, pág. 53), útil como modulador de la actividad del receptor 5HT2A de la serotonina."* (sic).

Además, el señor Examinador afirma que la diferencia entre la invención definida en la reivindicación 36 y la composición de D1 consiste en la inclusión de un compuesto de acuerdo con la reivindicación 1 en la composición; y debido a que no hay evidencia de un efecto técnico inesperado derivado de incluir un compuesto de acuerdo con la reivindicación 1 en la composición farmacéutica, dicha composición de la reivindicación 36 se considera obvia y por lo tanto no tiene nivel inventivo.

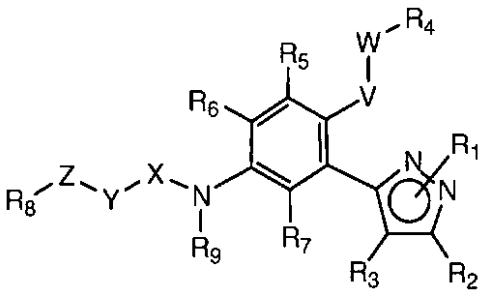
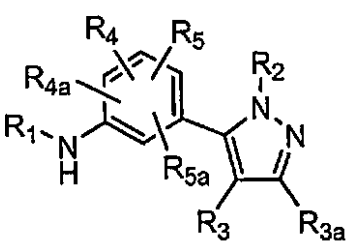
Por ultimo, con relación a la reivindicación 37 el señor Examinador señala que dicha reivindicación de proceso corresponde a una de las operaciones unitarias convencionales en ciencias farmacéuticas que no aporta ningún conocimiento adicional al esto de la técnica.

Teniendo en cuenta lo anterior, a continuación me permito exponer algunos argumentos técnicos por medio de los cuales se pone de manifiesto que la presente solicitud no resulta obvia a partir de las enseñanzas del documento D1 y por lo tanto sí goza de nivel inventivo:

4.1. Nivel Inventivo de la solicitud frente a las enseñanzas del documento WO 2004058722 (D1)

En primer lugar, el solicitante desea manifestar que el señor Examinador ha malinterpretado las características técnicas esenciales del estado del arte comprendido en el documento D1. En este sentido, me permito señalar que en el cuadro de la página 3 del Concepto Técnico emitido, en el cual se muestra el análisis comparativo de las características técnicas esenciales de la solicitud con las del estado de la Técnica, no se resumen las características técnicas esenciales del documento D1 correspondiente a la WO 2004058722, en vez de esto, en la columna derecha de dicho cuadro se resumen las características técnicas de un documento diferente que se cita en el Reporte Internacional de Búsqueda de esta solicitud, dicho documento corresponde a US 4,409,231.

Por lo tanto, a continuación me permito presentar un cuadro que muestra el análisis comparativo de las características técnicas esenciales de la presente invención con las del documento WO 2004058722 (D1), el cual es considerado por el señor Examinador como el documento del estado de la técnica más cercano a la invención:

Reivindicación 1	Documento WO 2004058722 (D1)
 (Ia)	 (A)
-X-Y-Z-R₈ X es C(=O), C(=S), o está ausente; Y es O, NR₁₁ o está ausente; Z es alquileo C₁₋₄ o cicloalquileo C₃₋₆, cada uno opcionalmente sustituido con 1 a 8 sustituyentes, o Z está ausente; R₈ es alquil C₁₋₈, alquenil C₂₋₆, aril, cicloalquil C₃₋₇ o heteroaril, cada uno opcionalmente sustituido; R₁₁ es H o alquil C₁₋₈	R₁ R₁ es arilo o heteroarilo opcionalmente sustituido con de 1 a 5 sustituyentes
-V-W-R₄ V es O W es alquileo C₁₋₄ opcionalmente sustituido o W está ausente; R₄ es heterobicíclico, heterocíclico, o heteroaril, cada uno opcionalmente sustituido	R₄, R_{4a}, R₅, R_{5a} R₄, R_{4a}, R₅, R_{5a} son H, acilo C₁₋₅, aciloxi C₁₋₅, alquileo C₂₋₆, alcoxi C₁₋₄, alquilo C₁₋₆, alquil C₁₋₅ carboxamida, alquileo C₂₋₆, alquil C₁₋₄ sulfonamida, alquil C₁₋₄ sulfinilo, alquil C₁₋₄ sulfonilo, alquiltio C₁₋₄, alquileilo C₁₋₆, carbo-alcoxi C₁₋₆, carboxamida, carboxi, ciano, cicloalquilo C₃₋₇, dialquil C₂₋₈ carboxamida, halogeno, haloalcoxi C₁₋₄, haloalquilo C₁₋₄, haloalquil C₁₋₄ sulfinilo, haloalquil C₁₋₄ sulfonilo, haloalquiltio C₁₋₄, hidroxilo, tior, heteroarilo de 5 o 6 miembros opcionalmente sustituido, nitro, fenilo o NR₆R₇
R₉ R₉ es H o alquil C₁₋₈	H

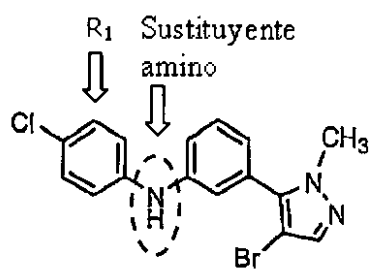
Así las cosas, me permito señalar que el documento D1 únicamente describe cierto tipo de diarilaminas y arilheteroarilaminas que actúan como moduladores del receptor 5-HT_{2A} (Resumen de D1). De hecho, del análisis del cuadro anterior la persona versada en la materia reconocerá que a pesar del alcance amplio del documento D1, la materia contenida en D1 no se solapa en absoluto con la materia de la reivindicación 1 ahora modificada de la solicitud en cuestión.

En este sentido, me permito indicar que una característica técnica esencial que presentan todos los compuestos de la reivindicación 1 del capítulo reivindicatorio ahora modificado de la solicitud en estudio, consiste en que todos los compuestos en su anillo de fenilo central deben estar sustituidos con un grupo -V-W-R₄, el cual

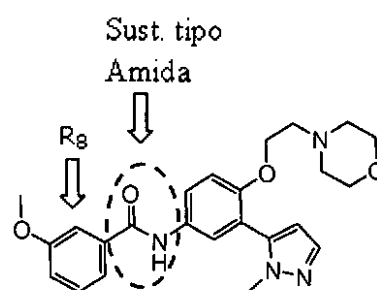
comprende un átomo de Oxígeno unido, directamente o vía un conector de alquileo C_{1-4} , a un sistema de heteroanillo. En contraste, los compuestos objeto de D1 no pueden tener como sustituyente en su anillo de fenilo central a un átomo de Oxígeno unido, directamente o vía un conector de alquileo, a un sistema de heteroanillo, debido a que tal sustituyente no puede obtenerse a partir de las definiciones de R_4 , R_{4a} , R_5 y R_{5a} descritas en D1 (véase el cuadro comparativo anterior).

Además, el alcance del documento D1 solo comprende compuestos que son diaril o arilheteroaril **aminas** debido a que el sustituyente R_1 que se une al nitrógeno de la anilina es definido únicamente como arilo o heteroarilo. (véase el cuadro comparativo anterior). En contraste con las enseñanzas de D1, **la reivindicación 1 de la presente solicitud, en virtud del grupo -X-Y-Z- R_8 unido al nitrógeno de la anilina, comprende no solo aminas sino también amidas, ureas y carbamatos, así como también sus derivados tiocarbonilo.** (véase el cuadro anteriormente expuesto)

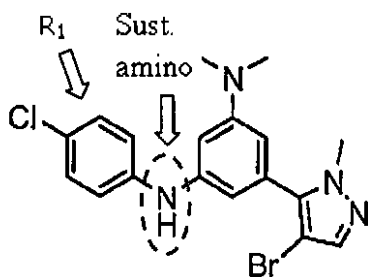
En consecuencia, me permito señalar que **mientras D1 revela solo 78 compuestos de los cuales todos tienen un sustituyente amino que conecta el anillo de fenilo central a R_1** (véanse, por ejemplo, los compuestos de la Tabla 2 de las páginas 29 a 39 de D1), **la presente solicitud revela al menos 836 compuestos de los cuales la gran mayoría (más del 95%) tiene un sustituyente tipo amida, urea o carbamato que conecta el anillo central de fenilo al sustituyente R_8 .** En este sentido, con el fin de ilustrar esta diferencia técnica esencial que existe entre los compuestos de la presente solicitud y aquellos revelados en el documento D1, me permito dirigir la atención del señor Examinador a las estructuras químicas de algunos de los compuestos de D1 y de la solicitud en estudio, en donde se aprecia que ninguno de los compuestos revelados en D1 tiene un sustituyente tipo amida, urea o carbamato que conecte el anillo central de fenilo al sustituyente R_1 , las cuales sí están presentes en los compuestos de la presente invención conectando el anillo central de fenilo al sustituyente R_8 :



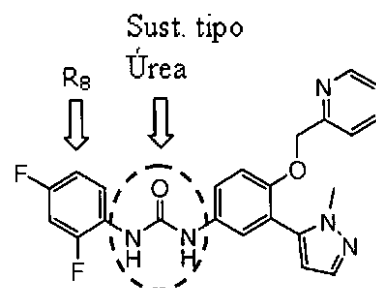
Compuesto 36 de D1



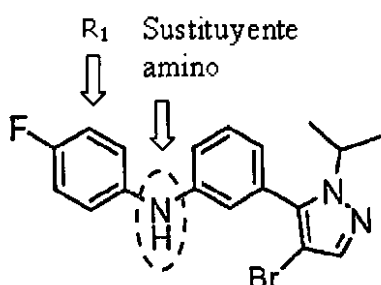
Ejemplo 1.88 de la solicitud
(Compuesto 733)



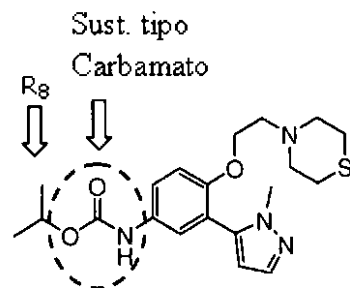
Compuesto 67 de D1



Ejemplo 1.14 de la solicitud
(Compuesto 28)



Compuesto 73 de D1



Ejemplo 1.84 de la solicitud
(Compuesto 214)

En vista de lo anterior, la persona versada en la materia reconocerá que **las diaril o arilheteroaril aminas del documento D1 se encuentran distantes de ser una enseñanza que permita la obtención de los compuestos de la invención en estudio ya que D1 no proporciona ninguna sugerencia o motivación que conduzca a la persona versada en la materia a realizar modificaciones de los compuestos allí revelados con la intención de producir los compuestos objeto de la presente solicitud.** En este sentido, me permito manifestar que **es a todas luces evidente que el documento D1 falla en proporcionar una motivación que conduzca a la persona versada en la materia a la modificación de los compuestos de D1 para incluir en el anillo de fenilo central el sustituyente -V-W-R₄ y el sustituyente -X-Y-Z-R₈ (que unido al nitrógeno de la anilina genera compuestos tipo amida, urea y carbamato que no se encuentran dentro del alcance de D1) que contienen los compuestos de la presente solicitud.**

Por lo tanto, debido a que **D1 no puede proporcionar una motivación para preparar los compuestos de la presente solicitud toda vez que estos compuestos tienen sustituyentes que no fueron enseñados y que ni siquiera fueron incluidos como referencia en D1, la presente invención no puede obtenerse de manera obvia a partir de las enseñanzas de D1 por lo que goza de nivel inventivo frente al estado del arte más cercano.**

Por otra parte, con respecto a los señalamientos realizados por el señor Examinador quien afirma que *"No hay evidencia de un efecto técnico inesperado derivado de incluir los radicales V,W;R4 y R8, ya que aunque presenta el protocolo para realizar la prueba de acumulación de fosfato de inositol, no presenta ninguna clase de resultados con los compuestos de la invención."* (sic) y que *"aunque D1 presenta diferencias estructurales tanto a la molécula de D1 como a la molécula de la invención se les atribuye la misma actividad por lo que no hay evidencia de un efecto técnico inesperado con las diferencias estructurales que se pretenden reivindicar."* (sic), a continuación me permito presentar argumentos técnicos por medio de los cuales quedan claramente demostrados los efectos técnicos sorprendentes e inesperados que exhiben los compuestos de la presente invención.

Para comenzar, me permito señalar que el documento D1 menciona que los compuestos allí descritos son usados para la profilaxis y tratamiento de, en otros, la agregación plaquetaria y los trastornos de sueño. Por otra parte, el Ejemplo 11 de D1 describe un ensayo de acumulación de fosfato de inositol para medir la actividad en el receptor 5-HT_{2A}, en donde **los valores de CI₅₀ de los compuestos revelados en D1 se encuentran en el rango de 113 nM a 1.77 nM y adicionalmente se menciona que la mayoría de los demás compuestos de D1 ensayados tenían valores de CI₅₀ entre 10 µM y 4 nM.** Por ultimo, me permito señalar que D1 no revela ningún dato de ensayos *in vivo*.

En contraste, **los compuestos de la presente invención en el mismo ensayo de acumulación de fosfato de inositol tuvieron valores de CI₅₀ en el rango de 10 µM a 0.1 nM** (ver Ejemplo 3 de la página 110 de la memoria descriptiva de la solicitud en estudio)

Por lo tanto, **el límite inferior de CI₅₀ de los compuestos de la invención es 40 veces menor que el límite inferior de CI₅₀ del rango general mostrado para los compuestos de D1, lo cual pone de manifiesto que los compuestos de la presente solicitud son más potentes que los compuestos de D1 en la inhibición del receptor 5-HT_{2A} de la serotonina.** Asimismo, me permito señalar que el compuesto de mayor actividad del documento D1 (el compuesto 70 que tiene un valor de CI₅₀= 1.77 nM) es más de 17 veces menos activo que los compuestos de mayor actividad de la presente invención (que tienen valores de CI₅₀ de 0.1 nM).

En vista de lo anterior, **salta a la vista que los compuestos de la presente solicitud sí tienen un efecto técnico inesperado frente a las enseñanzas de D1, toda vez que el introducir los sustituyentes -X-Y-Z-R₈ y -V-W-R₄ en la estructura del 3-amino fenil pirazol-3-ilo produjo compuestos que de manera sorprendente e inesperadamente pueden ser hasta 17 veces más**

activos que los compuestos de D1 en los ensayos de acumulación de fosfato de inositol, lo que indica que los compuestos de la presente solicitud tienen una mayor potencia que los compuestos de D1 para inhibir el receptor 5-HT_{2A} de la serotonina.

Adicionalmente, a diferencia del documento D1, la memoria descriptiva de la presente solicitud incluye otros ejemplos en los que se provee información adicional de los efectos técnicos de los compuestos de la invención, incluyendo datos in vivo. Por ejemplo, el Ejemplo 4 contenido en la página 111 de la descripción revela la capacidad de los compuestos de la solicitud para unirse a una preparación de membrana con ción de receptor 5-HT_{2A} usando un ensayo de unión a radioligando DOI, en donde se muestran los resultados de este ensayo para cuatro compuestos de la invención en valores de CI₅₀ y además se revela que otros compuestos de la invención tuvieron valores de actividad en el rango de aproximadamente 10 µM a aproximadamente 0.1 nM para este mismo ensayo.

Sumado a lo anterior, el Ejemplo 5 de la solicitud en trámite describe un ensayo de agregación de plaquetas humanas *in Vitro*, el cual enseña que ciertos compuestos de la invención tuvieron valores de actividad en el rango de aproximadamente 80 µM a aproximadamente 1 nM y que otros compuestos de la invención tuvieron valores de actividad de aproximadamente 8 µM a aproximadamente 2 nM.

Así también, el Ejemplo 11 de la solicitud en estudio revela un ensayo *in Vitro* de agregación de plaquetas de perro en el que se generaron curvas de CI₅₀ para cada compuesto ensayado, obteniéndose valores entre aproximadamente 2 nM hasta más de aproximadamente 10 µM.

Además, el Ejemplo 12 de la solicitud en estudio revela un ensayo de agregación de sangre entera de perro *ex-vivo* en el que algunos compuestos de la invención demostraron una inhibición significativa de la agregación plaquetaria producida por colágeno y amplificada por 5-HT a concentraciones entre 0.1 mg/kg y 5 mg/kg. Y por otra parte, en el ensayo *in vivo* de trombosis, sangrado, agregación y PK de rata, la DE₅₀ generada para el Compuesto 5 para el tiempo necesario para lograr la oclusión y el tiempo de sangrado en este modelo indicó que había una separación de aproximadamente 7 veces (en escala logarítmica) en este índice terapéutico.

Sumado a lo anterior, me permito señalar que existen datos adicionales publicados que también demuestran las ventajas técnicas de los compuestos de la invención sobre las enseñanzas del documento D1. Por ejemplo, en el documento Journal of Medicinal Chemistry (2010), 53(11), páginas 4412 – 4421 de Xiong y colaboradores (el cual se adjunta al presente memorial) se describen varios estudios

de la Relación Actividad-Estructura llevados a cabo por el Solicitante en relación con los compuestos de la invención. En este sentido, **la Tabla 1 de la página 4414 de este artículo además de mostrar las afinidades de unión al receptor 5-HT_{2A} humano de 21 compuestos (tanto descritos en la presente solicitud como cercanamente relacionados con los mismos), también muestra la capacidad de dichos compuestos para inhibir la amplificación de la agregación plaquetaria humana estimulada por ADP mediada por serotonina.**

Finalmente, me permito dirigir la atención del señor Examinador hacia el **compuesto 733 de la presente solicitud (Ejemplo 1.88) y que también se conoce como APD791.** Dicho compuesto recientemente fue el objeto de ensayos clínicos humanos Fase 1a y Fase 1b exitosos. En estos ensayos, que en primer lugar fueron diseñados para evaluar la seguridad y tolerabilidad de dosis únicas y múltiples ascendentes, el compuesto APD791 generalmente fue bien tolerado, y fue demostrada la inhibición dependiente de dosis de la amplificación de la agregación de plaquetas mediada por serotonina. Además, estos ensayos mostraron que el APD791 fue rápidamente absorbido y que las cantidades absorbidas estaban relacionadas con la dosis. Por último, **los estudios clínicos mostraron que debido a la capacidad del APD791 para bloquear la activación del receptor 5-HT_{2A} en las plaquetas y en otros tejidos cardiovasculares, el APD791 pudo frenar los efectos mediados por serotonina en el ámbito clínico, logrando de este modo reducir o prevenir la trombosis.**

En conclusión, la persona versada en la materia reconocerá que debido a que **nada en D1 enseña o sugiere la modificación de los compuestos allí descritos para la obtención de compuestos como los de la presente solicitud, que contienen específicamente los sustituyentes -X-Y-Z-R₈ y -V-W-R₄ sobre el anillo central de 3-amino fenil pirazol-3-ilo; ni D1 enseña o sugiere que los compuestos así modificados pudieran tener hasta 17 veces más potencia que los compuestos de D1 en los ensayos de acumulación de fosfato de inositol, lo que indica que los compuestos de la presente solicitud tienen una mayor potencia que los compuestos de D1 para inhibir el receptor 5-HT_{2A} de la serotonina; ni tampoco D1 enseña o sugiere que tales compuestos como los de la presente solicitud pudieran ser usados en ensayos clínicos humanos y que inhibieran satisfactoriamente la amplificación de la agregación plaquetaria y por lo tanto redujeran o previnieran la trombosis; es a todas luces evidente que los compuestos de la presente solicitud sí tienen efectos técnicos sorprendentes e inesperados frente a las enseñanzas de D1, y que además la solicitud en cuestión no puede ser obvia o evidente a partir de las enseñanzas del documento D1 y por lo tanto goza de nivel inventivo frente a las enseñanzas de este documento que es considerado el estado del arte más cercano a la invención.**

Dentro de este marco de ideas, me permito referirme al señalamiento que hace el Tribunal de Justicia de la Comunidad Andina con respecto a las definiciones de "obvio" y "evidente" en la evaluación del Nivel Inventivo de una solicitud de patente¹:

"Resulta pertinente, a los efectos del artículo 4 de la Decisión 344, definir qué se entiende por "obvio" y por "evidente" a fin de determinar qué se debe entender por nivel inventivo.

Lo obvio es aquello que salta a la vista de manera clara y directa; lo "que se encuentra o pone delante de los ojos, muy claro o que no tiene dificultad". Mientras que lo evidente consiste en la "certeza clara y manifiesta de la que no se puede dudar; cierto, claro, patente y sin la menor duda". De forma que algo que resulte obvio no es necesariamente evidente; empero lo que es evidente, es también obvio.

En este orden de ideas, se puede concluir que una invención goza de nivel inventivo cuando a los ojos de un experto medio en el asunto de que se trate, se necesita algo más que la simple aplicación de los conocimientos técnicos en la materia para llegar a ella, es decir, que de conformidad con el estado de la técnica el invento no sea consecuencia clara y directa de dicho estado."

De esta forma, para el caso que nos compete, claramente a partir de las enseñanzas del documento D1 no "saltan a la vista de manera clara y directa" compuestos derivados de 3-amino fenil pirazol-3-ilo que tengan específicamente los sustituyentes -X-Y-Z-R₈ y -V-W-R₄ contenidos en todos compuestos que son objeto de la presente invención, ni a partir de las enseñanzas de D1 se tiene "certeza clara y manifiesta" de que los compuestos así modificados pudieran tener hasta 17 veces más potencia que los compuestos de D1 en los ensayos de acumulación de fosfato de inositol, lo que indica que los compuestos de la presente solicitud tienen una mayor potencia que los compuestos de D1 para inhibir el receptor 5-HT_{2A} de la serotonina, ni se tiene "certeza clara y manifiesta" a partir de dicho documento D1 de que tales compuestos pudieran ser usados en ensayos clínicos humanos, y mucho menos se tiene "certeza clara y manifiesta" a partir de D1 de que en tales ensayos clínicos humanos dichos compuestos inhibieran satisfactoriamente la amplificación de la agregación plaquetaria y por lo tanto redujeran o previnieran la trombosis.

En vista de estos hechos es evidente que la solicitud en estudio conlleva nivel inventivo frente al estado del arte más próximo.

¹ Tribunal de Justicia de la Comunidad Andina, PROCESO 192-IP-2005

Por otra parte, me permito señalar que las reivindicaciones 36 y 37 gozan de nivel inventivo frente a las enseñanzas del documento D1 toda vez que dichas reivindicaciones son dependientes de la reivindicación 1 ahora modificada, la cual es inventiva frente a las enseñanzas de D1 por las razones discutidas anteriormente.

4.2. Consideraciones relativas al documento US 4,409,231

Por otro lado, en vista de que el señor Examinador en su Concepto Técnico antes mencionado hizo referencia al documento US 4,409,231 en el cuadro de comparación de las características técnicas esenciales de la solicitud con las del estado de la Técnica, me permito manifestar que es deseo del solicitante exponer algunos argumentos por medio de los cuales se pone de manifiesto que la presente solicitud tampoco resulta obvia a partir de las enseñanzas del documento US 4,409,231.

En primer lugar, el documento US 4,409,231 revela un pequeño grupo de ariloxiamino propanoles que presuntamente son usados como agentes anti-hipertensivos y β -adrenolíticos. Sin embargo, en ninguna parte de dicho documento se hace mención a los receptores 5-HT_{2A} o a la propia serotonina.

En segundo lugar, los compuestos reivindicados por el documento US 4,409,231 no se solapan con ninguno de los compuestos de la presente invención, entre otras cosas porque, como lo señala el señor Examinador en el cuadro comparativo de su Concepto Técnico (folio 542), no hay ningún sustituyente en los compuestos reclamados por US 4,409,231 que corresponda al grupo R₄ de la solicitud en trámite (el cual es heterobicíclico, heterocíclico, o heteroaril opcionalmente sustituido).

Adicionalmente, me permito señalar que una especie revelada en el documento US 4,409,231 contiene un anillo heterocíclico unido al anillo de fenilo central a través de un conector de alquileo. Sin embargo, esta especie no se encuentra dentro del alcance de la reivindicación 1 ahora modificada en virtud de la salvedad que esta contiene.

En conclusión, el documento US 4,409,231 no enseña o sugiere que alguno de los compuestos allí revelados podría ser usado para modular los receptores 5-HT_{2A} o para prevenir la trombosis en humanos y mucho menos enseña o sugiere los compuestos de la presente solicitud, por lo que es a todas luces evidente que la reivindicación 1 ahora modificada de la solicitud en cuestión es inventiva frente a las enseñanzas del documento US 4,409,231.

Aparte de lo planteado previamente en el presente escrito, atentamente me permito destacar ante ese Despacho que las solicitudes correspondientes a la presente solicitud, con el mismo objeto y alcance que la solicitud colombiana, han sido concedidas en la Oficina Europea de Patentes y en Chile a pesar que los documentos WO 2004058722 (D1) y US 4,409,231 fueron citados como anterioridades en contra de las solicitudes correspondientes. Sin embargo, teniendo en cuenta los argumentos anteriormente discutidos dichas Oficinas de Patentes consideraron que los mencionados documentos no afectan la novedad ni el nivel inventivo de la solicitud de patente en cuestión.

Ante todo, con el anterior comentario de ninguna manera pretendo desconocer la independencia de la que goza la Superintendencia de Industria y Comercio al momento de analizar los méritos de patentabilidad de una solicitud, de acuerdo con las disposiciones del Convenio de París para la protección de la propiedad industrial.

Sin embargo, sí considero de gran importancia resaltar el hecho de que la Oficina Europea de Patentes y su equivalente de Chile han resuelto que los documentos WO 2004058722 y US 4,409,231 no afectan la novedad ni el nivel inventivo de la solicitud de patente en estudio, hecho que indiscutiblemente representa un claro indicio de la patentabilidad de la presente invención.

5. Anexo me permito presentar:

- (a) Copia de las páginas **118** a **130** que corresponden al capítulo reivindicatorio ahora modificado que consta de **37** reivindicaciones.
- (b) Copia de las páginas 221 a 233 del libro editado por R. Hilfiker, *"Polymorphism in the Pharmaceutical Industry"*, Wiley-VCH, 2006, Chapter 8 "The importance of Solvates".
- (c) Copia de las páginas 183 a 227 del libro editado por Harry G. Brittain, *"Polymorphism in Pharmaceutical Solids"*, Vol. 95, Marcel Dekker, Inc. New York, 1999.
- (d) Copia del documento de Vippagunta y col., *Advanced Drug Delivery Reviews*, Vol 48 (2001), pages 3-26.
- (e) Copia de la publicación PCT WO2011/075596
- (f) Copia del documento de Xiong y col., *Journal of Medicinal Chemistry* (2010), Vol. 53, Numero 11, páginas 4412 - 4421.

JOSE LLOREDA CAMACHO & CO.

Calle 72 No. 5-83 Piso 5, Bogotá, D.C. Tel: 6069700 Fax 6069701

6. En vista de lo anterior, ruego a usted proceder a estudiar nuevamente la solicitud a la luz de los argumentos y modificaciones presentados y conceder el privilegio de patente solicitado.

Señor Superintendente,


ALICIA LLOREDA RICAURTE

T.P. 53.215

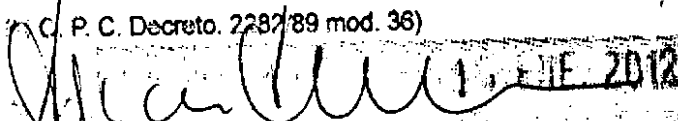
NOTARIA 41 DEL CIRCULO DE BOGOTÁ D.C.
DEPARTAMENTO DE CUNDINAMARCA
REPÚBLICA DE COLOMBIA

Mario Albeiro
Eliana Díaz
C.C. 20.056.778
Vello. *for*

El Notario 41 del círculo de Bogotá, da fe de que compareció personalmente Alicia Lloreda Ricaurte identificada (o) con: 39.690.713 y tarjeta profesional de abogado No. 53.215 del Consejo Superior de la Judicatura, quien autentica la firma puesta por ella (e) mismo en este escrito.

18 ENE. 2012

(Art. 10 C. P. C. Decreto. 2282/89 mod. 36)

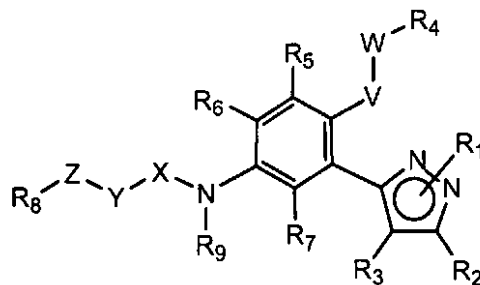

ALICIA LLOREDA RICAURTE

C.C. No. 39.690.713 de Usaquén



REIVINDICACIONES

1. Un compuesto seleccionado a partir de los compuestos de la fórmula (Ia):



(Ia)

y sales, hidratos, y solvatos farmacéuticamente aceptables de los mismos;

en donde:

V es O;

W es alquileo C₁₋₄ opcionalmente sustituido con 1 a 8 sustituyentes seleccionados independientemente a partir de: alquil C₁₋₃, alcoxi C₁₋₄, carboxi, ciano, haloalquil C₁₋₃, halógeno y oxo; o W está ausente;

X es C(=O), C(=S) o está ausente;

Y es O, NR₁₁ o está ausente;

Z es alquileo C₁₋₄ o cicloalquileo C₃₋₆, cada uno opcionalmente sustituido con 1 a 8 sustituyentes seleccionados independientemente a partir de: alquilo C₁₋₃, alcoxi C₁₋₄, carboxi, ciano, haloalquilo C₁₋₃, halógeno, hidroxil y oxo; o Z está ausente;

R₁ se selecciona a partir de: H, alquil C₁₋₆, alquenil C₂₋₆, alquinilo C₂₋₆ y cicloalquil C₃₋₇;

R₂ se selecciona a partir de: H, acil C₁₋₆, aciloxi C₁₋₆, alquenil C₂₋₆, alcoxi C₁₋₆, alquil C₁₋₆, alquilcarboxamida C₁₋₆, alquinil C₂₋₆, alquilsulfonamida C₁₋₆, alquilsulfinil C₁₋₆, alquilsulfonil C₁₋₆, alquiltio C₁₋₆, alquiltio C₁₋₆, alquiltio C₁₋₆, amino, alquilamino C₁₋₆, dialquilamino C₂₋₈, carbo-alcoxi C₁₋₆, carboxamida, carboxi, ciano, cicloalquil C₃₋₇, dialquilcarboxamida C₂₋₈, dialquilsulfonamida C₂₋₈, halógeno, haloalcoxi C₁₋₆, haloalquil C₁₋₆, haloalquilsulfinil C₁₋₆, haloalquilsulfonil C₁₋₆, haloalquiltio C₁₋₆, hidroxil, tiol, nitro y sulfonamida;

R₃ se selecciona a partir de: H, alquenil C₂₋₆, alquil C₁₋₆, alquilcarboxamida C₁₋₆, alquinil C₂₋₆, alquilsulfonamida C₁₋₆, carbo-alcoxi C₁₋₆, carboxamida, carboxi, ciano, cicloalquil C₃₋₇, dialquilcarboxamida C₂₋₈, halógeno, heteroaril y fenil;

en donde cada uno de dichos grupos alquenil C₂₋₆, alquil C₁₋₆, alquinil C₂₋₆, alquilsulfonamida C₁₋₆, cicloalquil C₃₋₇, heteroaril y fenil está opcionalmente sustituido con 1 a 5 sustituyentes seleccionados independientemente a partir de: acil C₁₋₅, aciloxi C₁₋₅, alquenil C₂₋₆, alcoxi C₁₋₄, alquil C₁₋₈, alquilamino C₁₋₆, dialquilamino C₂₋₈, alquilcarboxamida C₁₋₄, alquinil C₂₋₆, alquilsulfonamida C₁₋₄, alquilsulfinil C₁₋₄, alquilsulfonil C₁₋₄, alquiltio C₁₋₄, alquiltio C₁₋₄, alquiltio C₁₋₄, amino, carbo-alcoxi C₁₋₆, carboxamida, carboxi, ciano, cicloalquil C₃₋₆, dialquilcarboxamida C₂₋₆, halógeno, haloalcoxi C₁₋₄, haloalquil C₁₋₄, haloalquilsulfinil C₁₋₄, haloalquilsulfonil C₁₋₄, haloalquiltio C₁₋₄, hidroxil, nitro y sulfonamida;

R₄ es heterobicíclico, heterocíclico, o heteroaril,

cada uno opcionalmente substituido con substituyentes seleccionados independientemente a partir de: acil C₁₋₆, aciloxi C₁₋₁₂, alquenil C₂₋₆, alcoxi C₁₋₄, alcóxicarbonilamino C₁₋₆, alquil C₁₋₆, alquilamino C₁₋₆, dialquilamino C₂₋₈, alquilcarboxamida C₁₋₄, alquinil C₂₋₆, alquilsulfonamida C₁₋₄, alquilsulfinil C₁₋₄, alquilsulfonil C₁₋₄, alquiltio C₁₋₄, alquilureil C₁₋₄, amino, carbo-alcoxi C₁₋₆, carboxamida, carboxi, ciano, cicloalquil C₃₋₆, cicloalquilcarbonil C₃₋₇, dialquilcarboxamida C₂₋₆, formil, halógeno, haloalcoxi C₁₋₄, haloalquil C₁₋₄, haloalquilsulfinil C₁₋₄, haloalquilsulfonil C₁₋₄, haloalquiltio C₁₋₄, heteroaril, hidroxil, nitro, fenil y sulfonamida;

en donde dicho acil C₁₋₆, aciloxi C₁₋₁₂, alcoxi C₁₋₄, alquil C₁₋₆, alquilcarboxamida C₁₋₄, amino, carbo-alcoxi C₁₋₆ y heteroaril está cada uno opcionalmente substituido con substituyentes seleccionados independientemente a partir de: alquil C₁₋₆, acil C₁₋₆, alcoxi C₁₋₄, alquilamino C₁₋₆, dialquilamino C₂₋₈, alquilcarboxamida C₁₋₄, alquilsulfonil C₁₋₄, amino, carbo-alcoxi C₁₋₆, carboxamida, carboxi, ciano, cicloalquil C₃₋₆, halógeno, haloalcoxi C₁₋₄, haloalquil C₁₋₄, hidroxil y fenil;

R₅, R₆, y R₇ se seleccionan cada uno independientemente a partir de: H, acil C₁₋₆, aciloxi C₁₋₆, alquenil C₂₋₆, alcoxi C₁₋₆, alquil C₁₋₆, alquilcarboxamida C₁₋₆, alquinil C₂₋₆, alquilsulfonamida C₁₋₆, alquilsulfinil C₁₋₆, alquilsulfonil C₁₋₆, alquiltio C₁₋₆, alquilureil C₁₋₆, amino, alquilamino C₁₋₆, dialquilamino C₂₋₈, alquilimino C₁₋₆, carbo-alcoxi C₁₋₆, carboxamida, carboxi, ciano, cicloalquil C₃₋₇, dialquilcarboxamida C₂₋₈, dialquilsulfonamida C₂₋₈, halógeno, haloalcoxi C₁₋₆, haloalquil C₁₋₆, haloalquilsulfinil C₁₋₆, haloalquilsulfonil C₁₋₆, haloalquiltio C₁₋₆, heterocíclico, hidroxil, tiol, nitro, fenoxi y fenil;

R₈ es alquil C₁₋₈, alquenil C₂₋₆, aril, cicloalquil C₃₋₇ o heteroaril,

cada uno opcionalmente substituido con substituyentes seleccionados independientemente a partir de: acil C₁₋₆, aciloxi C₁₋₆, alquenil C₂₋₆, alcoxi C₁₋₆, alquil C₁₋₆, alquilcarboxamida C₁₋₆, alquinil C₂₋₆, alquilsulfonamida C₁₋₆, alquilsulfinil C₁₋₆, alquilsulfonil C₁₋₆, alquiltio C₁₋₆, alquilureil C₁₋₆, amino, alquilamino C₁₋₆, dialquilamino C₂₋₈, alquilimino C₁₋₆, carbo-alcoxi C₁₋₆, carboxamida, carboxi, ciano, cicloalquil C₃₋₇, dialquilcarboxamida C₂₋₈, dialquilsulfonamida C₂₋₈, halógeno, haloalcoxi C₁₋₆, haloalquil C₁₋₆, haloalquilsulfinil C₁₋₆, haloalquilsulfonil C₁₋₆, haloalquiltio C₁₋₆, heterocíclico, hidroxil, tiol, nitro, fenoxi y fenil,

o dos substituyentes adyacentes junto con dicho aril o dicho heteroaril forman un cicloalquil C₅₋₇ que opcionalmente comprende 1 a 2 átomos de oxígeno y opcionalmente está substituido con F, Cl o Br;

en donde dicho alquenil C₂₋₆, alquil C₁₋₆, alquinil C₂₋₆, alquilamino C₁₋₆, alquilimino C₁₋₆, dialquilamino C₂₋₈, heterocíclico y fenil está cada uno opcionalmente substituido con 1 a 5 substituyentes seleccionados independientemente a partir de: acil C₁₋₆, aciloxi C₁₋₆, alquenil C₂₋₆, alcoxi C₁₋₆, alquil C₁₋₆, alquilcarboxamida C₁₋₆, alquinil C₂₋₆, alquilsulfonamida C₁₋₆, alquilsulfinil C₁₋₆, alquilsulfonil C₁₋₆, alquiltio C₁₋₆, alquilureil C₁₋₆, amino, alquilamino C₁₋₆, dialquilamino C₂₋₈, carbo-alcoxi C₁₋₆, carboxamida, carboxi, ciano, cicloalquil C₃₋₇, dialquilcarboxamida C₂₋₈, halógeno, haloalcoxi C₁₋₆, haloalquil C₁₋₆, haloalquilsulfinil C₁₋₆, haloalquilsulfonil C₁₋₆, haloalquiltio C₁₋₆, hidroxil, tiol y nitro;

R₉ y R₁₁ son cada uno independientemente H o alquil C₁₋₈;

siempre que R₄ sea un grupo diferente de un grupo oxiranil;

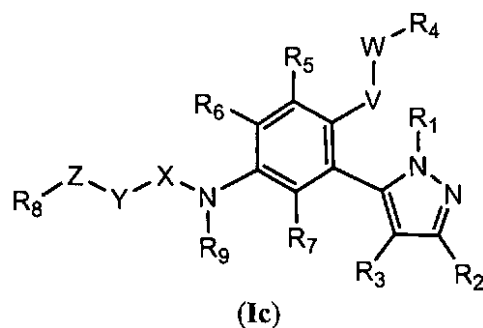
en donde;

"heteroaril" indica un sistema de anillos aromáticos que puede ser un solo anillo, dos anillos fusionados o tres anillos fusionados en donde por lo menos un carbono del anillo está remplazado por un heteroátomo seleccionado de: O, S y N; en donde N está opcionalmente sustituido con H, acilo C_{1-4} , o alquilo C_{1-4} ;

"heterobicíclico" indica un anillo bicíclico no aromático, en donde 1, 2 o 3 carbonos del anillo están remplazados por un heteroátomo seleccionado de: O, S, $S(=O)$, $S(=O)_2$ y NH; en donde el nitrógeno está opcionalmente sustituido con acilo C_{1-4} o alquilo C_{1-4} ; y 1 o 2 carbonos de anillo están opcionalmente sustituidos con oxo o tiooxo; y

"heterocíclico" indica un anillo de carbonos, no aromático en donde uno, dos o tres carbonos de anillo están remplazados por un heteroátomo seleccionado de: O, S, $S(=O)$, $S(=O)_2$, NH; en donde N está opcionalmente sustituido con acilo C_{1-4} o alquilo C_{1-4} ; y los átomos de carbono del anillo están opcionalmente sustituidos con oxo o tiooxo.

2. Un compuesto de acuerdo con la reivindicación 1 seleccionado a partir de los compuestos que tienen la Fórmula (Ic), y sales, hidratos, y solvatos farmacéuticamente aceptables de los mismos:



3. Un compuesto de acuerdo con la reivindicación 1 o la reivindicación 2, en donde W es - CH_2 -, - CH_2CH_2 -, - $CH_2C(=O)$ -, - $CH_2CH_2CH_2$ -, - $C(CH_3)_2C(=O)$ -, - $CH_2CH(CH_3)$ -, - $CH(CH_3)CH_2$ -, - $C(CH_3)_2CH_2$ -, o - $CH_2C(CH_3)_2$ -.
4. Un compuesto de acuerdo con la reivindicación 1 o la reivindicación 2, en donde W está ausente.
5. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 4, en donde X es $C(=O)$.
6. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 4, en donde X está ausente.
7. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 6, en donde Y es NH, O o está ausente.

8. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 7, en donde Z está ausente, o es $-\text{CH}_2-$, $-\text{CH}(\text{OH})-$, $-\text{CF}_2-$, $-\text{C}(\text{CH}_3)_2-$, 1,1-ciclopropil, $-\text{CH}_2\text{CH}_2-$, $-\text{CH}_2\text{CH}_2\text{CH}_2-$, $-\text{CH}(\text{CH}_3)-$ o $-\text{C}(=\text{O})-$.
9. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 8, en donde R_1 es $-\text{CH}_3$.
10. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 9, en donde R_2 es H.
11. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 10, en donde R_3 es H, F, Cl o Br.
12. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 11, en donde R_4 es heterobicíclico, heterocíclico, o heteroaril,

cada uno opcionalmente substituido con substituyentes seleccionados independientemente a partir de: acil C_{1-6} , aciloxi C_{1-12} , alcoxycarbonilamino C_{1-6} , alcoxi C_{1-4} , alquil C_{1-6} , alquilcarboxamida C_{1-4} , alquilsulfonamida C_{1-4} , alquilsulfonil C_{1-4} , alquiltireil C_{1-4} , amino, carbo-alcoxi C_{1-6} , carboxamida, carboxi, dialquilcarboxamida C_{2-6} , formil, halógeno, haloalquil C_{1-4} , heteroaril, hidroxil y fenil;

en donde dicho acil C_{1-6} , aciloxi C_{1-12} , alcoxi C_{1-4} , alquil C_{1-6} , alquilcarboxamida C_{1-4} , amino, carbo-alcoxi C_{1-6} y heteroaril está cada uno opcionalmente substituido con substituyentes seleccionados independientemente del grupo que consiste de alquil C_{1-6} , carbo-alcoxi C_{1-6} , carboxi y fenil;

siempre que R_4 sea un grupo diferente de un grupo oxiranil.

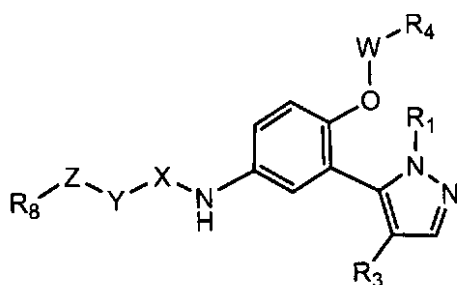
13. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 11, en donde R_4 se selecciona a partir de:

pirrolidin-1-il, pirrolidin-2-il, piperidin-1-il, piperidin-4-il, piperidin-3-il, morfolin-4-il, piperazin-1-il, piridin-3-il, piridin-2-il, piridin-4-il, azetidin-1-il, tiomorfolin-4-il, morfolin-2-il, 2,5-diaza-biciclo[2,2,1]hept-2-il, [1,4]oxazepan-4-il, 1,1-dioxo-1 λ^6 -tiomorfolin-4-il, piperidin-2-il, azepan-1-il, pirrolidin-3-il, 3-oxo-piperazin-1-il, 7-aza-biciclo[2,2,1]hept-7-il e imidazol-1-il,

cada uno opcionalmente sustituido con substituyentes seleccionados independientemente a partir de: CH_3 , $\text{C}(=\text{O})\text{O}-t\text{-butil}$, $\text{C}(=\text{O})\text{OH}$, $\text{C}(=\text{O})\text{OEt}$, $\text{NHC}(=\text{O})\text{O}-t\text{-butil}$, OH , $\text{C}(=\text{O})\text{NHCH}_2\text{C}(=\text{O})\text{OCH}_3$, $\text{NHC}(=\text{O})\text{CH}_2\text{C}(=\text{O})\text{OCH}_3$, $\text{C}(=\text{O})\text{NHCH}_2\text{C}(=\text{O})\text{OH}$, $\text{NHC}(=\text{O})\text{CH}_2\text{C}(=\text{O})\text{OH}$, $\text{C}(=\text{O})\text{OCH}_3$, $\text{OC}(=\text{O})\text{CH}_2\text{CH}_2\text{C}(=\text{O})\text{OCH}_3$, $\text{OC}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$, $\text{CH}_2\text{C}(=\text{O})\text{OCH}_2\text{CH}_3$, OCH_3 , $\text{CH}_2\text{C}(=\text{O})\text{OH}$, $\text{OC}(=\text{O})\text{CH}_2\text{CH}_2\text{C}(=\text{O})\text{OCH}_3$, $\text{CH}_2\text{CH}_2\text{C}(=\text{O})\text{OCH}_3$, $\text{C}(=\text{O})\text{CH}_3$, $\text{C}(=\text{O})\text{OCH}_2\text{-fenil}$, $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{C}(=\text{O})\text{OCH}_3$, $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{C}(=\text{O})\text{OH}$, F, fenil, $\text{CH}_2\text{C}(=\text{O})\text{OCH}_3$, $\text{S}(=\text{O})_2\text{CH}_3$, $\text{OCH}_2\text{-fenil}$, $\text{CH}_2\text{-fenil}$, $\text{C}(=\text{O})\text{NH}_2$, CHO, $-\text{NH}_2$, $\text{NHC}(=\text{O})\text{CH}_3$, $\text{C}(=\text{O})\text{N}(\text{CH}_3)_2$, $\text{NHS}(=\text{O})_2\text{CH}_3$, $-\text{CF}_3$, 3-metil-[1,2,4]oxadiazol-5-il y $\text{CH}(\text{CH}_3)_2$.

14. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 13, en donde R_5 , R_6 y R_7 son todos H.
15. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 14, en donde R_8 es alquil C_{1-6} , alquenil C_{2-6} , aril, cicloalquil C_{3-7} o heteroaril,
 cada uno opcionalmente sustituido con sustituyentes seleccionados independientemente a partir de: acil C_{1-6} , alcoxi C_{1-6} , alquil C_{1-6} , ciano, halógeno, haloalcoxi C_{1-6} , haloalquil C_{1-6} e hidroxil,
 o dos sustituyentes adyacentes junto con dicho aril o dicho heteroaril forman un cicloalquil C_{5-7} que opcionalmente comprende 1 a 2 átomos de oxígeno y opcionalmente está sustituido con F.
16. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 14, en donde R_8 se selecciona a partir de:
 metil, *iso*-propil, *iso*-butil, *n*-propil, *n*-butil, 2-metil-propenil, fenil, naftalen-1-il, ciclopropil, ciclobutil, ciclopentil, piridin-2-il, piridin-3-il, 1*H*-benzoimidazol-2-il, benzooxazol-2-il, benzotiazol-2-il, tiofen-2-il, furan-2-il, benzotiofen-2-il, tiazol-2-il, isoxazol-3-il, y piridin-4-il,
 cada uno opcionalmente sustituido con sustituyentes seleccionados independientemente a partir de: $C(=O)CH_3$, OCH_3 , CH_3 , F, Cl, Br, CF_3 , hidroxil, OCF_3 , y CN,
 o dos sustituyentes adyacentes junto con dicho fenil forman un cicloalquil C_5 que comprende 2 átomos de oxígeno y opcionalmente está sustituido con F.
17. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 14, en donde R_8 se selecciona a partir de:
 4-cloro-fenil, 2,4-difluoro-fenil, 4-fluoro-fenil, 3-cloro-fenil, 2,2-difluoro-benzo[1,3]dioxol-5-il, 4-hidroxi-fenil, 4-cloro-2-hidroxi-fenil, fenil, 3-fluoro-fenil, 2-fluoro-fenil, 2-cloro-fenil, 4-bromo-fenil, 4-metoxi-fenil, 4-trifluorometil-fenil, 3,5-bis-trifluorometil-fenil, 2-fluoro-5-metil-fenil, 3-metoxi-fenil, 3-acetil-fenil, 4-metil-fenil, 3-trifluorometil-fenil, 3,5-difluoro-fenil, 2,4-dicloro-fenil, 4-cloro-2-trifluorometil-fenil, 3,4-difluoro-fenil, 2,5-difluoro-fenil, 2,6-difluoro-fenil, naftalen-1-il, 4-trifluorometoxi-fenil, 3-ciano-fenil, 2-trifluorometoxi-fenil, 4-cloro-2-fluoro-fenil, 2,3-difluoro-fenil, 2,4,5-trifluoro-fenil, 2,3,4-trifluoro-fenil, 3,4-dicloro-fenil, 4-fluoro-3-trifluorometil-fenil, 5-fluoro-2-trifluorometil-fenil, 2-trifluorometil-fenil, 3-metil-fenil, 2-fluoro-4-trifluorometil-fenil, 4-cloro-3-fluoro-fenil, 3-fluoro-4-metil-fenil, 4-fluoro-3-metil-fenil, 3-fluoro-4-trifluorometil-fenil, 3-cloro-4-fluoro-fenil, 2,6-dicloro-fenil, 4-ciano-fenil, 2,5-dicloro-fenil y benzo[1,3]dioxol-5-il.
18. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 14, en donde R_8 se selecciona a partir de:
 metil, *iso*-propil, *iso*-butil, *n*-propil, *n*-butil, 2-metil-propenil, 3-metil-butyl, ciclopropil, ciclobutil y ciclopentil.

19. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 14, en donde R_8 se selecciona a partir de:
- piridin-3-il, 6-trifluorometil-piridin-3-il, 3-hidroxi-piridin-2-il, 6-metil-piridin-3-il, 6-hidroxi-piridin-3-il, 1*H*-benzoimidazol-2-il, benzooxazol-2-il, benzotiazol-2-il, tiofen-2-il, furan-2-il, 5-cloro-tiofen-2-il, benzotiofen-2-il, tiazol-2-il, 5-metil-isoxazol-3-il y piridin-4-il.
20. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 19, en donde R_9 es H.
21. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 20, en donde R_{11} es H.
22. Un compuesto de acuerdo con la reivindicación 1, seleccionado a partir de los compuestos que tienen la Fórmula (Ik):



(Ik)

y sales, hidratos, y solvatos farmacéuticamente aceptables de los mismos;
en donde:

W es $-\text{CH}_2-$, $-\text{CH}_2\text{CH}_2-$, $-\text{CH}_2\text{C}(=\text{O})-$, $-\text{CH}_2\text{CH}_2\text{CH}_2-$, $-\text{C}(\text{CH}_3)_2\text{C}(=\text{O})-$, $-\text{CH}_2\text{CH}(\text{CH}_3)-$, $-\text{CH}(\text{CH}_3)\text{CH}_2-$, $-\text{C}(\text{CH}_3)_2\text{CH}_2-$ o $-\text{CH}_2\text{C}(\text{CH}_3)_2-$, o W está ausente;

X es $\text{C}(=\text{O})$ o está ausente;

Y es NH, O o está ausente;

Z está ausente, o es $-\text{CH}_2-$, $-\text{CH}(\text{OH})-$, $-\text{CF}_2-$, $-\text{C}(\text{CH}_3)_2-$, 1,1-ciclopropil, $-\text{CH}_2\text{CH}_2-$, $-\text{CH}_2\text{CH}_2\text{CH}_2-$, $-\text{CH}(\text{CH}_3)-$ o $-\text{C}(=\text{O})-$;

R_1 es alquil C_{1-6} ;

R_3 es H o halógeno;

R_4 es heterobicíclico, heterocíclico, o heteroaril,

cada uno opcionalmente sustituido con sustituyentes seleccionados independientemente a partir de: acil C_{1-6} , aciloxi C_{1-12} , alcoxi C_{1-4} , alcoxicarbonilamino C_{1-6} , alquil C_{1-6} , alquilcarboxamida C_{1-4} , alquilsulfonamida C_{1-4} , alquilsulfonil C_{1-4} , alquiltireil C_{1-4} , amino, carbo-alcoxi C_{1-6} , carboxamida, carboxi, dialquilcarboxamida C_{2-6} , formil, halógeno, haloalquil C_{1-4} , heteroaril, hidroxil y fenil;

en donde dicho acil C_{1-6} , aciloxi C_{1-12} , alcoxi C_{1-4} , alquil C_{1-6} , alquilcarboxamida C_{1-4} , amino, carbo-alcoxi C_{1-6} y heteroaril está cada uno opcionalmente sustituido con sustituyentes seleccionados independientemente a partir de: alquil C_{1-6} , carbo-alcoxi C_{1-6} , carboxi y fenil; y

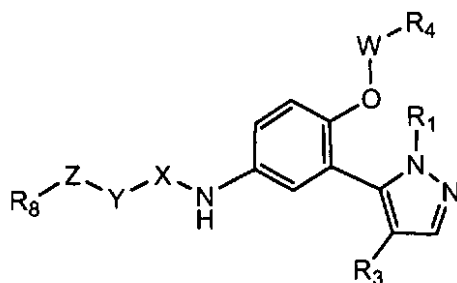
R_8 es alquil C_{1-6} , alquenil C_{2-6} , aril, cicloalquil C_{3-7} o heteroaril,

cada uno opcionalmente sustituido con sustituyentes seleccionados independientemente a partir de: acil C₁₋₆, alcoxi C₁₋₆, alquil C₁₋₆, alquilsulfonil C₁₋₆, amino, alquilamino C₁₋₆, dialquilamino C₂₋₈, alquilimino C₁₋₆, carbo-alcoxi C₁₋₆, carboxamida, carboxi, ciano, cicloalquil C₃₋₇, halógeno, haloalcoxi C₁₋₆, haloalquil C₁₋₆, heterocíclico, hidroxil, nitro y fenil,

o dos sustituyentes adyacentes junto con dicho aril o dicho heteroaril forman un cicloalquil C₅₋₇ que opcionalmente comprende 1 a 2 átomos de oxígeno y opcionalmente está sustituido con F;

siempre que R₄ sea un grupo diferente de un grupo oxiranil.

23. Un compuesto de acuerdo con la reivindicación 1, seleccionado a partir de los compuestos que tiene la Fórmula (Ik):



(Ik)

y sales, hidratos, y solvatos farmacéuticamente aceptables de los mismos;

en donde:

W es -CH₂-, -CH₂CH₂-, -CH₂C(=O)-, -CH₂CH₂CH₂-, -C(CH₃)₂C(=O)-, -CH₂CH(CH₃)-, -CH(CH₃)CH₂-, -C(CH₃)₂CH₂- o -CH₂C(CH₃)₂-, o W está ausente;

X es C(=O) o está ausente;

Y es NH, O o está ausente;

Z está ausente, o es -CH₂-, -CH(OH)-, -CF₂-, -C(CH₃)₂-, 1,1-ciclopropil, -CH₂CH₂-, -CH₂CH₂CH₂-, -CH(CH₃)- o -C(=O)-;

R₁ es alquil C₁₋₆;

R₃ es H o halógeno;

R₄ es heterobícíclico, heterocíclico, o heteroaril,

cada uno opcionalmente sustituido con sustituyentes seleccionados independientemente a partir de: acil C₁₋₆, aciloxi C₁₋₁₂, alcoxi C₁₋₄, alcoxycarbonilamino C₁₋₆, alquil C₁₋₆, alquilcarboxamida C₁₋₄, alquilsulfonamida C₁₋₄, alquilsulfonil C₁₋₄, alquileil C₁₋₄, amino, carbo-alcoxi C₁₋₆, carboxamida, carboxi, dialquilcarboxamida C₂₋₆, formil, halógeno, haloalquil C₁₋₄, heteroaril, hidroxil y fenil;

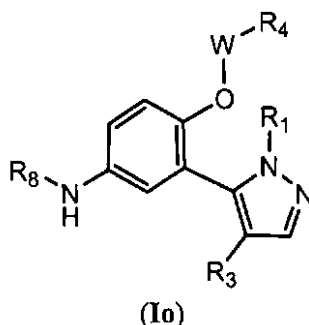
en donde dicho acil C₁₋₆, aciloxi C₁₋₁₂, alcoxi C₁₋₄, alquil C₁₋₆, alquilcarboxamida C₁₋₄, amino, carbo-alcoxi C₁₋₆ y heteroaril está cada uno opcionalmente sustituido con sustituyentes seleccionados independientemente a partir de: alquil C₁₋₆, carbo-alcoxi C₁₋₆, carboxi y fenil; y

R₈ se selecciona a partir de: 4-cloro-fenil, 2,4-difluoro-fenil, 4-fluoro-fenil, 3-cloro-fenil, 2,2-difluoro-benzo[1,3]dioxol-5-il, 4-hidroxi-fenil, 4-cloro-2-hidroxi-fenil, fenil, 3-fluoro-fenil, 2-fluoro-fenil, 2-cloro-fenil, 4-bromo-fenil, 4-metoxi-fenil, 4-trifluorometil-fenil, 3,5-bis-trifluorometil-fenil, 2-fluoro-5-metil-fenil, 3-metoxi-fenil, 3-acetil-fenil, 4-

metil-fenil, 3-trifluorometil-fenil, 3,5-difluoro-fenil, 2,4-dicloro-fenil, 4-cloro-2-trifluorometil-fenil, 3,4-difluoro-fenil, 2,5-difluoro-fenil, 2,6-difluoro-fenil, naftalen-1-il, 4-trifluorometoxi-fenil, 3-ciano-fenil, 2-trifluorometoxi-fenil, 4-cloro-2-fluoro-fenil, 2,3-difluoro-fenil, 2,4,5-trifluoro-fenil, 2,3,4-trifluoro-fenil, 3,4-dicloro-fenil, 4-fluoro-3-trifluorometil-fenil, 5-fluoro-2-trifluorometil-fenil, 2-trifluorometil-fenil, 3-metil-fenil, 2-fluoro-4-trifluorometil-fenil, 4-cloro-3-fluoro-fenil, 3-fluoro-4-metil-fenil, 4-fluoro-3-metil-fenil, 3-fluoro-4-trifluorometil-fenil, 3-cloro-4-fluoro-fenil, 2,6-dicloro-fenil, 4-ciano-fenil, 2,5-dicloro-fenil y benzo[1,3]dioxol-5-il;

siempre que R_4 sea un grupo diferente de un grupo oxiranil.

24. Un compuesto de acuerdo con la reivindicación 1, seleccionado a partir de los compuestos que tienen la Fórmula (Io):



y sales, hidratos, y solvatos farmacéuticamente aceptables de los mismos;

en donde:

W es $-\text{CH}_2-$, $-\text{CH}_2\text{CH}_2-$ o $-\text{CH}_2\text{C}(=\text{O})-$;

R_1 es alquil C_{1-6} ;

R_3 es H o halógeno;

R_4 es pirrolidin-1-il, pirrolidin-2-il, piperidin-1-il, piperidin-4-il, piperidin-3-il, morfolin-4-il, piperazin-1-il, piridin-3-il, piridin-2-il o piridin-4-il,

cada uno opcionalmente sustituido con substituyentes seleccionados independientemente a partir de: CH_3 , $\text{C}(=\text{O})\text{O}-t\text{-butil}$, $\text{C}(=\text{O})\text{OH}$, $\text{C}(=\text{O})\text{OEt}$, $\text{NHC}(=\text{O})\text{O}-t\text{-butil}$, OH , $\text{C}(=\text{O})\text{NHCH}_2\text{C}(=\text{O})\text{OCH}_3$, $\text{NHC}(=\text{O})\text{CH}_2\text{C}(=\text{O})\text{OCH}_3$, $\text{C}(=\text{O})\text{NHCH}_2\text{C}(=\text{O})\text{OH}$, $\text{NHC}(=\text{O})\text{CH}_2\text{C}(=\text{O})\text{OH}$, $\text{C}(=\text{O})\text{OCH}_3$, $\text{OC}(=\text{O})\text{CH}_2\text{CH}_2\text{C}(=\text{O})\text{OCH}_3$, $\text{OC}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$, $\text{CH}_2\text{C}(=\text{O})\text{OCH}_2\text{CH}_3$, OCH_3 , $\text{CH}_2\text{C}(=\text{O})\text{OH}$, $\text{OC}(=\text{O})\text{CH}_2\text{CH}_2\text{C}(=\text{O})\text{OCH}_3$, $\text{CH}_2\text{CH}_2\text{C}(=\text{O})\text{OCH}_3$, $\text{C}(=\text{O})\text{CH}_3$ y $\text{C}(=\text{O})\text{OCH}_2\text{-fenil}$; y

R_8 se selecciona a partir de: 1H-benzoimidazol-2-il, benzooxazol-2-il y benzotiazol-2-il.

25. Un compuesto de acuerdo con la reivindicación 1, seleccionado a partir de los siguientes compuestos, y sales, hidratos, y solvatos farmacéuticamente aceptables de los mismos:

(A1) 1-[3-(4-bromo-2-metil-2H-pirazol-3-il)-4-(2-pirrolidin-1-il-etoxi)-fenil]-3-(4-cloro-fenil)-urea (Compuesto 1);

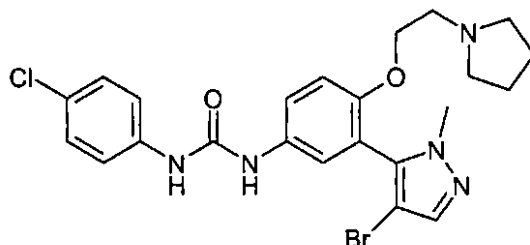
(A2) N-(3-(4-cloro-1-metil-1H-pirazol-5-il)-4-(2-morfolinoetoxi)fenil) ciclopropanocarboxamida (Compuesto 271);

- (A3) N-{3-(4-bromo-2-metil-2H-pirazol-3-il)-4-[2-(4-metoxi-piperidin-1-il)-etoxi]-fenil}-3-fluoro-benzamida (Compuesto 637);
- (A4) N-[3-(4-cloro-2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-3-fluoro-4-metil-benzamida (Compuesto 559);
- (A5) 1-(3-(4-cloro-1-metil-1H-pirazol-5-il)-4-(2-morfolinoetoxi)fenil)-3-(3-clorofenil)urea (Compuesto 231);
- (A6) 1-(4-cloro-bencil)-3-[3-(2-metil-2H-pirazol-3-il)-4-(3-morfolin-4-il-propoxi)-fenil]-urea (Compuesto 666);
- (A7) 3-cloro-N-[3-(2-metil-2H-pirazol-3-il)-4-(2-pirrolidin-1-il-etoxi)-fenil]-benzamida (Compuesto 512);
- (A8) 3-fluoro-N-[3-(2-metil-2H-pirazol-3-il)-4-(2-pirrolidin-1-il-etoxi)-fenil]-4-trifluorometil-benzamida (Compuesto 487);
- (A9) 1-{3-(4-cloro-2-metil-2H-pirazol-3-il)-4-[2-(3-hidroxi-azetidin-1-il)-etoxi]-fenil}-3-(4-cloro-fenil)-urea (Compuesto 298);
- (A10) 1-[4-[2-(7-aza-biciclo[2,2,1]hept-7-il)-etoxi]-3-(4-bromo-2-metil-2H-pirazol-3-il)-fenil]-3-(3-trifluorometil-bencil)-urea (Compuesto 710);
- (A11) 1-[4-[2-(7-aza-biciclo[2,2,1]hept-7-il)-etoxi]-3-(4-bromo-2-metil-2H-pirazol-3-il)-fenil]-3-(2,4-difluoro-bencil)-urea (Compuesto 734);
- (A12) 4-fluoro-N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-benzamida (Compuesto 280);
- (A13) N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-4-trifluorometil-benzamida (Compuesto 282);
- (A14) N-[3-(2-metil-2H-pirazol-3-il)-4-(2-pirrolidin-1-il-etoxi)-fenil]-3-trifluorometil-benzamida (Compuesto 435);
- (A15) N-[4-[2-(3,3-difluoro-pirrolidin-1-il)-etoxi]-3-(2-metil-2H-pirazol-3-il)-fenil]-4-fluoro-3-metil-benzamida (Compuesto 546);
- (A16) 4-fluoro-3-metil-N-[3-(2-metil-2H-pirazol-3-il)-4-(1-metil-pirrolidin-2-ilmetoxi)-fenil]-benzamida (Compuesto 547);
- (A17) N-{3-(4-bromo-2-metil-2H-pirazol-3-il)-4-[2-(2-metil-pirrolidin-1-il)-etoxi]-fenil}-3,4-difluoro-benzamida (Compuesto 579);
- (A18) N-[3-(4-bromo-2-metil-2H-pirazol-3-il)-4-(1-metil-pirrolidin-3-iloxi)-fenil]-3,4-difluoro-benzamida (Compuesto 585);
- (A19) N-[4-[2-(3-hidroxi-pirrolidin-1-il)-etoxi]-3-(2-metil-2H-pirazol-3-il)-fenil]-4-trifluorometil-benzamida (Compuesto 586);
- (A20) 3-fluoro-N-[4-[2-(4-formil-piperazin-1-il)-etoxi]-3-(2-metil-2H-pirazol-3-il)-fenil]-benzamida (Compuesto 752);
- (A21) N-[4-[2-(4-acetil-piperazin-1-il)-etoxi]-3-(2-metil-2H-pirazol-3-il)-fenil]-3-metoxi-benzamida (Compuesto 755);
- (A22) N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-2-fenil-acetamida (Compuesto 762);
- (A23) 2-(3-fluoro-fenil)-N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-acetamida (Compuesto 766);

- (A24) 2-(3-cloro-fenil)-N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-acetamida (Compuesto 767);
- (A25) N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-2-(3-trifluorometil-fenil)-acetamida (Compuesto 768);
- (A26) 2-(3-metoxi-fenil)-N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-acetamida (Compuesto 769);
- (A27) N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-2-m-tolil-acetamida (Compuesto 770);
- (A28) N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-2-p-tolil-acetamida (Compuesto 771);
- (A29) 2-benzo[1,3]dioxol-5-il-N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-acetamida (Compuesto 772);
- (A30) N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-2-(4-trifluorometil-fenil)-acetamida (Compuesto 794);
- (A31) N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-2-tiofen-2-il-acetamida (Compuesto 795);
- (A32) 1-[4-(2-azetidín-1-il-etoxi)-3-(2-metil-2H-pirazol-3-il)-fenil]-3-(2,4-difluoro-fenil)-urea (Compuesto 86);
- (A33) 1-[3-(4-bromo-2-metil-2H-pirazol-3-il)-4-[2-(3-metoxi-azetidín-1-il-etoxi)-fenil]-3-(4-cloro-fenil)-urea (Compuesto 267);
- (A34) N-[3-(4-bromo-2-metil-2H-pirazol-3-il)-4-[2-(3-metoxi-azetidín-1-il-etoxi)-fenil]-3-metil-butiramida (Compuesto 326);
- (A35) benzooxazol-2-il-[3-(4-bromo-2-metil-2H-pirazol-3-il)-4-(2-piperidín-1-il-etoxi)-fenil]-amina (Compuesto 199);
- (A36) [3-(4-cloro-2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-amida del ácido 5-cloro-tiofeno-2-carboxílico (Compuesto 333);
- (A37) 3,4-difluoro-N-[4-[2-(2-metil-piperidín-1-il-etoxi)-3-(2-metil-2H-pirazol-3-il)-fenil]-benzamida (Compuesto 459);
- (A38) N-[3-(4-cloro-2-metil-2H-pirazol-3-il)-4-(1-metil-piperidín-4-il-oxi)-fenil]-4-fluoro-3-metil-benzamida (Compuesto 745);
- (A39) 1-(2-fluoro-fenil)-3-[3-(2-metil-2H-pirazol-3-il)-4-(2-piperidín-1-il-etoxi)-fenil]-urea (Compuesto 65);
- (A40) N-[3-(4-bromo-2-metil-2H-pirazol-3-il)-4-(2-pirrolidín-1-il-etoxi)-fenil]-3-fluoro-benzamida (Compuesto 455);
- (A41) [3-(4-bromo-2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-amida del ácido ciclopentanocarboxílico (Compuesto 310);
- (A42) N-[3-(2-metil-2H-pirazol-3-il)-4-(2-pirrolidín-1-il-etoxi)-fenil]-4-trifluorometil-benzamida (Compuesto 343);
- (A43) N-[3-(4-bromo-2-metil-2H-pirazol-3-il)-4-[2-(3-fluoro-pirrolidín-1-il-etoxi)-fenil]-3-fluoro-benzamida (Compuesto 475);
- (A44) 3-cloro-4-fluoro-N-[4-[2-(4-fluoro-piperidín-1-il-etoxi)-3-(2-metil-2H-pirazol-3-il)-fenil]-benzamida (Compuesto 538);

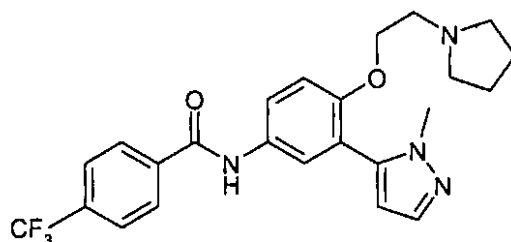
- (A45) N-[3-(4-bromo-2-metil-2H-pirazol-3-il)-4-(piperidin-3-iloxi)-fenil]-4-fluoro-benzamida (Compuesto 396);
- (A46) N-[4-[2-(4-acetil-piperazin-1-il)-etoxi]-3-(2-metil-2H-pirazol-3-il)-fenil]-3-fluoro-benzamida (Compuesto 660);
- (A47) N-[4-[2-(4,4-difluoro-piperidin-1-il)-etoxi]-3-(2-metil-2H-pirazol-3-il)-fenil]-3-fluoro-benzamida (Compuesto 778);
- (A48) 3-fluoro-N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-benzamida (Compuesto 729);
- (A49) 3-metoxi-N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-benzamida (Compuesto 733);
- (A50) 1-[3-(4-bromo-2-metil-2H-pirazol-3-il)-4-(2-pirrolidin-1-il-etoxi)-fenil]-3-(2,4-difluoro-fenil)-urea (Compuesto 5);
- (A51) 1-[3-(4-bromo-2-metil-2H-pirazol-3-il)-4-(2-piridin-4-il-etoxi)-fenil]-3-(4-cloro-fenil)-urea (Compuesto 145);
- (A52) 1-(2,4-difluoro-fenil)-3-[4-(2-imidazol-1-il-etoxi)-3-(2-metil-2H-pirazol-3-il)-fenil]-urea (Compuesto 219);
- (A53) Éster isopropilo del ácido [4-(2-azepan-1-il-etoxi)-3-(4-bromo-2-metil-2H-pirazol-3-il)-fenil]-carbámico (Compuesto 352);
- (A54) N-[3-(2-metil-2H-pirazol-3-il)-4-(2-[1,4]oxazepan-4-il-etoxi)-fenil]-3-trifluorometil-benzamida (Compuesto 568);
- (A55) N-[3-(4-bromo-2-metil-2H-pirazol-3-il)-4-(2-[1,4]oxazepan-4-il-etoxi)-fenil]-3-fluoro-4-trifluorometil-benzamida (Compuesto 606);
- (A56) Éster isopropilo del ácido [3-(2-metil-2H-pirazol-3-il)-4-(2-tiomorfolin-4-il-etoxi)-fenil]-carbámico (Compuesto 214);
- (A57) Éster isopropilo del ácido [3-(2-metil-2H-pirazol-3-il)-4-(2-[1,4]oxazepan-4-il-etoxi)-fenil]-carbámico (Compuesto 215);
- (A58) N-[3-(4-cloro-2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-3-trifluorometil-benzamida (Compuesto 527);
- (A59) 2,4-difluoro-N-[3-(2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-benzamida (Compuesto 281);
- (A60) 1-[3-(4-cloro-2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-3-(2,4-difluoro-fenil)-urea (Compuesto 16); y
- (A61) N-[3-(4-bromo-2-metil-2H-pirazol-3-il)-4-[2-(3-metoxi-azetidin-1-il)-etoxi]-fenil]-2-(4-cloro-fenil)-acetamida (Compuesto 269).

26. Un compuesto de acuerdo con la reivindicación 1, seleccionado a partir del siguiente compuesto y sales, hidratos, y solvatos farmacéuticamente aceptables del mismo:

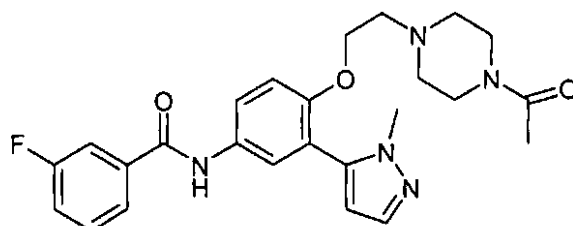


27. Un compuesto de acuerdo con la reivindicación 1, seleccionado a partir del siguiente compuesto y sales, hidratos, y solvatos farmacéuticamente aceptables del mismo:
 (A2) N-(3-(4-cloro-1-metil-1H-pirazol-5-il)-4-(2-morfolinoetoxi)fenil) ciclopropanocarboxamida (Compuesto 271).

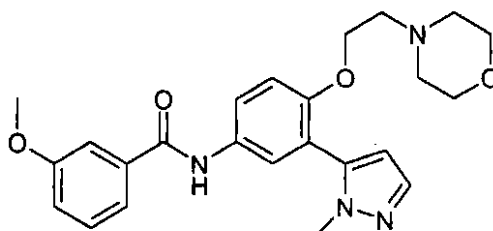
28. Un compuesto de acuerdo con la reivindicación 1, seleccionado a partir del siguiente compuesto y sales, hidratos, y solvatos farmacéuticamente aceptables del mismo:



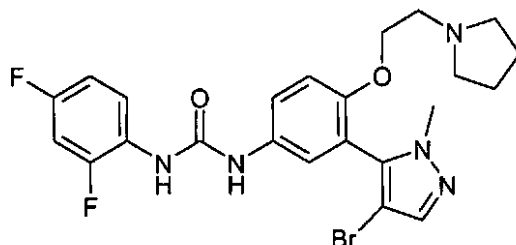
29. Un compuesto de acuerdo con la reivindicación 1, seleccionado a partir del siguiente compuesto y sales, hidratos, y solvatos farmacéuticamente aceptables del mismo:



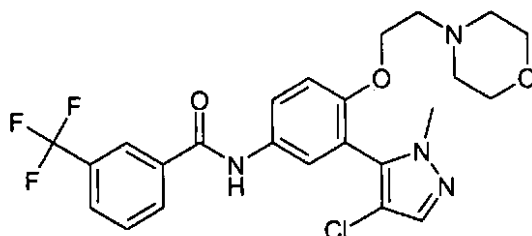
30. Un compuesto de acuerdo con la reivindicación 1, seleccionado a partir del siguiente compuesto y sales, hidratos, y solvatos farmacéuticamente aceptables del mismo:



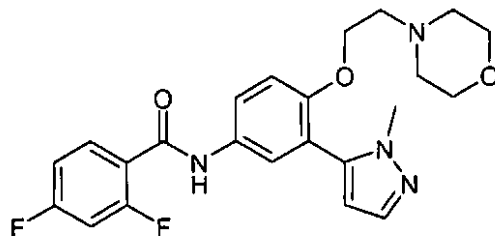
31. Un compuesto de acuerdo con la reivindicación 1, seleccionado a partir del siguiente compuesto y sales, hidratos, y solvatos farmacéuticamente aceptables del mismo:



32. Un compuesto de acuerdo con la reivindicación 1, seleccionado a partir del siguiente compuesto y sales, hidratos, y solvatos farmacéuticamente aceptables del mismo:



33. Un compuesto de acuerdo con la reivindicación 1, seleccionado a partir del siguiente compuesto y sales, hidratos, y solvatos farmacéuticamente aceptables del mismo:



34. Un compuesto de acuerdo con la reivindicación 1, seleccionado a partir del siguiente compuesto y sales, hidratos, y solvatos farmacéuticamente aceptables del mismo:

(A60) 1-[3-(4-cloro-2-metil-2H-pirazol-3-il)-4-(2-morfolin-4-il-etoxi)-fenil]-3-(2,4-difluoro-fenil)-urea (Compuesto 16).

35. Un compuesto de acuerdo con la reivindicación 1, seleccionado a partir del siguiente compuesto y sales, hidratos, y solvatos farmacéuticamente aceptables del mismo:

(A61) N-[3-(4-bromo-2-metil-2H-pirazol-3-il)-4-[2-(3-metoxi-azetidin-1-il)-etoxi]-fenil]-2-(4-cloro-fenil)-acetamida (Compuesto 269).

36. Una composición farmacéutica que comprende un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 35 y un portador farmacéuticamente aceptable.

37. Un proceso para preparar una composición que comprende mezclar un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 35 y un portador farmacéuticamente aceptable.

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of this study was that the use of solvent mixtures may improve the chances for obtaining crystals of organic compounds with high molecular volumes.

Over almost two decades we carefully collected data on the solid-state properties of a few thousand pharmaceutically relevant organic compounds, with special focus on those drug substances listed in the Pharmacopoeia European (PhEur). The 1997 edition of PhEur contained 559 well-defined organic drug compounds [50]. For more than 55% of them either polymorphs or solvates were known. In a newer evaluation of a larger set of data (PhEur edition 4.02, 808 solid organic compounds [51]) this fraction increased only slightly to 57%. As shown in Fig. 8.4, 29% of the compounds are known to form hydrates, 10% other solvates and 36% may occur in different polymorphic forms (i.e., different non-solvated forms). These statistics represent the present status of knowledge and the number of compounds that may appear in different crystal forms can only increase in the future when more experimental work is performed on these compounds. The relatively low occurrence of solvates with organic solvents coincides with the results of the CSD searches mentioned above, but the occurrence of hydrates among drug compounds is much higher. This can probably be attributed to the fact that drugs must have hydrophilic properties, at least to some extent, to dissolve in the body fluids and to be effective. Secondly, about 45% of the surveyed compounds are used as salts, preferentially as hydrochlorides (39% of all salts). Figure 8.5 illustrates that salts exhibit an expected higher occurrence of hydrates than non-salts, whereas the

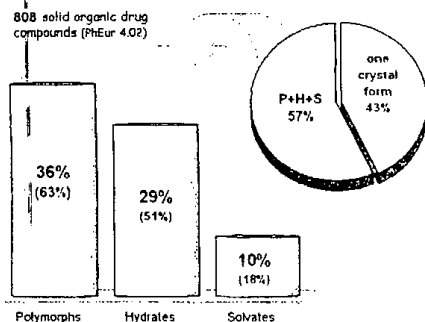


Fig. 8.4 Frequency of the occurrence of polymorphs (P), hydrates (H) and solvates containing organic solvents (S) among drug substances of the Pharmacopoeia Europaea. Upper percentages in the bars refer to the total number of compounds (808), those in

parenthesis refer to the 57% (pie diagram) of substances that can exist in more than one solid form. The latter ratio exceeds 100% because a substance can exist in different solid-state species.

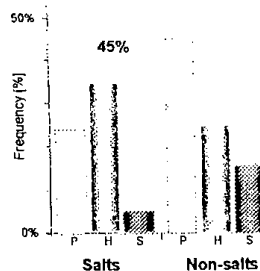


Fig. 8.5 Frequency of polymorphs (P), hydrates (H) and solvates with organic solvents (S) among substances of the Pharmacopoeia Europaea divided into salts and neutral compounds.

latter tend to form more solvates as well as polymorphs. This result clearly shows the propensity of water to interact and coordinate with ions and the lower ability of less polar solvents to bind strongly to the solute in the presence of counter-ions. The importance of hydrates in drug substances is also highlighted by the fact that almost 50% of the substances known to form adducts with water are specified (pharmacopoeial monograph) and used in a crystalline hydrate form. In 15 cases the PhEur specifies anhydrate and hydrate in extra monographs. On the contrary, for only 18% of the substances known to form polymorphs is it mentioned that "the substance may show polymorphism". Only three compounds are official in another solvate form: cefatrizin propylene glycol solvate, doxycycline hydrate (doxycycline hydrochloride hemihydrate hemihydrate) and warfarin sodium (which for some reason is called a "clathrate" in the pharmacopoeia but is a simple 2-propanol-solvate according to recent studies [91]). This analysis also confirms that solvate formation increases with molecular size. Furthermore, the likelihood of hydrate formation decreases with the lipophilicity of the drug molecule, whereas solvate formation with organic solvents increases. Further trends and interrelations between molecular properties and solvate/hydrate formation could be ascertained [51].

In rare cases, incredibly high numbers of solvates can be observed. The unsurpassed candidate is sulfathiazole with over one hundred solvates described [52]. For most of them the crystal structure could be determined, showing two types of structures, one where the solvent molecules are essential parts of the hydrogen-bonded framework structure and a second type in which the solvent basically fills the cavities (channels or layers) in the structural assembly of the sulfathiazole molecules. These types align well with the simple classification of stoichiometric and non-stoichiometric hydrates discussed above. Concerning isostructurality the author stated that the sulfathiazole solvate structures show unusual structural diversity. A class of substances prone to solvate formation is corticosteroids. For instance, flucortolone [53] has been reported to form 19 solvates (out of 31 tested) and one of its esters (flucortolone pivalate [54]) forms at

least 12. The formation of multiple solvates is not rare; however, it requires more attention and systematic work.

8.4 Generation and Characterization of Solvates

In the past, solvates were mostly obtained accidentally during crystallization from solvents by one of the various crystallization techniques (e.g., [55]) that are applied to purify or isolate a substance or, for instance, to grow single crystals for a crystal structure determination. Since it is imperative to establish the crystal forms of an active pharmaceutical ingredient (API) to satisfy the regulatory authorities [56, 57], solvates of drug compounds are now preferentially discovered in systematic polymorph screenings. The idea of such screens is to maximize the number of possible crystallization variables, which means that numerous crystallization experiments have to be performed properly to find all possible forms. Automated crystallization systems and strategies have been developed to speed up this process, allowing thousands of crystallization experiments in a short time [58–61]. Apart from the limited number of pharmaceutically acceptable solvents [1], hazardous and toxic solvents are also used because their specific features might increase the probability of finding new polymorphs or solvates.

Solvent crystallizations may yield highly unstable solvates that immediately desolvate on harvesting. Such unstable solvates survive only at low temperatures or when kept in the mother liquor and are often overlooked. Therefore, the statement that an individual polymorph was obtained by crystallization from a certain solvent does not necessarily mean that this polymorphic form nucleates directly in this solvent. The polymorph may have formed during the isolation procedure of an unstable solvate by desolvation. Attention to such "transient" solvates is important in order to understand formation principles of polymorphic forms.

It is unlikely that a stable hydrate will be overlooked by any of the screening programs, which always require careful and gentle drying procedures in the final stage to prevent desolvation. Since the presence of water can hardly be avoided during experimental work, unless specific efforts are undertaken, very stable hydrates may mask the existence of other forms and crystallize even with traces of water in a solvent or they form by the uptake of atmospheric moisture. Less stable or very unstable hydrates can sometimes be discovered only by chance or under rather extreme conditions. The recently discovered dihydrate of paracetamol, which crystallized at a pressure of 1.1 GPa from water, highlights these extremes [62]. Freeze-drying may also be a successful route to potential hydrate forms – as demonstrated by Yu et al. [63], who found a hemihydrate of the important pharmaceutical excipient mannitol, which was not detected by earlier solid-state screenings or freeze-drying experiments. That it is also possible to produce a solvate with this method was demonstrated by Otsuka et al. [64], who obtained a dioxane-solvate of phenobarbital by lyophilization.

Often, solvates can simply and quickly be generated by suspending the substance in the solvent or by wetting and grinding. Vapor sorption is a common method to generate hydrates. Exposure of a specific polymorph to water vapor may even result in a metastable hydrate form [10, 65] and it is also worth trying to transform solvates with organic solvents in moist air, with the aim of finding new hydrates or polymorphs.

The most common methods for characterizing solid-state forms, including solvates, are discussed in other chapters of this book and have been reviewed extensively (e.g., [13, 66, 67]). Compared to true polymorphs, the successful characterization of solvated crystal forms requires additional methods and strategies and doubtlessly needs more effort and time. The associated solvent is in most cases highly volatile and desolvation may already occur under mild conditions. Therefore, a comprehensive characterization of solvates often requires measurements under non-ambient conditions (dry atmosphere, certain partial pressure conditions, low and high temperature). Also, combinations of several methods can be very advantageous in the characterization of solvates, such as coupling of TGA or DSC with IR or mass spectrometry. These methods become particularly important for mixed solvates.

Moisture sorption experiments are an imperative part in any solid-state screening program and are a key technique in the characterization of hydrates (Chapter 9). Combination with powder X-ray diffraction allows the unequivocal recognition of stoichiometric and non-stoichiometric hydrates.

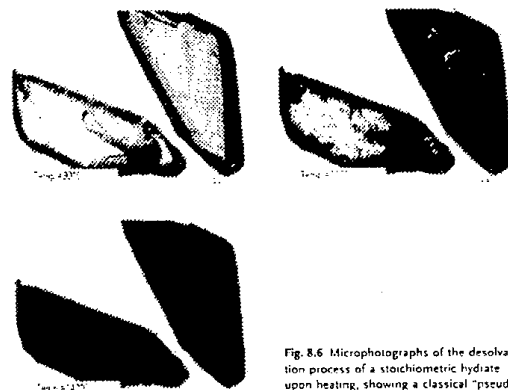


Fig. 8.6 Microphotographs of the desolvation process of a stoichiometric hydrate upon heating, showing a classical "pseudomorphosis".

Hot-stage polarized light microscopy is also helpful in the characterization of solvates. In many cases it even allows a simple, quick distinction between stoichiometric and non-stoichiometric solvates. As mentioned above, desolvation of a stoichiometric solvate is always associated with a significant structural change. Mostly, the solvate breaks up ("recrystallizes") into numerous small crystallites of the unsolvated crystal form during this process, whereas the original shape of the crystal is maintained. This can easily be recognized microscopically by the loss of transparency due to light scattering on the surfaces of the numerous crystallites. An example of this process called "pseudomorphosis" (NB not pseudopolymorphism!) is shown in Fig. 8.6 and further examples are given in [10]. Non-stoichiometric solvates, though, mostly desolvate without, or with only minor, observable changes. The crystals remain transparent on desolvation but fissures and cracks may appear. Therefore, it may be possible to determine crystal structures of the solvated and unsolvated state on one single crystal, as demonstrated recently [29].

8.5 Stability and Solubility of Solvates

The phase stability of solvates/hydrates, i.e., their potential to desolvate or to form, may differ extremely and is one of their most critical properties regarding handling and processing. Their stability depends on the temperature and the partial pressure of the solvent in the vicinity of the solid. In a solution of its own solvent, only temperature (and total pressure) must be considered. As the degree of solvation increases with decreasing temperature so the solvates stabilize as the temperature decreases. Thus crystallization experiments, at lower temperatures favor the formation of solvates. Outside the solvent the conditions are quite different for hydrates and solvates with organic molecules. The stabilizing solvent partial pressure becomes practically zero for solvates with organic solvents but not for hydrates due to atmospheric moisture.

There is no definition of what specifies a stable, moderately stable or unstable solvate. We may refer to the condition where the solvent partial pressure is at its maximum, i.e., their stability in the incorporated solvent (solubility experiments) or to an atmospheric condition at zero solvent partial pressure (thermogravimetry), both as a function of temperature. In contrast to organic solvents, the adjustment of a condition between these extremes is rather simple for water vapor and is routinely applied to characterize hydrates (moisture sorption experiments). Therefore, the stability of stoichiometric hydrates is often represented by partial pressure/composition phase diagrams at isothermal conditions, also called equilibrium moisture isotherms (Chapter 9). The relative humidity where a stoichiometric hydrate becomes unstable and starts to dehydrate (critical relative humidity) is an easily accessible criterion for the stability of a stoichiometric hydrate. However, the problem is the kinetics of desolvation, which usually do not allow us to reach the equilibrium in a reasonable time

and depend on many variables such as size of the crystals, crystal defects, the dynamics in the atmosphere, desolvation mechanism and more. Therefore, critical relative humidities determined by vapor sorption experiments may vary strongly, as has been demonstrated with caffeine hydrate [22]. Nevertheless, the information we can derive from partial pressure/composition phase diagrams allows us to derive valuable stability information about stoichiometric hydrates. The additional determination of a composition/temperature diagram as mentioned earlier [68] would characterize the stability range of a hydrate almost comprehensively and characterize the temperature above which a solvate cannot exist at atmospheric pressure and maximal partial pressure of the solvent. An exemplary and comprehensive study that conveys this was performed by De Kruif et al. [69] on citric acid. The determination of a composition/temperature phase diagram with differential scanning calorimetry (DSC) was demonstrated by Suzuki et al. [70]. Thermogravimetric analysis is an imperative part in the characterization and stability determination of solvates. The analysis gives the thermal stability of a solvate at dry atmospheric conditions but it may be also performed at elevated humidities.

Solubility studies at different temperatures in the solvent gives the stability range of solvate and unsolvated form and allow the determination of the transition temperature between these phases from a van't Hoff plot. This approach was demonstrated on various compounds forming hydrates or solvates with organic solvents by Shefter and Higuchi [71] in a classic paper and has been frequently applied since then. It became a rule that a solvate is always the most stable and therefore the least soluble form in its own solvent. Figure 8.7 demonstrates a typical situation, showing the order of solubility as stable hydrate $\rightarrow$ metastable hydrate $\rightarrow$ anhydrous form $\rightarrow$ solvate with organic solvent. From the intersection of the solubility lines of the anhydrous form and the hydrates the transition temperature can be established. In its own solvent the solvate becomes the most stable and least soluble form. However, in reality it is problematic to achieve equilibrium solubility values for metastable phases because they often transform faster into the solvate than they dissolve [65].

Van Tonder et al. [72] studied the solubility and intrinsic dissolution rates of several solvates of nicosamide in aqueous media. Both values were lower than that of the anhydrous form. This can be explained by the fast transformation of the solvates into the least-soluble hydrate. Conversely, the pentanol and toluene solvates of glibenclamide have higher solubility and dissolution rates than two non-solvated polymorphs [73]. This demonstrates the importance and the problems one has to expect when stable hydrates are involved.

Another approach to determine the stability of solvates is the use of solvent mixtures. Zhu and Grant have examined the influence of the water activity parameter a_w in organic solvent/water mixtures on the stability of the hydrates of theophylline and ampicillin [74]. A solvate is stabilized with increasing activity of the solvent of crystallization. Water activities, adjusted by mixing water with a water-miscible organic solvent, correlate with the relative humidity in the vapor. The advantage of this method is that it is often possible to achieve the equi-

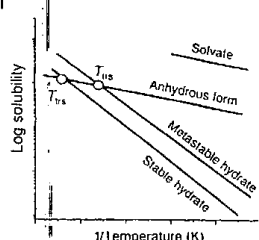


Fig. 8.7 Schematic representation of the solubilities of different solvates and an anhydrous form (succinylsulfathiazole) in an aqueous solution, plotted according to van't Hoff (Sheffer and Higuchi [71]). T_{HS} : transition temperatures between the anhydrous form and the hydrates.

librium in the liquid medium but usually not in water vapor for kinetic reasons. The activity parameter represents the principal thermodynamic factor that determines the stability of a solvate.

Solvates also often cause problems in solvent-mediated transformation experiments (also called slurry conversion experiments). Such experiments are applied to find or to verify the stable anhydrous form or the thermodynamic transition point of enantiotropically related polymorphs. Solvents forming a solvate with the substance under investigation are not suitable for such experiments because this solvate will very likely form. For polymorphic compounds that also form hydrates with strongly bound water this experimental approach becomes even less applicable, because the hydrate may form with the traces of water that are present in almost every solvent.

The stabilizing and protecting role of water in crystalline hydrates is well known for a series of β -lactam antibiotics. Cefixime trihydrate [75] is a very intriguing example. The partially dehydrated hydrate becomes disordered, which enables a hydrolytic reaction. Although the anhydrous form is chemically stable it cannot be used because it readily picks up water and rehydrates on storage. To stabilize the drug compound, the hydration state must be maintained during drug formulation, which requires a careful control of temperature, humidity and time. Similar reports exist of ampicillin trihydrate [76]. Vitamin B₁₂ (cyanocobalamin) is more stable to light and temperature in the hydrated form [77]. Byrn et al. [78] demonstrated on a series of non-stoichiometric solvates of prednisolone 21-*tert*-butylacetate that the presence of the solvent in voids (tunnels) protects the structure. Removing the solvent enables the penetration of oxygen into the voids and subsequent solid-state oxidation. Interestingly, water showed no protecting effect.

8.6 Processing of Solvates

Knowing the solubility difference between a solvate (almost exclusively a hydrate) and another solid-state candidate, the temperature and moisture dependent physical and chemical stability as well as the kinetics of these processes (which would deserve more attention than given here) allows us to estimate whether a solvate is a useful candidate for development. The solubility issue may become attractive in the case of poorly soluble compounds. Hydrate stability is critical for both classes of hydrates, but more for stoichiometric hydrates than non-stoichiometric ones. For a highly soluble, stable and bioavailable molecule, the risk of affecting the bioavailability by different crystal form is relatively low in general.

The next step is to consider the possible process that allows maintaining the features of the selected material (see also Chapter 13). Many steps in industrial processing may cause dehydration and rehydration cycles that may lead to a different polymorph, disordered and amorphous phases or another solvate. Theoretical approaches to processing-induced transformations (PITs) during pharmaceutical processing have been introduced by Morris et al. [68]. The examples given herein cover several hydrate problems and highlight the most problematic processes. In a later review Zhang et al. discussed the more practically oriented aspects of this topic [79]. Only a very few issues will be addressed here.

Literature on the compaction and tableting behavior of hydrates versus anhydrous forms is very rare. Burger et al. [80] compared the tableting behavior of modification I and a stable hemihydrate of sulfametrol. The hydrate showed better consolidation properties and yielded tablets with a higher tensile strength due to a better plastic deformation than the anhydrous form. Although the hemihydrate shows an about 20% lower solubility and thus also a slower dissolution rate, the liberation of the drug substance is about equal in tablets with a low excipient content (10%). This was explained by the faster disintegration time of the hemihydrate tablet. However, at higher excipient-to-drug ratios the liberation of the hemihydrate is worse than that of the anhydrous form. A study of the compaction behavior of thiamine-hydrochloride hydrates [81] also revealed that the less stable "monohydrate", which shows a non-stoichiometric behavior, possesses better bulk, consolidation and tablet properties than the stable hemihydrate due to a higher plasticity. Sun and Grant [82] showed that the monohydrate of 4-hydroxybenzoic acid shows a superior plastic deformation compared with the anhydrous form. The water molecules in the hydrate structure are arranged in layers, which represent slip planes and provide greater plasticity. Nevertheless, it seems that the differences of crystal forms in the compaction behavior can be balanced by excipients unless the active drug is not highly dosed.

Non-stoichiometric hydrates usually entail other problems than stoichiometric hydrates. The often weakly-bound water may become available ("free water") and interact with other components in a drug (e.g., excipients). Changes in

mass of several percent may also result in erroneous analytical results. Since no real phase change is connected with the variation in water/solvent content, the physical properties can be expected to be affected only moderately.

When a compound forms different solvates including a stable hydrate, the hydrate will press home an advantage in the widely unavoidable aqueous environment. At sufficient water activity, water can then strongly interact with the solvate, which is metastable in this environment, and induce a transformation by becoming a part of the structure. Such moisture-induced transformations proceed at a high rate, particularly in contact with an aqueous solution. Obviously, such a solvate can never be a suitable candidate for processing. Moisture may also catalyze the transformation of a solvate to be desolvated into an unsolvated form.

Pentamidine isothionate exemplifies that the nucleation of a solvate may be the critical process that determines whether one or another form results from a crystallization process [83]. In this case a trihydrate may form during freeze-drying, which dehydrates to a metastable form in the secondary drying stage. At lower concentrations and fast freezing rates the nucleation of the hydrate is less favored and the stable form with a low degree of order results.

8.7

Relevance, Problems and Potential Benefits

The observable trend [84] that new drug entities are becoming larger and therefore less soluble, as well as less absorbable and bioavailable, requires extra efforts and strategies in the pharmaceutical industry to obtain and market a (preferentially orally) active drug. The major solubility-improving strategy is doubtlessly the selection of a suitable salt. From the statistical data (see above) it becomes clear that both a larger molecular size as well as the change to a salt enhances the propensity of solvate (particularly hydrate) formation dramatically. New drug entities are, therefore, expected to show a higher ratio of solvates (hydrates). Considering that solvates that contain an organic solvate will play a subsidiary role compared to hydrates, we may overstate this as a "hydrate problem" in the pharmaceutical industry, reflecting the multilayered nature of hydrates and the extra effort needed to handle them.

Indinavir sulfate is an excellent example that stresses the problems we can meet when we administer salts of poorly soluble bases as well soluble salt [85]. The protease inhibitor is formulated as the sulfate salt in capsules for oral administration and is well bioavailable. The solubility in water at a pH <3.5 exceeds 100 mg mL⁻¹ but drops to about 0.02 mg mL⁻¹ at pH 7.0. This means that the solubility in the blood is 5000-fold lower than in the stomach. The concentration in urine right after the glomerular filtration in the kidney is close to saturation but becomes supersaturated during the following reabsorption of water in the loop of Henle. Finally, the base crystallizes as the worst soluble form, i.e., as the monohydrate in leaflet-like crystals and forms kidney stones.

Solvates of organic solvents that are regarded as unsafe are definitely not suitable candidates for the development of a final dosage form. But they can have technical features that can be used in manufacturing raw drug materials with desired or improved properties. Solvates can, for instance, be ideal precursors for the manufacturing of an individual polymorphic form or sometimes they can even be the only way. Zantosteron [86] and pilocain hydrochloride [87] are two representative examples.

Another potential technical feature is connected with the formation of small crystallites of often very homogeneous particle size distribution upon desolvation (pseudomorphosis, Fig. 8.6) of stoichiometric solvates. Particles of the original solvate crystals easily break apart to yield a very fine, highly crystalline and homogeneous powder. However, desolvation reactions are versatile and one may also get metastable or fully/partly disordered forms. The applicability of this particle size reduction method [88] as an alternative to often problematic milling processes depends on the structural features of the individual solvate.

Like other high-energy forms such as amorphous forms and metastable polymorphs, a solvate (with a non-toxic organic solvent) may also exhibit a significantly higher solubility than the stable form or an existing hydrate in aqueous media. This offers a viable strategy of enhancing the dissolution rate and bioavailability of poorly soluble drug compounds classified in class II or IV of the Biopharmaceutics Classification System (BCS) [89]. Possible problems of this strategy have been addressed above.

Finally, solvate formation, like polymorphism, opens perspectives for supramolecular chemistry and crystal engineering. Crystal structures of different solvates enable the study of molecular conformations, recurring patterns in hydrophobic aggregation and inclusion as well as molecular recognition phenomena in general [48, 90].

8.8

Patents

Because the crystalline forms of a substance cannot yet be predicted, and because of their potential industrial and technical benefits, solid-state forms can be protected as intellectual property by a patent (Chapter 14). A representative list of patented crystal forms of drug substances can be found in Byrn's classic book on solid-state chemistry [10]. Several past [11] and ongoing patent litigations concern solvates, particularly hydrates. In fact, more information about solid-state forms of newer drug compounds can now be found in patents than in peer-reviewed journals. Sometimes, amazingly high numbers (>20) of crystal forms are claimed for one compound. Such high numbers are only possible if the compound can form many different solvates, because the number of unsolvated forms (polymorphs) very rarely exceeds five. One striking problem is that solvates or hydrates are frequently claimed to be individual stoichiometric solvates with a defined solvent content, which on closer inspection are isostruc-

tural and represent only one non-stoichiometric solvate phase. One can of course artificially create (and claim?) an infinite number of forms from a non-stoichiometric solvate when the criteria for a discrete crystalline form is reduced to a measured solvent content in a sample. The generally enhanced complexity of the solid-state properties of solvates compared to polymorphs, connected with insufficient analytical data and the confusion in terminology, provides a lot of space for controversial issues and discussions. It is therefore extremely important to specify crystal forms and in particular solvates more accurately, based on several appropriate analytical techniques.

8.9

Conclusions

Solvate formation is a common phenomenon among drug substances and represents a challenging area for industry and basic research. Industry is faced with the problems of the complex behavior of these multicomponent crystals entities, although there are also potential benefits to exploit. The growing interest in supramolecular assemblies and principles of molecular recognition has attracted more attention to the relevance of solvent-molecule interactions in crystals. Except for water, this has often been ignored in the past in favor of the molecular structure.

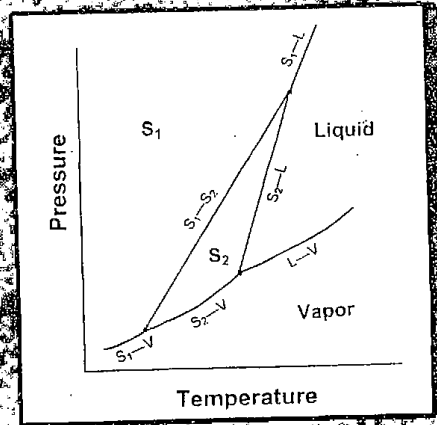
This chapter is far from comprehensive and there are certainly many solvate/hydrate systems that do not fit into the picture drawn here. However, the area requires more systematic work and the interplay of various scientific disciplines in order to draw the ultimate picture of this intriguing and important phenomenon.

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Polymorphism in Pharmaceutical Solids



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5

Generation of Polymorphs, Hydrates, Solvates, and Amorphous Solids

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I. METHODS EMPLOYED TO OBTAIN UNIQUE POLYMORPHIC FORMS

Organic medicinal agents that can exist in two or more solid phases often can provide some distinct advantages in particular applications. The metastable solid may be preferred in those instances where absorption of the drug is dissolution rate dependent. The stable phase may be less susceptible to chemical decomposition and may be the only form that can be used in suspension formulations. Often a metastable polymorph can be used in capsules or for tableting, and the thermodynamically stable form for suspensions. Factors related to processing, such as powder flow characteristics, compressibility, filterability, or hygroscopicity, may dictate the use of one polymorph in preference to another. In other cases, a particular form may be selected because of the high reproducibility associated with its isolation in the synthetic procedure.

It is essential to ascertain whether the crystalline material that results from a synthetic procedure is thermodynamically stable before conducting pivotal trials, since a more stable form may be obtained subsequently, and it may be impossible to produce the metastable form in future syntheses. Conversion from one polymorph to another can occur during processing or upon storage. An additional incentive for

isolating and identifying polymorphs that provides certain advantages is the availability of subsidiary patents for desirable polymorphic forms, or for retaining a competitive edge through unpublished knowledge. In 1990 Byrn and Pfeiffer found more than 350 patents on crystal forms granted on the basis of an advantage in terms of stability, formulation, solubility, bioavailability, ease of purification, preparation or synthesis, hygroscopicity, recovery, or prevention of precipitation [1].

One question that is likely to arise during the registration process is "What assurance can be provided that no other crystalline forms of this compound exist?" It is incumbent on the manufacturer of a new drug substance to show that due diligence has been employed to isolate and characterize the various solid-state forms of a new chemical entity. This may seem to be a daunting task, particularly in light of the widely quoted statement by Walter C. McCrone [2] that "Those who study polymorphism are rapidly reaching the conclusion that all compounds, organic and inorganic, can crystallize in different crystal forms or polymorphs. In fact, the more diligently any system is studied the larger the number of polymorphs discovered." On the other hand, one can take comfort from the fact that some important pharmaceuticals have been in use for many years and have, at least until now, exhibited only one stable form. Indeed, it seems to this author that there must be particular bonding arrangements of some molecules that are so favorable energetically as to make alternate arrangements unstable or nonisolatable.

In the future, computer programs using force-field optimization should be perfected to the point where it will be possible to predict, with confidence, that a particular crystalline packing arrangement is the most stable that is likely to be found. These programs also may make it possible to predict how many alternate arrangements having somewhat higher energy can potentially be isolated [3,4]. Until that time, the developmental scientist is handicapped in attempting to predict how many solid forms of a drug are likely to be found. The situation is further complicated by the phenomenon of "disappearing polymorphs" [5], or metastable crystal forms that seem to disappear in favor of more stable ones.

Some polymorphs can be detected, but not isolated. Hot stage microscopy has been used extensively to study polymorphic transfor-

mations. The microscopist can detect numerous polymorphic transformations, but the individual polymorphs often prove to be so unstable that they cannot be isolated by the usual methods. An excellent example of this is the work of Griebler and Burger on etofylline [6]. These authors identified five polymorphic forms by thermomicroscopy, but only stable Modification I could be obtained by recrystallization, even when seed crystals from the hot stage were used. Similarly, Kuhnert-Brandstätter, Burger, and Völlenkle [7] described six polymorphic forms of piracetam, only three of which could be obtained by solvent crystallization. All the others were found only by crystallization from the melt. What, then, is a careful investigator to do?

In this chapter, the various methods used to isolate polymorphs, hydrates, and solvates will be described. As Bernstein [8] has observed, "The conditions under which different polymorphs are obtained exclusively or together also can provide very useful information about the relative stability of different phases and the methods and techniques that might be necessary to obtain similar structures of different chemical systems." In this context, it is hoped that the following information will prove useful in devising a "screening" protocol for the preparation of the various solid state forms of pharmaceuticals. While one cannot be absolutely certain that no additional forms will be identified in the future, this approach should provide some assurance that "due diligence" has been exercised to isolate and identify crystalline forms that are likely to arise during the normal course of drug development and storage.

A. Sublimation

On heating, approximately two-thirds of all organic compounds are converted partially from the solid to the gaseous state and back to solid, i.e., they sublime [9]. While strictly speaking the term sublimation refers only to the phase change from solid to vapor without the intervention of the liquid phase, it is often found that crystals are formed on cooler surfaces in close proximity to the melt of organic compounds when no crystals were formed at temperatures below the melting point. The most comprehensive information concerning sublimation temperatures of compounds of pharmaceutical interest can be found in tables

in the textbook of Kuhnert-Brandstätter [9]. While the information in these tables is designed primarily for the microscopic examination of compounds, it is also possible to utilize it to determine which compounds might be susceptible to the application of techniques (such as vacuum sublimation) that can be carried out on larger scales and at lower temperatures.

The sublimation temperature and the distance of the collecting surface from the material undergoing sublimation have a great influence on the form and size of the crystals produced. The occurrence of polymorphic modifications depends on the temperature of sublimation. In general, it may be assumed that unstable crystals form preferentially at lower temperatures, while at higher temperatures stable forms are to be expected. Nevertheless, mixtures consisting of several modifications are frequently found together. This is the case for barbital and for estradiol benzoate. It should be obvious that the sublimation technique is applicable only to those compounds that are thermally stable.

A simple test can be used to determine if a material sublimes. A small quantity (10–20 mg) of the solid is placed in a petri dish that is covered with an inverted watch glass. The petri dish is heated gently on a hot plate and the watch glass is observed to determine if crystals are growing on it. According to McCrone [2], one of the best methods for obtaining a good sublimate is to spread the material thinly over a portion of a half-slide, cover with a large cover glass, and heat slowly using a Kofler block. When the sublimate is well formed, the cover glass is removed to a clean slide for examination. It is also possible to form good crystals by sublimation from one microscope slide to a second held above it, with the upper slide also being heated so that its temperature is only slightly below that of the lower slide. Cooling of the cover slip by placing drops of various low-boiling solvents on the top surface will cause condensation of the more unstable forms, the lower temperatures leading to the most unstable forms. On a larger scale, a glass cold finger or a commercial sublimator can be employed. Once crystals of various modifications have been obtained, they can be used as seeds for the solution phase crystallization of larger quantities.

Form I of 9,10-anthraquinone-2-carboxylic acid was obtained as needle-like crystals upon sublimation at temperatures exceeding 250°C [10]. Fokkens et al. have used sublimation to purify theophylline for

vapor pressure studies [11]. Sakiyama and Imamura found that stable phases of both 1,3-dimethyluracil and malonamide could be prepared by vacuum sublimation [12].

B. Crystallization from a Single Solvent

Slow solvent evaporation is a valuable method for producing crystals. Solutions of the material being crystallized, preferably saturated or nearly so, are filtered to remove most nuclei and then left undisturbed for a reasonable period of time. The rate of evaporation is adjusted by covering the solution with aluminum foil or Parafilm® containing a few small holes. For a solvent to be useful for recrystallization purposes, the solubility of the solute should be on the order of 5–200 mg/mL at room temperature. If the solubility exceeds 200 mg/mL, the viscosity of the solution will be high, and a glassy product is likely to be obtained. A useful preliminary test can be performed on 25–50 mg of sample, adding a few (5–10) drops of solvent. If all the solid dissolves, the solvent will not be useful for recrystallization purposes. Similarly, highly viscous solvents, and those having low vapor pressures (such as glycerol or dimethylsulfoxide) are not usually conducive to efficient crystallization, filtration, and washing operations. The solvents selected for recrystallization should include any with which the compound will come into contact during synthesis, purification, and processing, as well as solvents having a range of boiling points and polarities. Examples of solvents routinely used for such work are listed in Table 1 together with their boiling points.

The process of solution mediated transformation can be considered the result of two separate events, (a) dissolution of the initial phase, and (b) nucleation/growth of the final, stable phase. If crystals do not grow as expected from a saturated solution, the interior of the vessel can be scratched with a glass rod to induce crystallization by distributing nuclei throughout the solution. Alternatively, crystallization may be promoted by adding nuclei, such as seed crystals of the same material. For example, Suzuki showed that the α -form of inosine could be obtained by crystallization from water, whereas isolation of the β -form required that seeds of the β -form be used [13].

If two polymorphs differ in their melting point by 25–50°C, for

Table 1 Solvents Often Used in the Preparation of Polymorphs

Solvent	Boiling point (°C)
Dimethylformamide	153
Acetic acid	118
Water	100
1-Propanol	97
2-Propanol	83
Acetonitrile	82
2-Butanone	80
Ethyl acetate	77
Ethanol	78
Isopropyl ether	68
Hexane	69
Methanol	65
Acetone	57
Methylene chloride	40
Diethyl ether	35

monotropic polyinorphs the lower melting, more soluble, form will be difficult to crystallize. The smaller the difference between the two melting points, the more easily unstable or metastable forms can be obtained.

A commonly used crystallization method involves controlled temperature change. Slow cooling of a hot, saturated solution can be effective in producing crystals if the compound is more soluble at higher temperatures; alternatively, slow warming can be applied if the compound is less soluble at higher temperatures. Sometimes it is preferable to heat the solution to boiling, filter to remove excess solute, then quench cool using an ice bath or even a dry ice–acetone bath. High boiling solvents can be useful to produce metastable polymorphs. McCrone [2] describes the use of high boiling solvents such as benzyl alcohol or nitrobenzene for recrystallization on a hot stage. Belme et al. [14] showed that when buspirone hydrochloride is crystallized above 95°C the higher melting form is obtained; below 95°C the lower

melting form is obtained. Thus the lower melting polymorph could be converted to the higher melting polymorph by recrystallizing from xylene (boiling point 137–140°C).

To understand how temperature influences the composition of crystals that form, it is useful to examine typical solubility–temperature diagrams for substances exhibiting monotropic and enantiotropic behavior [15]. In Fig. 1a, Form II, having the lower solubility, is more stable than Form I. These two noninterchangeable polymorphs are monotropic over the entire temperature range shown. For indomethacin, such a relationship exists between Forms I and II, and between Forms II and III.

In Fig. 1b, Form II is stable at temperatures below the transition temperature T_i , and Form I is stable above T_i . At the transition temperature the two forms have the same solubility, and reversible transformation between enantiotropic Forms I and II can be achieved by temperature manipulation. The relative solubility of two polymorphs is a

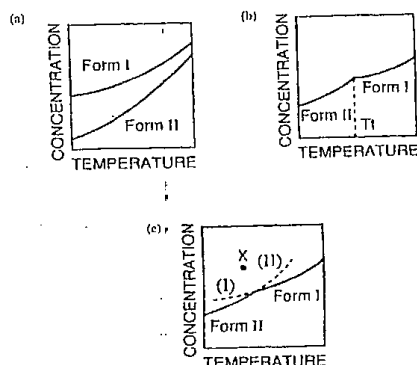


Fig. 1 Solubility curves exhibiting (a) monotropy, (b) enantiotropy, and (c) enantiotropy with metastable phases. (Reprinted with permission of the copyright holder [15].)

convenient measure of their relative free energies. The polymorph having the lower solubility is the more thermodynamically stable form, i.e., the form with the lower free energy at the temperature of the solubility measurement. At room temperature, carbamazepine Form I (m.p. 189°C) is more soluble than is Form III (m.p. 174°C), so the form with the higher melting point is more soluble. The polymorphs are enantiotropic with respect to each other [16].

There are situations in which kinetic factors can for a time override thermodynamic considerations. Figure 1c depicts the intervention of metastable phases (the broken line extensions to the two solubility curves). If a solution of composition and temperature represented by point X (supersaturated with respect to both I and II) is allowed to crystallize, it would not be unusual if the metastable Form I crystallized out first even though the temperature would suggest that Form II would be the more stable (i.e., less soluble) form. This is an extension of Ostwald's law of stages [17], which states that "when leaving an unstable state, a system does not seek out the most stable state, rather the nearest metastable state which can be reached with loss of free energy." This form then transforms to the next most soluble form through a process of dissolution and crystallization. Crystallization of Form I when Form II is more stable would be expected if Form I had the faster nucleation and/or crystal growth rate. However, if the crystals of Form I were kept in contact with the mother liquor, transformation could occur as the more soluble Form I crystals dissolve and the less soluble Form II crystals nucleate and grow. For crystals that exhibit this type of behavior, it is important to isolate the metastable crystals from the solvent by rapid filtration so that phase transformation will not occur.

In the general case, if there are any other polymorphic forms with solubilities below that of Form II, the above-described process will continue between each successive pair of forms until the system finally contains only the most stable (the least soluble) form. The implication of this hypothesis is that, by controlling supersaturation and by harvesting crystals at an appropriate time, it should be possible to isolate the different polymorphic forms. Furthermore, the theory predicts that at equilibrium the product of any crystallization experiment must be the stable form, regardless of the solvent system. It is apparent, however,

from the literature that for some solutes it is the choice of solvent rather than the effects of supersaturation that determines the form that crystallizes [18].

Crystallization of mannitol as a single solute was found to be influenced by both the initial mannitol concentration and by the rate of freezing [19]. In the range of 2.5% to 15%, the δ -polymorph is favored by higher concentrations, whereas the β -polymorph is favored at lower concentrations. At constant mannitol concentration (10%), the α -polymorph is favored by a slow freezing rate, whereas the δ -polymorph is favored by a fast freezing rate.

Kaneko et al. [20] observed that both the cooling rate and the initial concentration of stearic acid in *n*-hexane solutions influenced the proportion of polymorphs A, B, C, and E that could be isolated. Garti et al. [21] reported that for stearic acid polymorphs crystallized from various organic solvents, a correlation was observed between the polymorph isolated and the extent of solvent-solute interaction.

The reason for using crystallization solvents having varying polarities is that molecules in solution often tend to form different types of hydrogen-bonded aggregates, and that these aggregate precursors are related to the crystal structures that develop in the supersaturated solution [22]. Crystal structure analysis of acetanilide shows that a hydrogen-bonded chain of molecules is aligned along the needle axis of the crystals. This pattern is characteristic of secondary amides that crystallize in a *trans* conformation so that the carbonyl acceptor group and the -NH hydrogen bond donor are anti to one another. The morphology of acetanilide crystals can be controlled by choosing solvents that promote or inhibit the formation of this hydrogen-bond chain. Hydrophobic solvents such as benzene and carbon tetrachloride will not participate in hydrogen-bond formation, so they will induce the formation of rapidly growing chains of hydrogen-bonded amides. Crystals grown by evaporation methods from benzene or carbon tetrachloride are long needles. Solvents that are proton donors or proton acceptors inhibit chain formation by competing with amide molecules for hydrogen-bonding sites. Thus acetone inhibits chain growth at the -NH end, and methanol inhibits chain growth at the carbonyl end of the chain. Both solvents encourage the formation of rod-like acetanilide crystals, while

mixtures of benzene and acetone give hybrid crystals that are rod-shaped, with fine needles growing on the ends [23].

Some solvents favor the crystallization of a particular form or forms because they selectively adsorb to certain faces of some polymorphs, thereby either inhibiting their nucleation or retarding their growth to the advantage of others. Among the factors affecting the types of crystal formed are (a) the solvent composition or polarity, (b) the concentration or degree of supersaturation, (c) the temperature, including cooling rate and the cooling profile, (d) additives, (e) the presence of seeds, (f) pH, especially for salt crystallization, and (g) agitation [22].

Martínez-Ohárriz et al. [24] found that Form III of diflunisal is obtained from polar solvents, whereas Forms I and IV are obtained from nonpolar solvents. Likewise, Wu et al. [25] observed that when moricizine hydrochloride is recrystallized from relatively polar solvents (ethanol, acetone, and acetonitrile), Form I is obtained, whereas nonpolar solvents (methylene chloride or methylene chloride/ethyl acetate) yield Form II.

In determining what solvents to use for crystallization, one should be careful to select those likely to be encountered during formulation and processing. Typically these are water, methanol, ethanol, propanol, isopropanol, acetone, acetonitrile, ethyl acetate, and hexane. Matsuda employed 27 organic solvents to prepare two polymorphs and six solvates of piretanide [26].

According to McCrone [27], in a poor solvent the rate of transformation of a metastable to a more stable polymorph is slower. Hence a metastable form once crystallized can be isolated and dried before it is converted to a more stable phase by solution phase mediated transformation. In some systems the metastable form is extremely unstable and may be prepared only with more extreme supercooling. This is usually performed on a very small scale with high boiling liquids so that a saturated solution at a high temperature that is suddenly cooled to room temperature will achieve a high degree of supersaturation [28].

There are many examples in the literature of the use of single solvents as crystallization screens. Slow crystallization from acetone, acetonitrile, alcohols, or mixtures of solvents yields the Form A of

fosinopril sodium, but rapid drying of a solution of this compound yields Form B, sometimes contaminated with a small amount of Form A [29]. A rotary evaporator can be used to maintain a solution at the appropriate temperature as solvent is being removed.

Form I of dehydroepiandrosterone was obtained by recrystallization from warm ethyl acetate, acetone, acetonitrile, or 2-propanol. Form II was obtained by rapid evaporation, using a vacuum from solutions in dioxane, tetrahydrofuran, or chloroform (which are higher boiling, less polar solvents) [30].

C. Evaporation from a Binary Mixture of Solvents

If single-solvent solutions do not yield the desired phase, mixtures of solvents can be tried. Multicomponent solvent evaporation methods depend on the difference in the solubility of the solute in various solvents. In this approach, a second solvent in which the solute is sparingly soluble is added to a saturated solution of the compound in a good solvent. Often a solvent system is selected in which the solute is more soluble in the component with the higher vapor pressure. As the solution evaporates, the volume of the solution is reduced and, because the solvents evaporate at different rates, the composition of the solvent mixture changes.

Occasionally, crystals are obtained by heating the solid in one solvent and then pouring the solution into another solvent or over cracked ice. Otsuka et al. [31] obtained phenobarbital Form B by adding dropwise a saturated solution of the compound in methanol to water at room temperature. Form E was obtained by the same technique, but by using a saturated solution of phenobarbital in dioxane.

Kitamura et al. have shown that the fraction of Form A of *L*-histidine decreases quickly when the volume fraction of ethanol in an ethanol-water solvent system increases above 0.2, and that pure Form B is obtained at a 0.4 volume fraction of ethanol [32]. The transformation rate for conversion of Form B to Form A decreases with ethanol concentration. The authors postulated that the concentration of the conformer that corresponds to Form A decreases more with ethanol concentration than that of Form B, and so the growth rate of Form A will also decrease.

An example of precipitation in the presence of a second solvent is seen in the case of indomethacin. The γ -crystal form of indomethacin can be obtained by recrystallization from ethyl ether at room temperature, but the α -form is prepared by dissolution in methanol and precipitation with water at room temperature [33]. Precipitation can also result from the addition of a less polar solvent. Form II of midodrine hydrochloride, metastable with respect to Form I, can be prepared by precipitation from a methanolic solution by means of a less polar solvent such as ethyl acetate or dichloromethane [34].

In Fig. 2, three crystalline modifications of thalidomide are illustrated. These were obtained by solvent recrystallization techniques and differ both in crystal habit and in crystal structure. Two of the forms were obtained from a single solvent, and one from a binary mixture.

D. Vapor Diffusion

In the vapor diffusion method, a solution of the solute in a good solvent is placed in a small, open container that is then stored in a larger vessel containing a small amount of a miscible, volatile nonsolvent. The larger vessel (often a desiccator) is then tightly closed. As solvent equilibrium is approached, the nonsolvent diffuses through the vapor phase into the solution, and saturation or supersaturation is achieved. The solubility of the compound in a precipitant used in a two-solvent crystallization method such as vapor diffusion should be as low as possible (much less than 1 mg/mL), and the precipitant (the solvent in which the compound is poorly soluble) should be miscible with the solvent and the saturated solution. The most frequent application of this technique is in the preparation of single crystals for crystallographic analysis. An illustration of the technique is provided in Fig. 3 [35].

E. Thermal Treatment

Frequently when using differential scanning calorimetry as an analysis technique, one can observe an endothermic peak corresponding to a phase transition, followed by a second endothermic peak corresponding to melting. Sometimes there is an exothermic peak between the two endotherms, representing a crystallization step. In these cases it is often

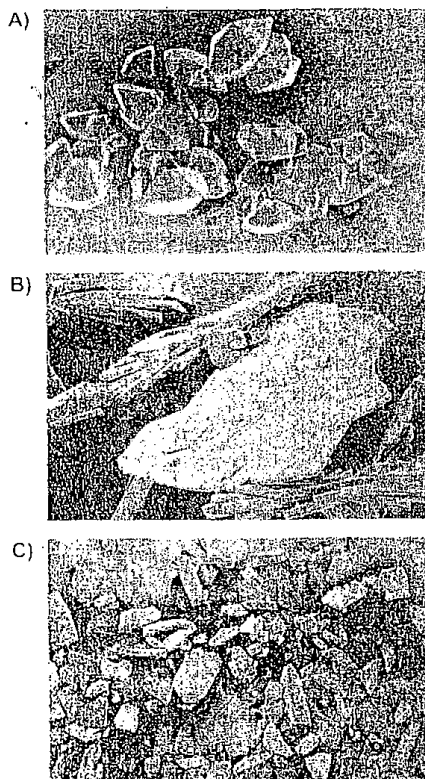


Fig. 2 Three crystalline modifications of thalidomide obtained by solvent recrystallization. (A) Form I obtained as bipyramids by slow crystallization of thalidomide in 1:1 dimethylformamide:ethanol at room temperature. (B) Form II obtained by immersing a saturated solution of thalidomide in acetonitrile in an ice bath. (C) Form III prepared as tabular crystals from a solution in boiling 1,4-dioxane, filtered, then allowed to cool to room temperature. (Photomicrographs courtesy of Dr. S. A. Botha, the University of Iowa.)

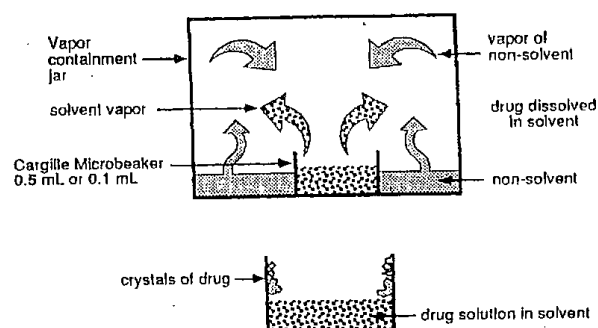


Fig. 3 Crystallization by vapor diffusion. (Reproduced with permission of the author [35] and the copyright holder, Pfizer, Inc.)

possible to prepare the higher melting polymorph by thermal treatment. Thus chlorpropamide Form A is obtained by recrystallization from ethanol solution, but Form C is obtained by heating Form A in an oven maintained at 100°C for 3 hours [36]. While the β -form of tegafur is obtained by the evaporation of a saturated methanol solution, the γ -form is obtained by heating the β -form at 130°C for one hour [37]. Form II of caffeine is prepared by recrystallization from distilled water, but Form I is prepared by heating Form II at 180°C for 10 hours [38].

F. Crystallization from the Melt

In accordance with Ostwald's rule [17], the cooling of melts of polymorphic substances often first yields the least stable modification, which subsequently rearranges into the stable modification in stages. Since the metastable form will have the lower melting point, it follows that supercooling is necessary to crystallize it from the melt. After melting, the system must be supercooled below the melting point of the metastable form, while at the same time the crystallization of the more stable form or forms must be prevented. Quench cooling a melt can

sometimes result in formation of an amorphous solid that on subsequent heating undergoes a glass transition followed by crystallization [39].

On a somewhat larger scale, one can use a vacuum drying pistol and a high boiling liquid such as chlorobenzene to achieve the desired end. Form II of *p*-(1*R*,3*S*)-3-thioanisoyl-1,2,2-trimethylcyclopentane carboxylic acid was obtained by recrystallization from a 50:50 v/v benzene:petroleum ether mixture. Form I then was obtained by melting Form II in the vacuum drying pistol [40]. Caffeine Form I is prepared by heating Form II at 180°C for 10 hours [38]. Yoshioka et al. [41] observed that when the amorphous solidified melt of indomethacin was stored at 40°C, it partly crystallized as the thermodynamically stable γ -form. Yet at 50°C, 60°C, and 70°C, mixtures of the α - and the γ -form were obtained. Sulfathiazole Form I is obtained by heating Form III crystals (grown from a dilute ammonium hydroxide solution at room temperature) at 170°C for 30–40 minutes [42].

G. Rapidly Changing Solution pH to Precipitate Acidic or Basic Substances

Many drug substances fall in the category of slightly soluble weak acids, or slightly soluble weak bases, whose salt forms are much more soluble in water. Upon addition of acid to an aqueous solution of a soluble salt of a weak acid, or upon addition of alkali to an aqueous solution of a soluble salt of a weak base, crystals often result. These crystals may be different from those obtained by solvent crystallization of the weak acid or weak base. Nucleation does not necessarily commence as soon as the reactants are mixed, unless the level of supersaturation is high, and the mixing stage may be followed by an appreciable time lag before the first crystals can be detected. Well-formed crystals are more likely to result in these instances than when rapid precipitation occurs.

Form I of the x-ray contrast agent iopanoic acid was prepared [43] by dissolving the acid in 0.1 N NaOH, adjusting the pH to 12.5, bubbling nitrogen into the solution, and adding 0.1 N hydrochloric acid until the pH reached 2.15. The resulting precipitate was vacuum filtered

and stored *in vacuo* (380 torr) for 12 hours at 35°C. Similarly, Form III of hydrochlorothiazide was precipitated from sodium hydroxide aqueous solution by the addition of hydrochloric acid [44].

When piretanide was dissolved in 0.1 N NaOH at room temperature and acid was added in a 1:1 ratio (to pH 3.3), piretanide Form C precipitated. However, when the base:acid ratio used was 1:0.95, a mixture of amorphous piretanide and Form C precipitated [45].

H. Thermal Desolvation of Crystalline Solvates

The term "desolvated solvates" has been applied to compounds that were originally crystallized as solvates but from which the solvent has been removed (generally by vaporization induced by heat and vacuum). Frequently, these "desolvated solvates" retain the crystal structure of the original solvate form and exhibit relatively small changes in lattice parameters. For this reason, these types have been referred to as pseudopolymorphic solvates. However, in instances where the solvent serves to stabilize the lattice, the process of desolvation may produce a change in lattice parameters, resulting in the formation of either a new crystal form or an amorphous form. These solvates have been referred to as polymorphic solvates. Byrn [46] has characterized the desolvation of polymorphic solvates as occurring in four steps, (a) molecular loosening, (b) breaking of the host-solvent hydrogen bonds (or other associations), (c) solid solution formation, and (d) separation of the product phase.

The process of desolvating pseudopolymorphic solvates is simpler, involving only the two steps of (a) molecular loosening and (b) breaking of host-solvent hydrogen bonds or associations. Byrn [46] has summarized the desolvation studies performed on caffeine hydrate, theophylline hydrate, thymine hydrate, cytosine hydrate, dihydro-phenylalanine hydrate, dialuric acid hydrate, cycloserine hydrate, erythromycin hydrate, fenoprofen hydrate, manganous formate hydrate, bis(salicylaldehyde) ethylenediamine cobalt (II) chloroformate, cephatoglycine hydrates and solvates, and cephalixin solvates and hydrates. Among factors that influence the desolvation reaction are the appearance of defects, the size of tunnels in the crystal packing arrange-

ment, and the strength of hydrogen bonding between the compound and its solvent of crystallization [46].

Rocco et al. [47] obtained Form II of zanoterone by recrystallization from ethanol and vacuum drying at 45°C. Form III was isolated by desolvating the acetonitrile solvate form at 80°C under vacuum, and this was the form chosen for use in the clinical drug product due to the high reproducibility of its isolation during manufacture. Similarly, Forms I and II of stanozolol were obtained by heating solvates of the compound to 205°C and 130°C, respectively [48].

The benzene solvate of iopanoic acid was prepared by rapidly freezing a warm benzene solution of iopanoic acid in a dry ice-acetone mixture [43]. The solid obtained was permitted to melt at room temperature, yielding crystals of the solvate suspended in benzene. When these were vacuum filtered and stored *in vacuo* (380 torr) for 12 hours at 70°C, Form II was obtained free of benzene.

Dehydration of hydrates can also lead to the formation of unique crystals. Caffeine Form II was prepared by recrystallizing caffeine from water, drying for 8 days at 30°C, and then heating for 4 hours at 80°C [38]. Chloroquine diphosphate 3:1 hydrate was converted to the anhydrous form at temperatures above 188°C [49]. Etoposide Form I (a monohydrate) was found to undergo a dehydration reaction in the temperature range of 85–115°C to yield etoposide Form Ia. This form could be melted at 198°C and transformed to etoposide Form IIa, which itself melted at 198°C and crystallized to still another polymorph, etoposide Form IIa at 206°C. Etoposide Form IIa was found to melt at 269°C and convert to its hydrated form, etoposide Form II, when exposed to the atmosphere at room temperature. This hydrate was also found to undergo a dehydration reaction at 90–120°C to yield etoposide Form IIa [50].

Differential scanning calorimetry (DSC) curves of levofloxacin hemihydrate measured under various conditions showed different thermograms. This behavior was attributed to the dehydration process that resulted in a multiple-phase transition. Dehydration at higher temperatures (above 70°C) gave a sharp endothermic peak in the DSC thermogram due to the melting of the γ -form, and at a lower temperature (50°C) it led to the observation of a sharp endothermic peak due to the

melting of the α -form. In contrast, the thermal behavior of levofloxacin monohydrate was not affected by dehydration [51].

I. Growth in the Presence of Additives

The presence of impurities can have a profound effect on the growth of crystals. Some impurities can inhibit growth completely, and some may enhance growth. Still others may exert a highly selective effect, acting only on certain crystallographic faces and thus modifying the crystal habit. Some impurities can exert an influence at very low concentrations (less than 1 part per million), whereas others need to be present in fairly large amounts to have any effect [15].

Additives can be designed to bind specifically to the surfaces of particular polymorphs and so inhibit their achieving the critical size for nucleation, allowing a desired phase to grow without competition [52]. Lahav and coworkers have shown that additives at levels as low as 0.03% can inhibit nucleation and crystal growth of a stable polymorph, thus favoring the growth of a metastable polymorph [53]. They also showed that it is possible to design crystal nucleation inhibitors to control polymorphism.

Davey et al. found that Form I crystals of terephthalic acid could be obtained by crystallization only in the presence of *p*-toluic acid [54]. Form II, the more stable polymorph at ambient temperatures, was recovered from a hydrothermal recrystallization experiment.

Ikeda et al. [55] determined that indomethacin can exist in three different crystal forms, denoted α -, β -, and γ -, with the α -form possessing a higher solubility than the γ -form. On recrystallization, crystals of the α -form were the first to be deposited, but these converted gradually to the less soluble γ -form. However, in the presence of hydroxypropyl methylcellulose, conversion from the α -form to the γ -form was inhibited, leading to an increase in the solubility of indomethacin.

While the α -form of glycine normally is obtained by recrystallization from water, 3% of racemic hexafluorovaline leads to the precipitation of the γ -polymorph as trigonal pyramids [56]. This additive was designed to be strongly adsorbed at the four {011} crystal faces of the α -form and to bind at only one pole of the polar crystal, thus leaving

the crystal free to grow at the opposite pole. Since it is bound at the slow growing NH_3^+ end of the polar axis, it does not interfere with the fast growing CO_2^- end.

J. Grinding

Polymorphic transformations have been observed to occur on grinding of certain materials, such as sulfathiazole, barbital, phenylbutazone, cephalixin, chloramphenicol palmitate, indomethacin, and chlorpropamide. Byrn [46] has stated that polymorphic transformations in the solid state require the three steps of (a) molecular loosening (nucleation by separation from the lattice), (b) solid solution formation, and (c) separation of the product (crystallization of the new phase). Depending on the material and the conditions employed, grinding can result in conversion to an amorphous substance. With the exercise of care, different polymorphic forms can be obtained. Otsuka et al. [57] showed that metastable Forms B and C of chloramphenicol palmitate were transformed into stable Form A upon grinding at room temperature. Indomethacin was transformed into a noncrystalline solid during grinding at 4°C, and into metastable Form A by grinding at 30°C. Caffeine Form II is converted into Form I with grinding, and a 95% phase conversion was obtained following 60 hours of grinding time [38].

II. METHODS EMPLOYED TO OBTAIN HYDRATE FORMS

Pharmaceutical solids may come into contact with water during processing steps, such as crystallization, lyophilization, wet granulation, aqueous film-coating, or spray-drying. Moreover, they may be exposed to water during storage in an atmosphere containing water vapor, or in a dosage form consisting of materials that contain water (e.g., excipients) and are capable of transferring it to other ingredients. Water may be adsorbed onto the solid surface and/or may be absorbed in the bulk solid structure. When water is incorporated into the crystal lattice of the compound in stoichiometric proportions, the molecular adduct or adducts formed are referred to as hydrates [58]. More than 90 hydrates

are described in various USP monographs. Hydrates can be prepared by recrystallization from water or from mixed aqueous solvents. They can also result, in some instances, from exposure of crystal solvates (such as methanolates or ethanolates) to an atmosphere containing water vapor.

Crystalline substances often form with water molecules located at specific sites in the crystal lattice, which are held in coordination complexes around lattice cations. This type of water is denoted as water of crystallization and is common for inorganic compounds. For example, nickel sulfate forms a well-defined hexahydrate, where the waters of hydration are bound directly to the Ni(II) ion. Extraneous inclusion of water molecules can occur if a coprecipitated cation carries solvation molecules with it. Water also can be incorporated into random pockets as a result of physical entrapment of the mother liquor. Well-defined multiple hydrate species can also form with organic molecules. For example, raffinose forms a pentahydrate.

Although most hydrates exhibit a whole-number-ratio stoichiometry, an unusual case is the metastable hydrate of caffeine, which contains only 0.8 moles of water per mole of caffeine. Only in a saturated water vapor atmosphere will additional amounts of water be adsorbed at the surface of the 4/5-hydrate to yield a 5/6 hydrate [59].

In some instances, a compound of a given hydration state may crystallize in more than one form, so that the hydrates themselves exhibit polymorphism. One such example is nitrofurantoin, which forms two monohydrates that have distinctly different temperatures and enthalpies of dehydration. The monohydrates have quite different packing arrangements, with Form I possessing a layer structure and Form II exhibiting a herringbone motif. The included water molecules play a major role in stabilizing the crystal structures. Whereas water molecules are contained in isolated cavities in Form II, in Form I they are located in continuous channels, and this apparently facilitates the escape of water when these crystals are heated [60].

Another example of hydrate polymorphism is amiloride hydrochloride [61], which can be obtained in two polymorphic dihydrate forms. These forms are indistinguishable by techniques other than x-ray powder diffraction.

It is interesting that scopolamine hydrobromide has been reported

to exist as the anhydrous form, a "hemihydrate," a sesquihydrate, and a trihydrate [62], while the unit cell parameters and the molecular geometry of these are all the same as those of the hemihydrate. This finding suggests that the "hemihydrate" is actually a partially desolvated sesquihydrate.

Ouabaine is another example of a compound that exhibits many different hydration levels, the most hydrated form being stable at the lowest temperature. Thus the nonahydrate phase of ouabaine is obtained from water at 0–15°C, the octahydrate phase at 15–28°C, and the dihydrate phase at 28–90°C. In addition, ouabaine phases corresponding to 4.5 H₂O, 4 H₂O, and 3 H₂O may be obtained from mixtures of water with other solvents. The anhydrous phase of ouabaine anhydrate is crystallized from ethanol at high temperatures [63].

Typically, hydrates are obtained by recrystallization from water. For example, trazodone hydrochloride tetrahydrate was prepared by dissolving the anhydrate in hot distilled water, allowing the solution to remain at room temperature overnight, and storing the collected crystals at 75% relative humidity and 25°C until they reached constant weight [64].

Hydrates can sometimes be obtained by simply suspending the anhydrous material in water, whereupon a form of Ostwald ripening occurs. For instance, aqueous suspensions of anhydrous metronidazole benzoate are metastable, and storage at temperatures lower than 38°C leads to monohydrate formation accompanied by crystal growth [65]. Sorbitol provides another example of this behavior, where slow cooling of a saturated aqueous solution yields long thin needles of sorbitol hydrate [66]. When suspended in water, anhydrous carbamazepine is transformed to carbamazepine dihydrate [67]. In other instances, hydrates can be obtained from mixed solvent systems. Acemetacin monohydrate can be obtained by slow evaporation from a mixture of acetone and water at room temperature [68].

Simply exposing an anhydrous powder to high relative humidity can often lead to formation of a hydrate. On exposure to a relative humidity of 100%, dexmedetomidine hydrochloride is converted to a monohydrate [69]. Droloxifene citrate is an example of a compound that is not very hygroscopic and yet forms a hydrate. Only after storage of the anhydrous form at 85% relative humidity does some sorption of

water occur. The monohydrate phase can be formed by exposing the anhydrous form to 98% relative humidity for ten days at 24°C [70].

III. METHODS EMPLOYED TO OBTAIN SOLVATE FORMS

Often, when solvents are employed in the purification of new drug substances by recrystallization, it is observed that the isolated crystals include solvent molecules, either entrapped within empty spaces in the lattice or interacting via hydrogen bonding or van der Waals force with molecules constituting the crystal lattice. Solvent molecules also can be found in close association with metal ions, completing the coordination sphere of the metal atom. Coordinated solvent molecules are considered as part of the crystallized molecule. A crystal with large empty channels or cavities is not stable because of packing demands. The size and chemical environment of the cavity or channel determine what kind of solvent molecule can be included in the structure and what kind of interaction occurs between solvent and structure.

Depending on the nature of molecular packing arrangements, it may happen that the inclusion of solvent is necessary to build a stable crystal structure. van Geerestein et al. [71] found during numerous crystallization attempts of 11β-[4-(dimethylamino)phenyl]-17β-hydroxy-17α-(1-propynyl)estra-4,9-diene-3-one that crystals were only obtainable in the presence of *n*-butyl acetate or *n*-propyl acetate. The crystal structure of the compound crystallized from *n*-butyl acetate/methylcyclohexane was solved, and one solvent molecule was found in the crystal structure that showed no strong interactions with the rest of the structure. Apparently, this solvent molecule was necessary to fill empty space resulting after the molecular packing. Solvates in which the solvent fills empty space are generally nonstoichiometric, such as the nonstoichiometric solvates formed by droloxifene citrate with acetonitrile, 2-propanol, ethanol, 1-propanol, and 1-butanol. Typically such solvates exhibit the same x-ray diffraction pattern as does the nonsolvated compound.

When solvent molecules increase the strength of the crystal lattice, they can affect the stability of the compound to solid-state decom-

position. It has been observed that the four solvated and one nonsolvated structures of prenisolone *tert*-butyl acetate affect the flexibility of the steroid nucleus and the structure-dependent degradation of the compound when exposed to air and light [72].

van der Sluis and Kroon found 1,247 different compounds with cocrystallized solvents in the Cambridge Crystallographic Database [73]. Out of 46,460 total structures, they found 9,464 solvate structures, and 95% of these contained one of the 15 solvents given in Table 2.

The most commonly encountered solvates among pharmaceuticals are those of 1:1 stoichiometry, but occasionally mixed solvate species are encountered. For structures containing more than one solvent type, one generally finds nonpolar solvents crystallizing together on the one hand and polar solvents on the other. For example, the most common solvents found cocrystallizing with water are (in order of im-

Table 2 Distribution of the 15 Most Abundant Solvents in the Cambridge Crystallographic Database, as the Percentage of Solvate Structures

Solvent	Occurrence (%)
Water	61.4
Methylene dichloride	5.9
Benzene	4.7
Methanol	4.1
Acetone	2.8
Chloroform	2.8
Ethanol	2.6
Tetrahydrofuran	2.3
Toluene	2.2
Acetonitrile	1.9
<i>N,N</i> -dimethylformamide	0.9
Diethyl ether	0.9
Pyridine	0.7
Dimethyl sulfoxide	0.5
Dioxane	0.5

Source: From Ref. 73. Reproduced with permission of the copyright owner.

portance) ethanol, methanol, and acetone. An interesting example of a structure containing a polar and a nonpolar solvent is the sodium salt of the antibiotic K-41, *p*-bromobenzoate monohydrate *n*-hexane solvate [74], which is crystallized from *n*-hexane saturated with water. Perhaps the best known mixed solvate is doxycycline hyclate: (doxycycline · HCl)₂C₂H₆O · H₂O. Triamterene also forms a mixed solvate, containing one *N,N*-dimethylformamide molecule and one water molecule within the crystal lattice [75].

The techniques used to obtain solvates are generally similar to the solvent methods used to obtain polymorphs, i.e. crystallization from a single solvent, from mixed solvents, or by vapor diffusion. Sometimes, it is possible to exchange one solvent within the crystal structure for another. When one recrystallizes a hydrate from dry methanol, in most cases one is left with either a methanol solvate or an anhydrous, unsolvated form of the compound.

A large number of solvates have been reported, especially for steroids and antibiotics. It has been observed that cortisone acetate and dexamethasone acetate can be crystallized as 10 different solvates. Dirithromycin, a semisynthetic macrolide antibiotic, crystallizes in two anhydrous polymorphic forms and in at least nine stoichiometric solvate forms. Six of the known solvates are isomorphous, having nearly identical x-ray powder diffraction patterns [76]. In addition to the anhydrate and dihydrate, erythromycin also forms solvates with acetone, chloroform, ethanol, *n*-butanol, and *i*-propanol [77].

It may be instructive to consider some examples of solvate formation. The compound 5-methoxysulphadiazine forms 1:1 host-guest solvates with dioxane, chloroform, and tetrahydrofuran [78]. These were prepared by heating to boiling a solution of the sulfonamide in the appropriate solvent, followed by slow cooling to obtain large crystals. Spironolactone forms 1:1 solvates with methanol, ethanol, ethyl acetate, and benzene. It also forms a 2:1 spironolactone-acetonitrile solvate [79,80]. The spironolactone solvates were prepared by crystallization in a refrigerator from solutions that were nearly saturated at room temperature.

Another steroid that forms solvates is stanozolol [81]. Solvates having 1:1 stoichiometry were prepared by recrystallization from methanol, ethanol, and 2-propanol, by heating the compound in the

appropriate solvent to 60–70°C and then cooling to 0°C in an ice bath to induce crystallization. The compound also forms a monohydrate and two polymorphs. The polymorphs were prepared by heating the solvates to either 130°C (Form II) or 205°C (Form I).

Mefloquine hydrochloride is an interesting case of a compound that forms stoichiometric 1:1 solvates on cooling hot (50°C) saturated acetone solutions (Form B, acetone solvate 1:1), hot (50°C) saturated isopropanol (Form I, isopropanol solvate 1:1), and a nonstoichiometric ethanol solvate (2.12% ethanol) from hot (50°C) saturated ethanol, Form E, whose x-ray powder pattern does not change following heating to 80°C, in spite of a decrease in the ethanol level to 0.12%. Mefloquine hydrochloride can also be obtained in a nonsolvated form from hot (70°C) saturated acetonitrile (Form A) and as two hemihydrates from water (Forms D and C) prepared at room temperature and at 30°C [82].

IV. METHODS EMPLOYED TO OBTAIN AMORPHOUS MATERIALS

Solids can exist in crystalline or amorphous form. Crystalline materials have defined structures, stoichiometric compositions, and melting points and are characterized by their chemical, thermal, electrical, optical, and mechanical properties [83]. By contrast, amorphous materials have no clearly defined molecular structure and no long-range order, so their structure can be viewed as being similar to that of a frozen liquid but without the thermal fluctuations observed in the liquid phase. As a result, amorphous materials exhibit the classical diffuse "halo" x-ray powder diffraction pattern rather than the sharp peaks observed in the pattern of a crystalline substance. When the halo is broad, it is often difficult to distinguish between a material that is truly amorphous (e.g., a true glass) and one that is merely microcrystalline. This situation exists because when microcrystallites have diameters less than about 50 Å in diameter, a similar "halo" effect is observed.

While crystalline solids offer the advantages of chemical and thermodynamic stability, amorphous solids are occasionally preferred because they undergo dissolution at a faster rate. Rapid dissolution is desirable in the case of solids, which must be dissolved prior to paren-

teral administration. Faster dissolution is also important for poorly soluble compounds administered orally, since there is often a correlation between dissolution rate and bioavailability. In fact, there are instances in which only the amorphous form has adequate bioavailability.

Amorphous solids can be precipitated from solution or obtained from melts of compounds by carrying out the solidification in such a way as to avoid the thermodynamically preferred crystallization process. They also can be prepared by disrupting an existing crystal structure. Excess free energy and entropy are incorporated into solids as they are converted into the amorphous state, since solidification occurs without permitting the molecules to reach their lowest energy states.

A. Solidification of the Melt

Amorphous solids are often created by rapidly cooling a liquid so that crystallization nuclei can neither be created nor grow sufficiently, whereupon the liquid then remains in the fluid state well below the normal freezing point. In principle, a liquid should freeze (crystallize) when cooled to a temperature below its freezing point. However, if the rate of cooling is high relative to the rate of crystallization, then the liquid state can persist well below the normal freezing point. As cooling continues there is a rise in the rate of increase of the viscosity of the supercooled liquid per unit drop in temperature. The initially mobile fluid turns into a syrup, then into a viscoelastic state, and finally into a brittle glass. A glass is, therefore, a supercooled liquid, and is characterized by an extremely high viscosity (typically of the order of 10^{14} Pa · s). Mechanically, if not structurally, glasses can be regarded as solids.

The characteristic temperature below which melted solids must be cooled to form a glass is the glass transition temperature T_g . The glass transition is a dynamic event that occurs at a temperature below which coordinated molecular motion becomes so slow that a liquid can be considered to take on the properties of a solid. While the exact value of this transition temperature depends on the heating rate, the glass transition temperature is generally found to be about two-thirds that of the melting temperature T_m . Glass transition temperatures reported for pharmaceuticals also follow this general rule, as can be seen in the

listing of ten pharmaceuticals that form glasses (Table 3). It is often found that the presence of impurities that facilitate glass formation increases the ratio T_g/T_m either by raising T_g or by lowering T_m . Hence one might wonder if some of the high values in the last column of Table 3 are due to partial decomposition of the drug substance upon melting. Of course, this is an important concern when employing the melt solidification procedure for the preparation of amorphous materials.

There are many examples given in the monograph *Thermomicroscopy in the Analysis of Pharmaceuticals* [9] of other compounds that solidify on the microscope hot stage to form glasses. However, Table 4 contains examples from the literature in which solidification from the melt (either by slow cooling to room temperature or by quench cooling with liquid nitrogen) has been employed as the specific method for obtaining amorphous material.

B. Reduction of Particle Size

Reduction of the particle size of crystalline materials to the microcrystalline level can yield a material incapable of exhibiting an x-ray pow-

Table 3 Pharmaceuticals Forming Glasses above Room Temperature

Compound	T_g (K)	T_m (K)	T_g/T_m
Cholecalciferol	296	352	0.84
Sulfisoxazole	306	460	0.67
Stilbestrol	308	439	0.70
Phenobarbital	321	443	0.72
Quinidine	326	445	0.73
Salicin	333	466	0.71
Sulfathiazole	334	471	0.71
Sulfadimethoxine	339	465	0.73
Dehydrocholic acid	348	502	0.69
17- β -Estradiol	354	445	0.80

Source: Ref. 84.

Table 4 Amorphous Pharmaceuticals Obtained by Solidification from the Melt

Compound	Method used	Reference
Phenylbutazone	Solidification from the melt	[85]
Indomethacin	Quench cooling using liquid nitrogen or slow cooling from the melt over 30 min	[86,87]
Felodipine	Cooling of the melt in liquid nitrogen or at ambient temperature	[88,89]
Nifedipine	Melting at 180°C followed by immersion in liquid nitrogen	[90]
Benperidol	Melt in an oven at 277°C then cool to room temperature	[91]
Acetaminophen	Solidification of the melt at -5°C/min	[92]
Sulfapyridine	Melting any crystalline form and slowly cooling the melt	[93]
Lovostatin	Melting under nitrogen, rapid cooling to 20°C below the glass transition point	[94]

der diffraction pattern. Dialer and Kuessner [95] found that when sucrose was milled in a vibratory ball mill, the ordered crystal was transformed into a glass-like structure. The increase in surface energy of milled sucrose, as measured by heat of solution, could not be accounted for by an increase in surface area alone. Hence milling disrupts the crystal lattice and imparts the excess free energy and entropy associated with amorphous substances.

Particle size reduction can be achieved using a variety of methods. Sometimes it is helpful to carry out the particle size reduction at reduced temperatures, such as at 4°C or at liquid nitrogen temperature, -196°C. In other instances, grinding with an excipient has been employed as a means of obtaining amorphous materials. Cyclodextrins and microcrystalline cellulose have been used for this purpose. It is also possible that the use of polymeric excipients may inhibit crystal growth when the amorphous solid is dissolved in water. Table 5 contains a list of compounds that have been obtained in amorphous, or partly amorphous, form by milling.

Table 5 Amorphous Pharmaceuticals Obtained by Milling

Compound	Method used	Reference
Cimetidine	Milling	[96]
PR76505	Grinding in a ball mill	[97]
Cephalexin	Grinding in an agate centrifugal ball mill for 4 hours	[98]
Indomethacin	Grinding for 4 hours at 4°C in a centrifugal ball mill; grinding the γ-form at 4°C	[57,99]
(E)-6-(3,4-Dimethoxyphenyl)-1-ethyl-4-mesitylimino-3-methyl-3,4-dihydro-2(1H)-pyrimidinone	Grinding in a stainless steel shaker ball mill for 60 minutes	[100]
9,3"-Diacetyl-midecamycin	Mixed grinding with polyvinylpyrrolidone or polyvinylpyrrolidone + hydroxypropylmethylcellulose for 9 hours	[101]
Chloramphenicol stearate	Milling in a Pulverisette 5 grinder (Fritsch) (agate mortar and balls) with colloidal silica or microcrystalline cellulose	[102,103]
Calcium gluceptate	Milling in a Pulverisette 2 grinder (Fritsch) (agate mortar and balls) for 4 hours	[104]
Chloramphenicol palmitate	Milling in a Pulverisette 0 grinder (Fritsch) (agate mortar and balls) for 85 hours	[105]
Aspirin	Grinding with adsorbents under reduced pressure	[106]
Ibuprofen	Grinding with β-cyclodextrin	[107]
	Roll mixing with β-cyclodextrin	[108]
Hydrocortisone acetate	Grinding with crystalline cellulose	[109]

Table 5 Continued

Compound	Method used	Reference
Digoxin	Milling in a Glen Creston Model M270 ball mill for 8 hours	[110]
	Comminution of 1 g at 196°C for 15 minutes in a freezer mill	[111]
Amobarbital	Ball-milling with methylcellulose, microcrystalline cellulose, or dextran 2000	[112,113]
Acetaminophen	Ball milling for 24 hours with α- and β-cyclodextrin	[114]
6-Methyleneandrosta-1, 4-diene-3,17-dione	Co-grinding with β-cyclodextrin for 2 hours	[115]

C. Spray-Drying

In the pharmaceutical industry, spray-drying is used to dry heat-sensitive pharmaceuticals, to change the physical form of materials for use in tablet and capsule manufacture, and to encapsulate solid and liquid particles. This methodology is also used extensively in the processing of foods [116]. In the spray-drying process, a liquid feed stream is first atomized for maximal air spray contact. The particles are then dried in the airstream in seconds owing to the high surface area in contact with the drying gas. Spray-drying can produce spherical particles that have good flow properties, and the process can be optimized to produce particles of a range of sizes required by the particular application. The process can be run using either aqueous or nonaqueous solutions. Examples of pharmaceuticals obtained in the form of amorphous powders by spray-drying are found in Table 6.

D. Lyophilization

Lyophilization (also known as freeze-drying) is a technique that is widely employed for the preparation of dry powders to be reconstituted at the time of administration. It is a particularly useful technique in the

Table 6 Amorphous Pharmaceuticals Obtained by Spray-Drying

Compound	Method used	Reference
YM022	Spray-drying a methanol solution	[117]
α -Lactose monohydrate	Spray-drying in a Buchi 190	[118]
	Spray-drying a solution or suspension	[119]
4"-O-(4-methoxy-phenyl) acetytylosin	Spray drying a dichloromethane solution	[120]
Salbutamol sulfate	Spray-drying of an aqueous solution in Buchi 90 spray dryer	[121]
Lactose	Spray-drying an aqueous solution	[118,122]
Furosemide	Spray-drying from a 4:1 chloroform: methanol solution at 50 and 150°C inlet temperature	[123,124]
Digoxin	Spray-drying an aqueous solution containing hydroxypropyl methylcellulose	[125]
Cefazolin sodium	Spray-drying from a 25% aqueous solution with an inlet temperature of 150°C and an outlet temperature of 100°C	[126]
9,3"-Diacetyl-midecamycin	Spray-drying of aqueous solution in the presence and absence of ethylcellulose	[127]

case of compounds that are susceptible to decomposition in the presence of moisture but that are more stable as dry solids. The physical form, chemical stability, and dissolution characteristics of lyophilized products can be influenced by the conditions of the freeze-drying cycle. In most pharmaceutical applications, lyophilization is performed on aqueous solutions containing bulking agents, and these often are chosen so as to form a coherent cake after completion of the freeze-drying process. However, lyophilization also can be employed to convert crystalline materials into their amorphous counterparts. The lyophilization process usually consists of the three stages of freezing, primary drying,

and secondary drying. For the preparation of amorphous materials, rapid freezing is employed so as to avoid the crystallization process. Both aqueous solutions and solutions containing organic solvents have been lyophilized. The primary drying phase involves sublimation of frozen water or vaporization of another solvent. This step is carried out by reducing the pressure in the chamber and supplying heat to the product. The secondary drying phase consists of the desorption of moisture (or residual solvent) from the solid.

Recently, excipients of various types have been employed in frozen solutions so as to inhibit crystallization. Cyclodextrins appear to be particularly useful for this purpose, although it is generally necessary to employ rapid freezing to liquid nitrogen temperatures to ensure that the freeze-dried product is noncrystalline. When α -cyclodextrin, which has a larger cavity than does β -cyclodextrin, is frozen at a relatively slow rate, it will cocrystallize with compounds such as benzoic acid, salicylic acid, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, and methyl *p*-hydroxybenzoate [128]. However, rapid freezing of a methyl *p*-hydroxybenzoate solution containing α -cyclodextrin at a benzoate/cyclodextrin ratio of 0.33 yields an amorphous solid after freeze-drying [29].

β -Cyclodextrin and its derivatives have been shown to form amorphous lyophilized products with a number of compounds, principally nonsteroidal antiinflammatory agents. Examples from the literature of excipients and pharmaceuticals prepared as amorphous materials by lyophilization are given in Table 7.

E. Removal of Solvent from a Solvate or Hydrate

Solids can sometimes be rendered amorphous by the simple expedient of allowing solvent molecules of crystallization to evaporate at modest temperatures. If the solvent merely occupies channels in the crystal structure, the structure often remains intact, but when the solvent is strongly bonded to molecules of the host, the structure frequently will collapse when the solvent is removed and one obtains an amorphous powder. A few examples of amorphous solids obtained in this manner are found in Table 8.

Table 7 Amorphous Pharmaceuticals Obtained by Lyophilization

Compound	Method used	Reference
Lactose	Lyophilization of a 5% Aqueous Solution	[130]
MK-0591	Lyophilization	[131]
Raffinose	Lyophilization of a 10% aqueous solution frozen at -45°C	[132]
Sucrose	Lyophilization of 10% aqueous solutions	[133]
Dirithromycin	Freeze-drying from methylene chloride solution	[134]
Cefalexin	Aqueous solution frozen at -196°C , then freeze-dried	[135]
	Lyophilization of a saturated aqueous solution	[136]
Calcium gluceptate	Freeze-drying from 2% aqueous solution	[137]
Griseofulvin	Freeze-drying of solutions of griseofulvin or of solutions of mixtures of griseofulvin and mannitol in dioxane or 1:1 dioxane-water with fast freezing in liquid nitrogen	[138]
Tolobuterol hydrochloride	Freeze-drying of aqueous solution	[139]
E1040	Freeze-drying of aqueous solution	[140]
Glutathione	Freeze-drying of a 5% aqueous solution	[141]
Aspirin	Freeze drying of an aqueous solution in the presence of 1.0% hydroxypropyl- β -cyclodextrin	[142]
Ketoprofen	Freeze-drying in the presence of heptakis-(2,6-O-dimethyl)- β -cyclodextrin	[143]
	Freeze-drying with β -cyclodextrin (rapid freezing with liquid nitrogen)	[144]
Glibenclamide	Freezing at liquid nitrogen temperature, freeze-drying over 24 hours	[145]

Table 7 Continued

Compound	Method used	Reference
Naproxen	Copolyphilization (223K and 0.013 torr) of naproxen and hydroxyethyl- β -cyclodextrin, or hydroxypropyl- β -cyclodextrin	[146]
Sodium ethacrylate	Rapid freezing of an aqueous solution to -50°C , followed by freeze-drying	[147]
<i>p</i> -Aminosalicylic acid	Copolyphilization of <i>p</i> -aminosalicylic acid in aqueous solution with pullulan	[148]
Ceftazidime	Freeze-drying a nearly saturated aqueous solution of the free acid	[149]
Cefaclor	Freeze-drying from a nearly saturated aqueous solution	[149]
Cephalothin sodium	Freeze-drying from a 25% aqueous solution	[149]
Cefamandol sodium	Freeze-drying from a 25% aqueous solution	[149]
Cefazolin sodium	Freeze-drying an aqueous solution at low temperature	[149]
Nicotinic acid	Freeze-drying in the presence of β -cyclodextrin (fast-freezing); and heptakis (2,6-O-dimethyl)- β -cyclodextrin	[150]

F. Precipitation of Acids or Bases by Change in pH

If the level of supersaturation is carefully controlled, it is often possible to avoid crystallization when a water-soluble salt of a weak acid is precipitated with a base, or when a water-soluble salt of a weak base is precipitated with an acid. When crystalline topanoic acid is dissolved in 0.1 N NaOH, and 0.1 N HCl is added, an amorphous powder is precipitated [43]. A similar phenomenon is observed in the case of the precipitation of piretanide [155]. Another example in this genre is the

Table 8 Amorphous Pharmaceuticals Obtained by Solvent Removal

Compound	Method used	Reference
Tranilast anhydrate	Dehydration of the monohydrate over P_2O_5	[151]
Raffinose	Lyophilization and heat drying of the pentahydrate	[132]
Erythromycin	Heating the dihydrate for 2 hours at 135°C in an oven, and then cooling to room temperature	[152, 153]
Calcium DL-pantothenate	Drying the methanol:water 4:1 solvate <i>in vacuo</i> at 50–80°C	[154]

precipitation of amorphous calcium carbonate, which occurs when a calcium chloride solution is combined with a sodium carbonate solution at 283K [156].

G. Miscellaneous Methods

Earlier during the discussion on the preparation of polymorphs, the doping of crystals was mentioned as a technique for encouraging the formation of one type of polymorph over another. Similarly, if a dopant is employed at levels that will disrupt the crystal lattice, the substance can be made to solidify as an amorphous material. Duddu and Grant [157] observed changes in the enthalpy of fusion of (–)-ephedrinium 2-naphthalenesulfonate when the opposite enantiomer, (+)-ephedrinium 2-naphthalenesulfonate, was added as a dopant.

When *m*-cresol was added to a suspension of insulinotropin crystals grown from a normal saline solution, the crystals were immediately rendered amorphous. It was postulated [158] that the *m*-cresol molecules diffused into the crystals through solvent channels and disturbed the lattice interactions that ordinarily maintained the integrity of the crystal. When zinc acetate or zinc chloride was added to the suspension, the zinc ion stabilized the crystal lattice so that the subsequent addition of *m*-cresol did not alter the integrity of the crystals.

Sometimes solvents exert a similar effect. When a small amount of ethyl acetate is added to a calcium chloride solution prior to addition

of sodium fenoprofen, the calcium fenoprofen that precipitates has a low degree of crystallinity [159]. Similarly, when calcium DL-pantothenate is precipitated from methanol or ethanol solution by the addition of acetone, ether, ethyl acetate, or other solvents, the precipitate obtained is found to be amorphous [154].

V. SUMMARY

The pharmaceutical development scientist who is assigned the task of demonstrating that a substance exhibits only one crystalline form, or that of discovering whether additional forms exist, can utilize the techniques outlined in this chapter as a starting point. Upon completion of this program, one can certainly conclude that due diligence has been employed to isolate and characterize the various solid-state forms of any new chemical entity. One should always be aware that nuclei capable of initiating the crystallization of previously undiscovered forms might be lurking around the laboratory, ready to confound the investigator should their effects become known. In addition, the phenomenon of "disappearing polymorphs" can come into play, and techniques that formerly yielded the same crystals every time may subsequently yield crystals of another, more stable form. In the future, the use of computer simulations of alternative crystallographic structures will suggest how much laboratory work might be required to isolate the polymorphs or solvates of a given compound. Until then, the empirical approach remains superior.

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6

Methods for the Characterization of Polymorphs and Solvates

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Crystalline solids

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Abstract

Many drugs exist in the crystalline solid state due to reasons of stability and ease of handling during the various stages of drug development. Crystalline solids can exist in the form of polymorphs, solvates or hydrates. Phase transitions such as polymorph interconversion, desolvation of solvate, formation of hydrate and conversion of crystalline to amorphous form may occur during various pharmaceutical processes, which may alter the dissolution rate and transport characteristics of the drug. Hence it is desirable to choose the most suitable and stable form of the drug in the initial stages of drug development. The current focus of research in the solid-state area is to understand the origins of polymorphism at the molecular level, and to predict and prepare the most stable polymorph of a drug. The recent advances in computational tools allow the prediction of possible polymorphs of the drug from its molecular structure. Sensitive analytical methods are being developed to understand the nature of polymorphism and to characterize the various crystalline forms of a drug in its dosage form. The aim of this review is to emphasize the recent advances made in the area of prediction and characterization of polymorphs and solvates, to address the current challenges faced by pharmaceutical scientists and to anticipate future developments. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Crystallinity; Polymorphs; Hydrates; Solvates; Formulation; Drug substance; Phase transformation; Characterization

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1. Introduction

Most organic and inorganic compounds of pharmaceutical relevance can exist in one or more crystalline forms. When applied to solids, the adjective, *crystalline*, implies an ideal crystal in which the structural units, termed *unit cells*, are repeated regularly and indefinitely in three dimensions in space. The unit cell has a definite orientation and shape defined by the translational vectors, a , b , and c , and hence has a definite volume, V , that contains the atoms and molecules necessary for generating the crystal. Each crystal can be classified as a member of one of seven possible crystal systems or crystal classes that are defined by the relationships between the individual dimensions, a , b , and c , of the unit cell and between the individual angles, α , β , and γ of the unit cell [1,2]. The structure of a given crystal may be assigned to one of the seven crystal systems, to one of the 14 Bravais lattices, and to one of the 230 space groups [1]. All the 230 possible space groups, their symmetries, and the symmetries of their diffraction patterns are compiled in the International Tables for Crystallography [3].

The common crystalline forms found for a given drug substance are polymorphs and solvates. Crystalline polymorphs have the same chemical composition but different internal crystal structures and, therefore, possess different physico-chemical properties. The different crystal structures in polymorphs arise when the drug substance crystallizes in different crystal packing arrangements and/or different conformations. The occurrence of polymorphism is quite common among organic molecules, and a large number of polymorphic drug compounds have been noted and catalogued [4–7].

Solvates, also known as pseudopolymorphs, are

crystalline solid adducts containing solvent molecules within the crystal structure, in either stoichiometric or nonstoichiometric proportions, giving rise to unique differences in the physical and pharmaceutical properties of the drug. If the incorporated solvent is water, a solvate is termed a hydrate. Adducts frequently crystallize more easily because two molecules often can pack together with less difficulty than single molecules. While no definite explanations can be given, possible reasons include adduct symmetry, adduct-induced conformation changes, and the ability to form hydrogen bonds through the solvent molecules [2,8,9]. Desolvated solvates are produced when a solvate is desolvated and the crystal retains the structure of the solvate [10]. Desolvated solvates are less ordered than their crystalline counterparts and are difficult to characterize, because analytical studies indicate that they are unsolvated materials (or anhydrous crystal forms) when, in fact, they have the structure of the solvated crystal form from which they were derived [11].

Because different crystalline polymorphs and solvates differ in crystal packing, and/or molecular conformation as well as in lattice energy and entropy, there are usually significant differences in their physical properties, such as density, hardness, tabletability, refractive index, melting point, enthalpy of fusion, vapor pressure, solubility, dissolution rate, other thermodynamic and kinetic properties and even color [12]. Differences in physical properties of various solid forms have an important effect on the processing of drug substances into drug products [13], while differences in solubility may have implications on the absorption of the active drug from its dosage form [14], by affecting the dissolution rate and possibly the mass transport of the molecules. These concerns have led to an increased regulatory

interest in understanding the solid-state properties and behavior of drug substances. For approval of a new drug, the drug substance guideline of the US Food and Drug Administration (FDA) states that “appropriate” analytical procedures need to be used to detect polymorphs, hydrates and amorphous forms of the drug substance and also stresses the importance of controlling the crystal form of the drug substance during the various stages of product development [11]. It is very important to control the crystal form of the drug during the various stages of drug development, because any phase change due to polymorph interconversions, desolvation of solvates, formation of hydrates and change in the degree of crystallinity can alter the bioavailability of the drug. When going through a phase transition, a solid drug may undergo a change in its thermodynamic properties, with consequent changes in its dissolution and transport characteristics [15].

Various pharmaceutical processes during drug development significantly influence the final crystalline form of the drug in the dosage form. The various effects of pharmaceutical processing on drug polymorphs, solvates and phase transitions have been described in detail by Brittain and Fiese [16] and will be discussed in later chapters. Briefly, processes such as lyophilization and spray drying may lead to the formation of the amorphous form of drug, which tends to be less stable and more hygroscopic than the crystalline product. Also, processing stresses, such as drying, grinding, milling, wet granulation, oven drying and compaction, are reported to accelerate the phase transitions in pharmaceutical solids. The degree of polymorphic conversion will depend on the relative stability of the phases in question, and on the type and degree of mechanical processing applied. Keeping these factors in mind, it is desirable and usual to choose the most stable polymorphic form of the drug in the beginning and to control the crystal form and the distributions in size and shape of the drug crystals during the entire process of development. The presence of a metastable form during processing or in the final dosage form often leads to instability of drug release as a result of phase transformation [17].

Crystallization plays a critical role in controlling the crystalline form and the distribution in size and shape of the drug. The significance of crystallization

mechanisms and kinetics in directing crystallization pathways of pharmaceutical solids and the factors affecting the formation of crystals have been reviewed in detail by various researchers [12,18,19]. A crystalline phase is created as a consequence of molecular aggregation processes in solution that lead to the formation of nuclei, which achieve a certain size during the nucleation phase to enable growth into macroscopic crystals to take place during the growth phase. The factors affecting the rate and mechanisms by which crystals are formed are: solubility, supersaturation, rate at which supersaturation and desupersaturation occur, diffusivity, temperature, and the reactivity of surfaces towards nucleation. The various forces responsible for holding the organic crystalline solids together, such as nonbonded interactions and hydrogen bonding, have been discussed in detail by Byrn et al. [2] and Etter [20].

Various analytical methods are being currently used to characterize the crystalline form of the drug during the various steps of processing and development. These methods have been reviewed recently in detail by many authors [7,10,21–25]. The single most valuable piece of information about the crystalline solid, including the existence of polymorphs and solvates, is the molecular and crystalline structure, which is determined by single-crystal X-ray diffractometry [2]. Powder X-ray diffractometry provides a “fingerprint” of the solid phase and may sometimes be used to determine crystal structure. Once the existence of polymorphism (or solvate formation) is definitely established by single-crystal and powder X-ray diffractometry, spectral methods, such as Fourier transform infrared absorption (FTIR) spectroscopy, Fourier transform Raman scattering (FT Raman) spectroscopy, solid-state nuclear magnetic resonance (SSNMR) spectroscopy, ultraviolet and visible (UV–Vis) and/or fluorescence spectroscopy [23] may be employed for further characterization. Of special significance are thermal methods, such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and optical microscopy using a hot stage [24]. These methods are almost always employed for further characterization. Modulated (temperature) differential scanning calorimetry (MDSC) in combination with DSC and optical microscopy are able to identify the glass

transition of amorphous forms with much greater clarity and allow unique insights into the glass transitional and polymorphic behavior of drug substances [26].

Because solid-state NMR spectroscopy can be used to study crystalline solids, as well as pharmaceutical dosage forms, this powerful method is finding increasing application in deducing the nature of polymorphic variations [27], such as variations in hydrogen bonding network and molecular conformations among polymorphs [28,29] and for the determination of molecular conformations and mobility of drugs in mixtures and dosage forms [2]. Solid-state ^{13}C -NMR in conjunction with the techniques, known as high power proton decoupling, cross polarization (CP), and magic-angle spinning (MAS) offers information not obtained readily by other techniques. Recently, two-dimensional ^{13}C -solid-state NMR spectroscopy has been used to study the three conformational polymorphs of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile [30]. Use of two-dimensional NMR and total suppression of spinning side bands (TOSS) pulse sequences allowed the separation of isotropic and anisotropic chemical shifts for the three forms. This is a very powerful method for analyzing differences in the chemical environment and is finding increased application in the study of conformational polymorphism.

With advances in analytical methods, the current focus of research in the solid-state area is to understand polymorphism and pseudopolymorphism at the molecular level. Knowledge of the crystal packing arrangements and the various intermolecular forces involved in the different packing arrangements will help in the prediction and preparation of the most stable polymorphs of a given compound well in advance, to avoid surprises during product development. A current emphasis is on the development of software to predict crystal structures of polymorphs from molecular structures. A thorough understanding of the physicochemical properties of polymorphs and solvates (hydrates) is of primary importance to the selection of a suitable crystalline form and development of a successful pharmaceutical product. Bray et al. [31] have shown that, by thorough characterization of four different crystalline forms of L-738,167, a fibrinogen receptor antagonist by various analytical

techniques, it was possible to determine the suitability of one or two forms for the development of pharmaceutical oral dosage forms.

The present review aims to emphasize the recent advances made in the area of prediction and characterization of polymorphs and solvates, attempts to address the current challenges and problems faced by pharmaceutical scientists and intends to anticipate future development. This review does not attempt to provide solutions to the problems but attempts to review comprehensively the advances made in recent years to help address these problems.

2. Recent advances in the identification, prediction and characterization of polymorphs

2.1. Types of polymorphism

Based on differences in the thermodynamic properties, polymorphs are classified as either enantiotropes or monotropes, depending upon whether one form can transform reversibly to another or not. In an enantiotropic system, a reversible transition between polymorphs is possible at a definite transition temperature below the melting point. In a monotropic system, no reversible transition is observed between the polymorphs below the melting point. Four useful rules have been developed by Burger and Ramburger [32,33] to determine qualitatively the enantiotropic or monotropic nature of the relationship between polymorphs. These rules are the heat of transition rule, heat of fusion rule, infrared rule and density rule.

If, by use of the above rules, it is established that the polymorphs of a particular drug are enantiotropic or monotropic, then the next goal is to define the thermodynamically stable (or metastable) domain of each crystalline phase of a substance as a function of temperature. The plot of the Gibbs free energy difference, ΔG , against the absolute temperature, T , gives the most complete and quantitative information on the stability relationship of polymorphs [22], with the most stable polymorph having the lowest Gibbs free energy. The ΔG between the polymorphs may be obtained using several techniques operating at

different temperatures, such as solubility [34] and intrinsic dissolution rate. Yu [35] has derived thermodynamic equations to calculate ΔG between two polymorphs and its temperature slope from the melting data. This method is essentially an extension of the heat of fusion rule, which is based on statistical mechanics. Extrapolating ΔG to zero gives an estimate of the transition temperature, from which the existence of monotropy or enantiotropy is inferred. The integration of different types of data provides the ΔG vs. T curve over a wide temperature range and allows the consistency between techniques to be checked [22]. Another approach to establish the order of stability among various polymorphs has been studied using pressure versus temperature plots, e.g., for sulfanilamide and piracetam [36]. This approach is based upon Ostwald's principle of least vapor pressure, according to which the stable polymorph exhibits the lowest vapor pressure. The accuracy of this approach to establish the stability hierarchy among the polymorphs has been shown to be very much dependent on the accuracy of the experimental data.

In recent years, the main focus of research has been the characterization of polymorphs arising from structural differences in the crystal lattice. It has been established for some time that organic molecules are capable of forming different crystal lattices through two different mechanisms. One of the mechanisms is termed *packing polymorphism*, and represents instances where conformationally relatively rigid molecules can be assembled into different three-dimensional structures through the invocation of different intermolecular mechanisms. The other mechanism is termed *conformational polymorphism* and arises when a nonconformationally rigid molecule can be folded into different arrangements, which subsequently can be packed into alternative crystal structures. The distinction between *packing polymorphism* and *conformational polymorphism* is somewhat artificial because different packing arrangements impose different conformations on the molecules, however slight, and different conformations will inevitably pack differently. The structural aspects associated with polymorphs have been reviewed recently [2], as have the analogous features of solvate and hydrate systems [9]. In the next

section, the results of some more recent investigations are discussed.

2.2. Packing polymorphism

An investigation into the structures and charge densities of two polymorphs of *p*-nitrophenol has been performed with the aim of deducing the different modes of inter-molecular hydrogen bonding that lead to the formation of the two structures shown in Fig. 1a and b [37]. A detailed analysis of the charge density of the two forms indicates charge migration from the benzene ring region to the nitro and hydroxyl groups that accompanies the transformation of one form into the other. In addition, polarization of the oxygen lone-pair electrons was found to be substantially larger in the crystal forms than in the free molecule, resulting in considerably larger dipole moments in the solid state.

During the study of a new crystal form (form I) of

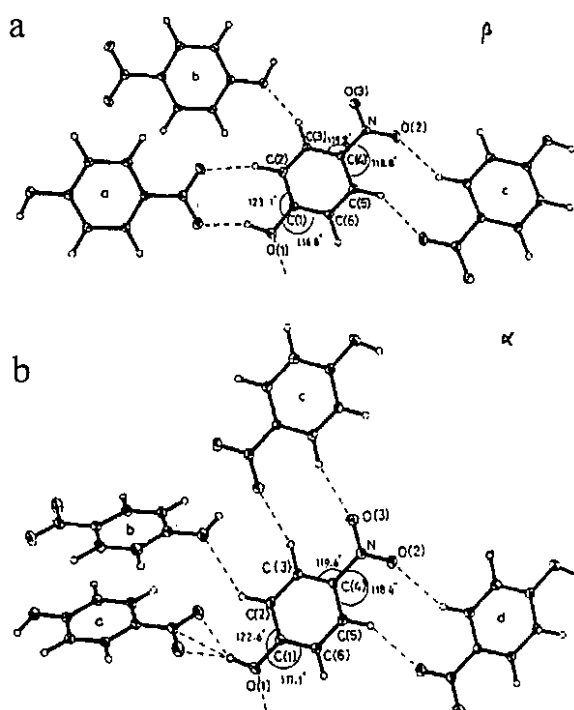


Fig. 1. Molecular packing diagrams of the (a) β polymorph of *p*-nitrophenol, (b) α polymorph of *p*-nitrophenol, showing 50% probability displacement ellipsoids ([37], reproduced with the permission of the American Chemical Society).

chlordiazepoxide, it was found that the heat of transition between the two forms (forms I and II) is rather modest, and kinetic factors permit the existence of the metastable phase [38]. Both structures contain four crystallographically independent molecules linked in dimers through hydrogen bonding, but the dimers are packed differently to yield the two crystal forms. Because the dimers in the fundamental units are spaced differently in the two forms, it was proposed that the solid-state enantiotropic transformation entailed rearrangement of the dimer units.

A different approach has been taken during an evaluation of the different structures formed by sulfathiazole [39]. Using a graph set approach to classify the known structural differences and similarities among the various forms, it became possible to identify packing motifs common to three of the four crystal structures. Fig. 2 shows the unit cells of the polymorphs I, II, III and IV, where molecules are paired as hydrogen-bonded dimers. At the end of the process, the authors were able to deduce possible links between the observed patterns of hydrogen bonding, processes of nucleation, and the crystal growth observed from a number of solvent systems. Interestingly, the analysis did not indicate a relationship between the appearance of a particular polymorph from solution and the growth of its fastest

growing surface. Rather, it appeared as if the different solvents affected the process of polymorph formation through their effects on nucleation of the various forms.

2.3. Conformational polymorphism

The conformational polymorphism of the two forms of piroxicam pivalate has been studied in detail [40]. This compound is distinctive in that the high-melting form (polymorph 1) contains an unanticipated array of associated molecules bound as centrosymmetric dimers through hydrogen bonding, with the amido nitrogen atom acting as the donor and the pyridine nitrogen as the acceptor (Scheme 1, structure I). The low-melting form (polymorph 2) contains molecules of two distinct conformational states coexisting in the same crystal (Fig. 3), but linked through different hydrogen bonding arrangements. This latter finding represents another unusual aspect of the crystallography of the substance.

The inclusion of different solvent molecules in a crystal lattice can lead to the existence of different packing patterns, and has also been found to influence the molecular conformation of paroxetine hydrochloride in two solvate forms [41]. One form

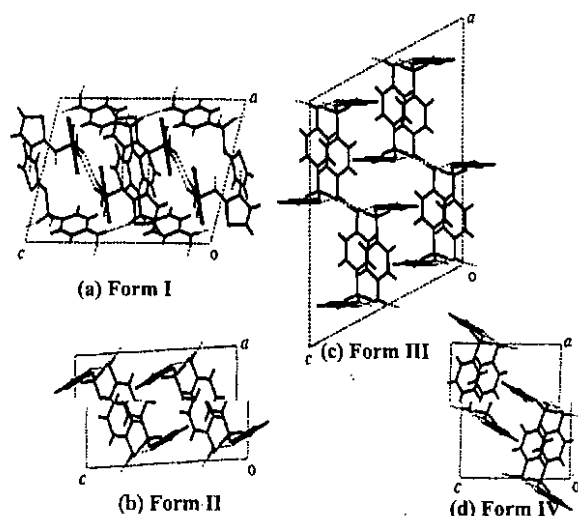
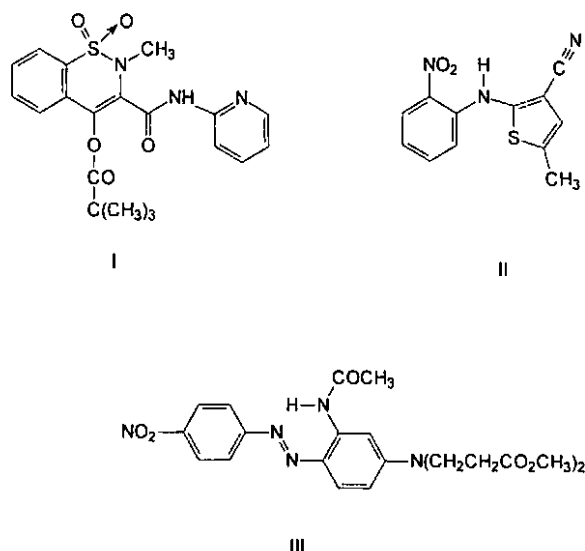


Fig. 2. Unit cells of four polymorphs (I, II, III and IV) of sulfathiazole showing hydrogen bonds, with the dimer structure clearly discernible ([39], reproduced with the permission of the Royal Society of Chemistry).



Scheme 1. Molecular structure of piroxicam pivalate (I) [40], 5-methyl-2-[2-(nitrophenyl)amino]-3-thiophenecarbonitrile (II) [30], 2'-acetamido-4'-[N,N-bis(2-methylcarbonylethyl)amino]-4-nitroazobenzene (III) [48].

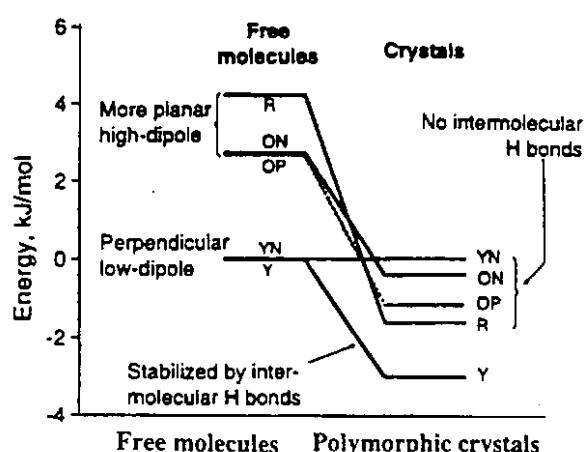


Fig. 4. Comparison of conformational energies and crystal energies of the various polymorphs of 5-methyl-2-[2-(nitrophenyl)amino]-3-thiophenecarbonitrile (II). Form R (red prisms, m.p. 106.2°C), form Y (yellow prisms, m.p. 109.8°C), form OP (orange plates, m.p. 112.7°C), form ON (orange needles, m.p. 114.8°C), form YN (yellow needles, m.p. not measurable) ([42], reproduced with the permission of the American Chemical Society).

physical transition, four steps have been proposed: (a) molecular loosening in the initial phase; (b) formation of an intermediate solid solution; (c) nucleation of the new solid phase and (d) growth of the new phase [2]. In an interesting study, Skwierczynski [44] has proposed a two-environment model to describe the decomposition reaction kinetics of a crystalline solid, aspartame. The decomposition reaction of aspartame is a simple unimolecular thermally-induced aminolysis and the reaction proceeds under anhydrous conditions, i.e., water is not a reactant [45]. This model links the chemistry of the solid-state reaction with the molecular mobility of the reactant as the reaction proceeds. The advantage of this model is that it can be used to determine the shelf life of a product from kinetic data gathered at elevated temperatures. Apart from solid–solid physical transformations, solution-mediated physical transformations among polymorphs are also known to occur in processes, such as wet granulation and during dissolution testing.

While the majority of studies have probed the equilibrium properties of polymorphic and solid-state solvated systems, relatively few have been concerned with the dynamics of phase transformation. Byrn et

al. [2] have reviewed briefly the aspect of polymorphic interconversions and the factors affecting the transformations. In one study, the contribution of hydrogen bonding to the $\alpha \rightarrow \beta$ phase change of resorcinol has been detailed [46]. The α form is more stable than the β form at room temperature, but is less dense than the β form. The transition of $\alpha \rightarrow \beta$ at an estimated transition temperature of 337 ± 1 K is accompanied by an increase in crystal density, with the structure shifting from an open array of molecules (linked through hydrogen bonding) to a denser structure resembling molecular crystals. Through the use of a simple potential model, it was concluded that, during the phase transformation, the energy of the hydrogen bonds decreases along with the extent of such bonding. The energy liberated by this process is almost offset by the enhanced Van der Waals energies associated with the increase in crystal density, and consequently the transition enthalpy is rather small. Accompanying the shifts in hydrogen bonding is a number of effective proton transfers, altering the covalent and ionic portions of the crystal. It was also learned that the increase in entropy produced from the redistribution of protons was of the same order of magnitude as the entropy of the phase transition.

A number of spectroscopic techniques have been used to study the processes associated with a polymorphic transition of 2-(2,4-dinitrobenzyl)-3-methylpyridine [47]. The two interconverting structures coexisted over a temperature range of at least 8–9°C. The phase change was associated with a molecular tautomerization that translated through the collective changes of a large number of molecules, yielding domains having definite short-range order. The slowly evolving spectroscopy that took place above the transition temperature was interpreted as the annealing of domains into a long-range ordered system. The process of phase transformation appeared to consist of an initial fast redistribution of the mole ratio of the coexisting phases, followed by a much slower process involving a macroscopic relaxation of the system. Although local thermodynamic equilibrium was thought to exist in individual domains, the magnitude noted for the temperature range of the phase transition was proposed to arise from nonequilibrium conditions existing among the various types of domain.

A combination of solid-state ^{15}N -NMR spectroscopy and X-ray crystallography was used to study polymorphic transitions in an azobenzene dyestuff, 2'-acetamido-4'-[*N,N*-bis(2-methoxycarbonylethyl)amino]-4-nitroazobenzene (Scheme 1, structure III) [48]. This work established that the structure of one polymorph was disordered, and that the process of phase transformation entailed a crankshaft-type motion of the azo linkage. The ORTEP plots of the two molecular conformations for the X-ray structure determination of structure III at 293 K are shown in Fig. 5. Selective polarization inversion and band shape-fitting experiments were used to deduce the thermodynamic parameters of the exchange process.

Raman spectroscopy was used to study the effect of pressure on the phase transitions in hexamethylbenzene and hexa(methyl- d_3)benzene [49]. The form II $\rightarrow$ form III transition of the partially deuterated substance was found to take place at a lower pressure relative to that of the analogous hexamethylbenzene compound, which was attributed to differences in the energies of the intramolecular methyl torsional vibration in the two crystal forms. In another study performed by the same group, the effects of both temperature and pressure on the phase transitions of tetrafluoro-1,4-benzoquinone were considered [50]. In this system, the changes in en-

vironmental conditions were found to influence a number of intermolecular and intramolecular vibrational modes, yielding conformational changes that in turn produced the observed phase transitions.

2.5. Prediction of polymorphs

The main challenge in managing the phenomenon of multiple solid forms of a drug is the inability to predict the number of forms that can be expected in a given case. This prediction would involve quantification of the myriad intermolecular forces within any proposed crystal structure as well as the ability to postulate the likely packing modes for a given molecule in all its configurations [10]. Accurate theoretical prediction of polymorphs from studies of molecular dynamics and crystal structure generation would be of outstanding importance in drug research [36].

More research is now being directed towards developing computational tools to understand the nature of polymorphism and to predict polymorphic forms at an early stage in the drug development process. The recent developments in computational chemistry allow the prediction of possible polymorphic forms based only on the molecular structure of the drug. The Polymorph Predictor, from Molecular Simulations, is currently the only commercial software package that can predict the possible polymorphs of an organic compound from its molecular structure [51]. The package developed by Karfunkel and co-workers [52–54] uses a Monte Carlo simulated annealing approach to generate thousands of possible crystal packing alternatives for a given molecule. Each of the unique crystal structures is then subjected to a lattice energy minimization to obtain the relative stability ranking of the various packing possibilities and the resulting lowest-energy structures are the potential polymorphs. This method has been successfully employed to generate known polymorphs of primidone (Fig. 6A and B) and progesterone, starting from the molecular structures alone [55]. It has also been used to predict polymorphs for a range of small molecules and to predict unknown polymorphic structures of 4-amidinoin-danone guanyldiazide, a selective inhibitor of *S*-adenosylmethionine decarboxylase [56], and of aspirin [57].

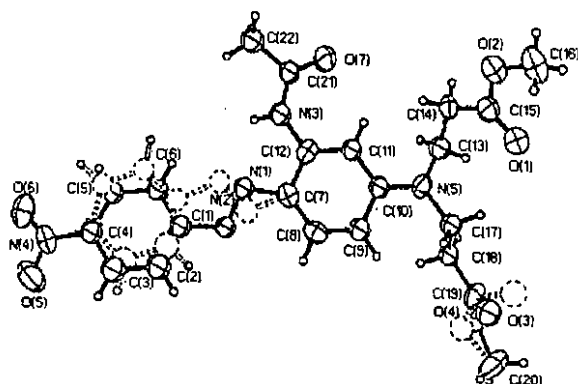


Fig. 5. ORTEP plots of the two molecular conformations (1 and 2) for the X-ray structure determination of 2'-acetamido-4'-[*N,N*-bis(2-methoxycarbonylethyl)amino]-4-nitroazobenzene (Scheme I Structure) at 293 K. Thermal ellipsoids are shown at 30% for clarity, with conformer 2 being represented by the solid lines and Conformer 1 by the dotted lines ([48], reproduced with the permission of the American Chemical Society).

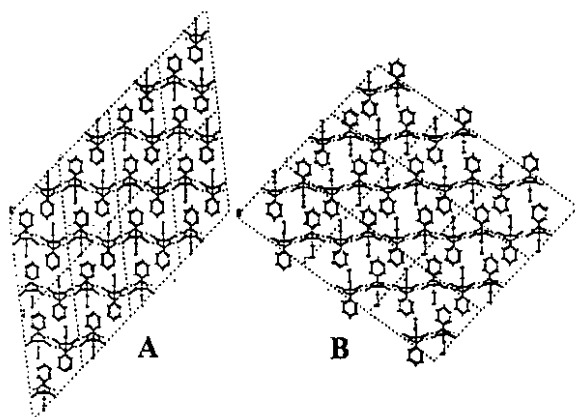


Fig. 6. Comparison of the crystal structure of primidone A (A) versus the most likely packing arrangement, frame 7 (B) ([55], reproduced with the permission of Elsevier Science).

The theoretical predictions of lattice energies, entropies, morphologies and polymorphs should stimulate experimental activities and vice versa. The current crystal-modeling efforts have the potential of producing more quantitative tools for bridging structures and properties, which could help in creating solid forms with desired properties [22]. There are many limitations in using computational methods for predicting polymorphs theoretically. The first limitation is that the *ab initio* screening is useful only for nonionic rigid molecules. For more complex systems, the method is very useful for generating plausible crystal structures, but it is not accurate enough to determine which of these possible structures can actually be crystallized [58]. In addition, the limitations in computer power can restrict the use of this method for predicting polymorphs of complex molecules. An issue of concern is that the existing methods only predict the lattice energies, which relate to internal energies or enthalpies of the crystals. However, the relative thermodynamic stability of polymorphs is determined by the Gibbs free energy, which is a linear function of both enthalpy and entropy. Predictions of the relative stability of polymorphs will be more accurate when the entropies, as well as lattice energies, are considered. Application of molecular dynamics may enable the entropies to be calculated. Hence, no general method is currently available for the prediction or interpretation of the properties of complicated polymorphic or pseudopolymorphic systems.

2.6. Directing the crystallization of specific polymorphs

Complementing the different computational methods for predicting the stable polymorphs of a given compound, various experimental methods are also being employed extensively to control the type of polymorph formed during the crystallization process. Many studies have reported the role of additives in controlling the outcome of the crystallization process. Some of the preselected additives are capable of inhibiting the nucleation and/or growth of the unwanted polymorphs. For the first time, the role of reaction by-products in controlling polymorph appearance of a drug has been reported [59]. This drug is sulfathiazole that is known to exist as polymorphs, forms I, II, III and IV, that differ in the hydrogen-bond network. Form I was found to be different from the other three forms as a result of a different hydrogen bonding at the aniline moiety of the molecule. From studies of the hydrogen-bonding pattern, it was predicted that the ethamido derivative of sulfathiazole could selectively control the formation of form I over other forms by entering the growing face of form I without disrupting the structure (Fig. 7a). Because a similar effect was not possible with the other forms, incorporation of the ethamido derivative in the other forms should inhibit their growth (Fig. 7b). Experimentally, it was shown that the ethamido by-product stabilized form I over the other polymorphs. This study clearly shows that the combination of crystal morphology and the hydrogen-bond network analysis of the different polymorphs offer a new and powerful approach to understanding and controlling polymorph appearance and stability in the presence of additives.

A similar approach was also applied to stabilize a metastable α conformational polymorph of L-glutamic acid using additives [60]. Methods such as DREIDING and TRIPOS force fields were used to select appropriate additives which could mimic the α and β conformations. Four additives were chosen for this study of which two were present exclusively in the β conformation and theoretically should selectively inhibit the crystallization of the β phase and thus stabilize the metastable α phase. Experimentally, it was proven that the additives, by virtue of their conformation, were able to selectively inhibit the

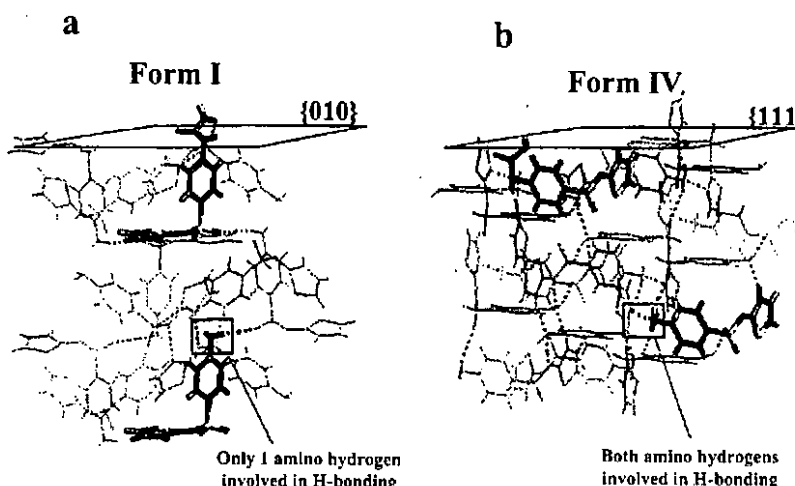


Fig. 7. Possible binding interaction of ethamidosulfamide in the fastest growing faces of (a) form I and (b) form IV of sulfathiazole ([59], reproduced with the permission of Elsevier Science).

appearance of the stable β polymorph of L-glutamic acid by interfering with either the nucleation rates or the growth rates and thus stabilize the metastable form. These studies demonstrate clearly that the molecular packing and intermolecular hydrogen bonds are the main features, which make possible the conformational discrimination. The use of conformational mimicry to stabilize the metastable structures of conformational polymorphs now offers a powerful tool for the prediction and development of robust processes for the control of polymorphic systems.

2.7. Characterization of polymorphs using a combination of analytical techniques

The common techniques often fail to differentiate definitively between two structurally similar polymorphs. Hence more advanced techniques or a combination of techniques need to be used to avoid errors of interpretation and in the identification of polymorphs [24]. Combinations of techniques are being employed currently for the characterization of crystalline pharmaceutical solids. For example, conventional single-crystal X-ray diffractometry and polarized microscopy were of no use in distinguishing the two forms I and II of roxifiban, a very promising cardiovascular drug, because of the relatively small crystallite sizes of the polymorphs. Hence, transmission electron microscopy (TEM) and

synchrotron X-ray diffraction techniques were employed to characterize the unit cells of the two forms. By coupling the highly resolved synchrotron powder X-ray diffraction data shown in Fig. 8, with in-

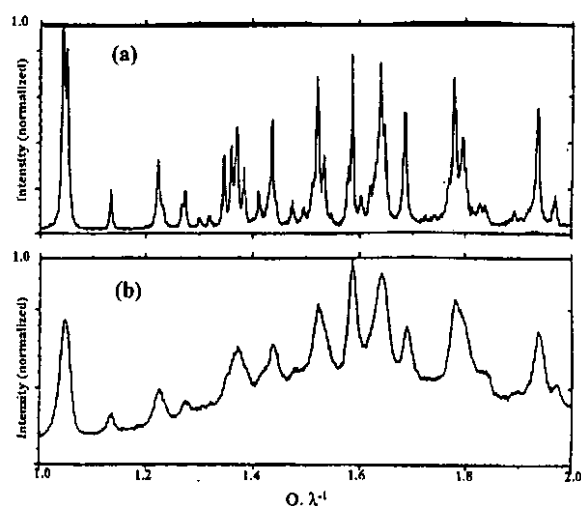


Fig. 8. A partial comparison of (a) a synchrotron pattern of polymorph II of roxifiban, collected using a wavelength of 1.00006 Å with (b) a conventional X-ray diffraction pattern using CuK α radiation in a region where there are many overlapping peaks. The patterns are plotted as a function of $Q = 2\pi/d = 4\pi \sin \theta/\lambda$ to remove the effects of different wavelengths ([61], reproduced with the permission of the American Pharmaceutical Association).

formation obtained from TEM diffraction patterns, the unit cell parameters of the two forms of roxifiban were determined [61]. Similarly, the three modifications, I, II and III, of the nonsteroidal antiinflammatory drug, tiaprofenic acid, could not be distinguished by the two traditional spectroscopic methods, FTIR and FT-Raman spectroscopy. The modifications can only be distinguished by a combination of thermoanalytical and powder X-ray diffractometric methods [62].

Another example, to which a combination of techniques has been successfully applied to identify the various conformational polymorphs of a drug, is the characterization of the solid forms of neotame [29]. Neotame, *N*-(3,3-dimethylbutyl)-L-aspartyl-L-phenylalanine methyl ester, a new high-potency sweetener exists in the following phase-pure crystalline forms: monohydrate, the most stable crystalline form of neotame under ambient conditions, a methanol + water solvate [63], a methanol solvate [64], an amorphous anhydrate [29] and a crystalline anhydrate (form A; [65]). The authors conducted a systematic study of the conversion of the monohydrate under vacuum to a mixture of anhydrate forms followed by the reconversion of the anhydrate to the monohydrate upon exposure to moisture under ambient conditions. No significant changes were observed in the powder X-ray diffraction patterns during part of the reconversion process, suggesting that no change in lattice structure had occurred. However, the solid-state ^{13}C -CP-MAS NMR spectra, indicated the presence of several forms of neotame during the reconversion (Fig. 9). This discrepancy in the results between the two techniques was attributed to the conformational change of neotame molecules during reconversion, without significant change in unit cell parameters. This example indicates that both solid-state ^{13}C -CP-MAS NMR spectroscopy and powder X-ray diffractometry are needed to analyze mixtures of solid forms of conformationally flexible molecules, such as neotame.

A combination of solid-state ^{13}C -NMR spectroscopy and single crystal X-ray diffractometry also has been used to examine the solid-state tautomerism of acetohexamide [66,67]. Polymorphism of the anti-diabetic drug acetohexamide has been investigated by numerous techniques. On the basis of FTIR data, form A of acetohexamide has been proposed to exist

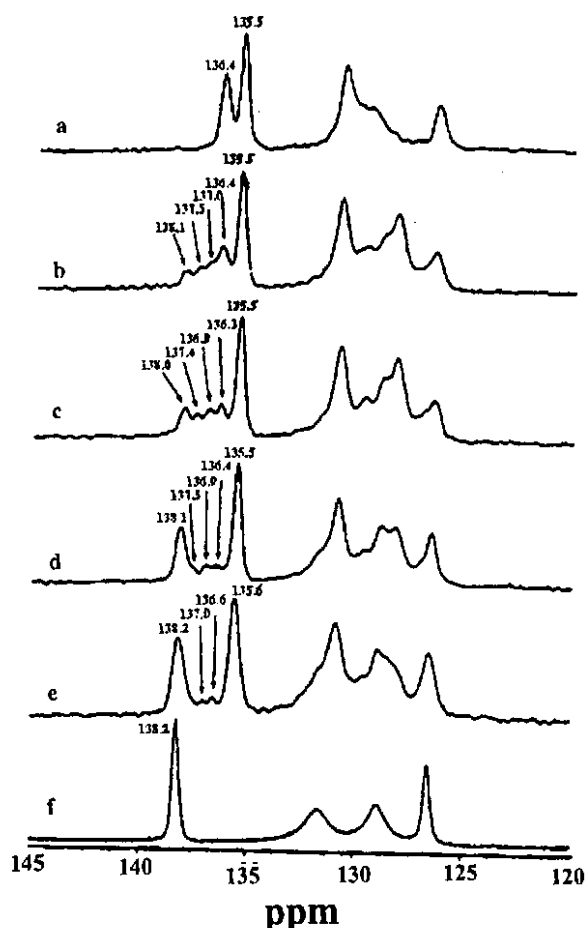


Fig. 9. Resonance signal of the phenyl carbon (C-15) attached to the side chain in the ^{13}C -CP-MAS NMR spectra of neotame anhydrate: (a) sample generated by placing the original monohydrate under vacuum (~ 1 Torr) for 3 days; (b–e) sample after being sealed in a jar for 2, 4, 6 and 8 days, respectively; (f) sample after being exposed to a relative humidity environment of 84% for 12 days ([29], reproduced with the permission of the American Chemical Society).

in the enol-tautomeric state, whereas form B has been proposed to be in the keto-tautomeric state. Using NMR and crystal structure data it was firmly established that both these acetohexamide polymorphic forms are present in the keto-form. Hence the combination of solid-state NMR spectroscopy and X-ray crystallography provided strong evidence that both forms of acetohexamide exist in the keto-tautomeric state and are truly polymorphic.

3. Recent advances in the identification and characterization of hydrates and solvates

3.1. Introduction to solvates and hydrates

It has been estimated that approximately one-third of the pharmaceutically active substances are capable of forming crystalline hydrates [68]. The water molecule, because of its small size, can easily fill structural voids and because of its multidirectional hydrogen bonding capability, is also ideal for linking a majority of drug molecules into stable crystal structures [2]. The mere presence of water in a system is not a sufficient reason to expect hydrate formation, because some compounds, though they are soluble in water, do not form hydrates. It is the activity of water in the medium that determines whether a given hydrate structure will form. Solvates may be formed when a pure organic solvent or a mixture of solvents is used as the solvent for crystallizing the compound. Guillory [69] has discussed the various methods of preparation of hydrates and solvates in detail. Because solvates behave similarly to hydrates, common analytical techniques can be used for characterization of solvates and hydrates.

3.2. Structural aspects

Crystalline hydrates, based on their structure may be classified into three categories. The first category (class I) are the isolated site hydrates, where the water molecules are isolated from direct contact with other water molecules by intervening drug molecules, e.g., cephadrine dihydrate. The second category (class 2) are channel hydrates where the water molecules included in the lattice lie next to other water molecules of adjoining unit cells along an axis of the lattice, forming channels through the crystal, e.g., ampicillin trihydrate. The channel hydrates can be subclassified into two subcategories. One category comprises the expanded-channel or nonstoichiometric hydrates, which may take up additional moisture in the channels when exposed to high humidity and for which the crystal lattice may expand or contract as the hydration or dehydration proceeds effecting changes in the dimensions of unit cells, e.g. cromolyn sodium. The other subcategory comprises

the planar hydrates, which are channel hydrates in which water is localized in a two-dimensional order, or plane, e.g., sodium ibuprofen. The third category (class 3) of crystalline hydrates are the ion-associated hydrates, in which the metal ions are coordinated with water, e.g., calteridol calcium [8,9].

In this section, some examples of nonstoichiometric hydrates and their characterization will be discussed in detail because these forms pose a special challenge in dosage form development due to unpredictability of water content in the crystals. Following the work of Cox et al. [70] the unusual water uptake and formation of nonstoichiometric hydrates of cromolyn sodium was reinvestigated using single crystal X-ray diffractometry, PXRD, as well as by molecular modeling [71]. Cromolyn sodium, an antiasthmatic drug, exists as two liquid crystalline phases and a crystalline hydrate phase that sorbs and liberates water continuously and reversibly to give a continuous range of nonstoichiometric hydrates [70]. The changes in the PXRD patterns of the crystalline hydrate phase of cromolyn sodium in response to the surrounding relative humidity (RH) were explained in the light of the molecular and crystal structure of cromolyn sodium. Single crystal X-ray diffractometry indicated the space group for cromolyn sodium as *P*1, a chiral space group, even though the molecule itself is achiral. The crystal structure of cromolyn sodium with five or six water molecules per cromolyn sodium molecule, solved at room temperature by Hamodrakas et al. [72], revealed the positions of only one sodium ion and two water molecules and showed that the second sodium ion and the other water molecules are disordered. Recently, the single crystal structure of cromolyn sodium at 76% RH, with 6.44 molecules of water was solved at 173 K by Chen et al. [71]. This work showed that the second undetermined sodium ion is disordered over three sites and that four of the eight water positions are partially occupied. Comparison of the crystal structures determined by Hamodrakas et al. [72] and Chen et al. [71] indicated that the cromolyn anion is flexible. In particular, the bond and torsional angles of the 2-hydroxypropane linking the two cyclic moieties, changed to accommodate lattice expansion or contraction resulting from water sorption and desorption by the crystals. As water is taken up, the relative occupancies of the sites of the

second sodium ion and that of water molecules change. As a result, the triclinic structure with $\alpha > 90^\circ$ approaches the monoclinic form with $\alpha \approx 90^\circ$. To summarize, the presence of large water channels, the flexibility of the 2-hydroxypropane link, the disorder of the second sodium ion (Fig. 10) and the disorder of the surrounding water molecules in the crystal lattice explain the reversible and nonstoichiometric water sorption and desorption by cromolyn sodium. This study emphasizes the importance of the detailed single crystal structure in explaining many unusual physico-chemical properties of drug hydrates.

The muscarinic agonist, LY297802 tartarate [i.e., (+)-3-[3-(butylthio)-1,2,5-thiadiazol-4-yl]-1-azabicyclo[2.2.2]octane monohydrogentartrate}, was also found to exhibit an unusual tendency to form nonstoichiometric hydrates of variable, but specific composition, ranging from 0 to 0.5 mol of water [73]. Solid-state ^{13}C -NMR spectroscopy, in conjunction with moisture sorption analysis and X-ray crystallography was used to provide unique insights into nonstoichiometric moisture sorption behavior. The PXRD patterns of the drug exposed to different RH values (0 to 75%), indicated neither a peak shift nor the presence of any new peaks, suggesting that

the anhydrous and the hydrated forms of the drug are isomorphic. Fig. 11 shows the significant changes in the SSNMR peaks on exposure to different relative humidities and temperature, indicating that water incorporated into the crystal lattice changes the local chemical environment and causes the observed NMR changes. The incorporation of water into the crystal lattice of the drug was also confirmed by X-ray crystallography. The considerable hygroscopicity of the drug was rationalized in terms of the similar crystal structures of the hydrated and nonhydrated forms, and hence no significant structural modifications are needed for the reabsorption of water into the solids. The rates of dehydration and rehydration are largely determined by the size of the water channels and the strength of the hydrogen-bonding interactions that bind the water molecules in the channels.

Another interesting study with different solvated forms of L-lysine monohydrochloride (LH) was conducted by Bandyopadhyay et al. [74]. LH was found to form: a pure methanol solvate at water activity, $a_w < 0.34$, with methanol activity, $a_m > 0.7$; a dihydrate at $a_w > \sim 0.65$ with $a_m < 0.45$; and mixed solvates at intermediate values of a_w and a_m . It was found that the dihydrate and the mono-

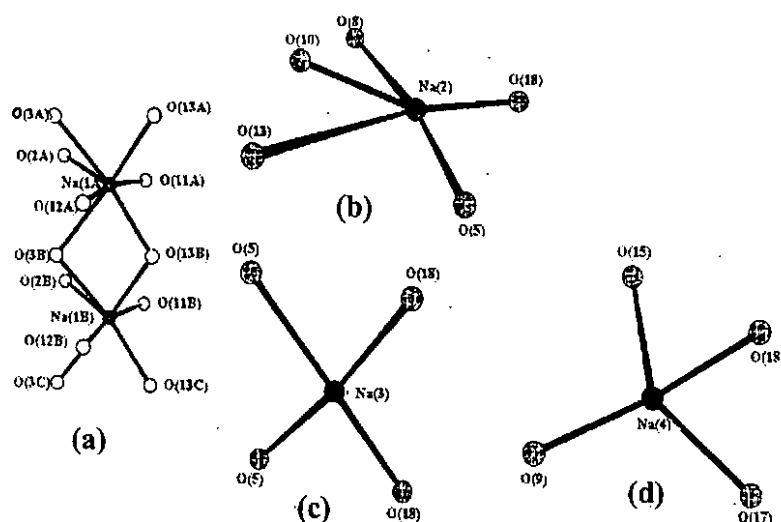


Fig. 10. In hydrated cromolyn sodium, coordination environment of: (a) the first (ordered) sodium ion, Na(1), shown in two neighboring unit cells (A and B); and the second (disordered) sodium ion at the three partially occupied sites, (b) Na(2), (c) Na(3), and (d) Na(4). The striped circles represent the sodium sites. The open circles represent the oxygen atoms coordinated to Na(1). The dotted (gray) circles represent the oxygen atoms coordinated to Na(2), Na(3), or Na(4) ([71], reproduced with the permission of the American Pharmaceutical Association).

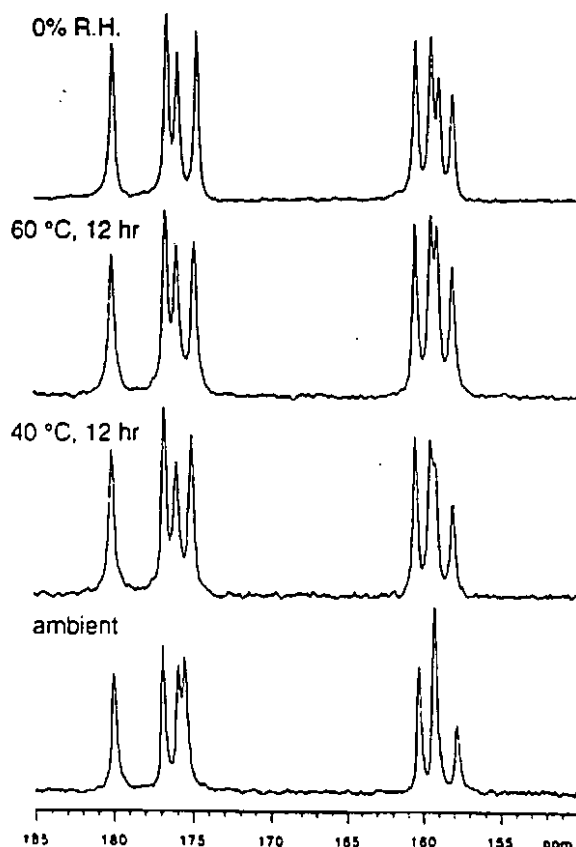


Fig. 11. Solid-state ^{13}C -NMR spectra of LY297802 tartrate ((+)-3-[3-(butylthio)-1,2,5-thiadiazol-4-yl]-1-azabicyclo[2.2.2]octane monohydrogentartrate) after storage at 0% humidity and before and after drying at elevated temperatures ([73], reproduced with the permission of the American Pharmaceutical Association).

methanol solvated forms of LH possessed similar crystal structures, similar PXRD patterns, but differed in the crystal habit. The crystal structures of LH hydrate and methanol solvate indicate that one molecule of methanol in the methanol solvate occupies approximately the same volume as the two water molecules in the dihydrate. During dehydration in the presence of methanol in the crystallization medium, the loss of one or more water molecules from the crystal lattice was compensated by the gradual uptake of methanol into the crystal structure to satisfy the hydrogen bonding pattern within the lattice with minor rearrangements, giving rise to mixed solvates. The similarities of the crystal lattices of the dihydrate and monomethanol solvate explain the similarity of the PXRD patterns.

3.3. Phase transformation of hydrates and solvates

Phase changes due to hydration/dehydration and solvation/desolvation of pharmaceutical compounds during processing or in the final product may result in an unstable system that would effect the bioavailability of drug from solid dosage forms. Various types of phase changes are possible in solid-state hydrated or solvated systems in response to changes in environmental conditions, such as relative humidity, temperature and pressure. For example, some hydrated compounds may convert to an amorphous phase upon dehydration and some may convert from a lower to a higher state of hydration yielding forms with lower solubility. Alternatively, a kinetically favored but thermodynamically unstable form may be converted during pharmaceutical processing to a more stable and less soluble form [8]. The phase transitions in hydrates and solvates can occur at various stages of dosage form development. Morris [9] has discussed the behavior of hydrates during processing, handling and storage of formulations in detail.

The phase transformations associated with exposure to water, such as during solubility measurements, wet granulation processes, dissolution studies and accelerated stability tests are likely to occur via solution mediation. Solution mediated phase transformations depend upon the solution phase to provide the mobility necessary to rearrange in the most stable form and hence are much faster than solid-state transformations. The rate of a solution-mediated transformation is proportional to the solubility of the species involved. Temperature, pressure and relative humidity may increase the rate of phase transformation of hydrates by inducing mobility in the system.

Solution-mediated phase transformations have been reported for many hydrate systems, such as theophylline crystals [17], eprosartan mesylate [75] and nedocromil sodium [76]. Ghosh and Grant [77] have addressed a common problem associated with the characterization of solvates which centers around the determination of solubilities of solvates and of nonsolvates that undergo phase transformation in the presence of an interacting solvent, such as solvation of nonsolvates in the solvent of crystallization or the desolvation of solvates in water. A thermodynamic cycle analogous to Hess's law but based on free

energies has been developed to predict the theoretical solubilities of 1,2-dialkyl-3-hydroxy-4-pyridones, which form 1:1 formic acid solvates in the presence of formic acid, and of the 1:1 formic acid solvates which produce the corresponding unsolvated compounds in the presence of water. A good correlation was obtained between the solubility values measured by the standard extrapolation method and that calculated by means of the thermodynamic cycle.

Apart from identifying and characterizing the phases during various stages of drug development, it is very important to gain an understanding of the dehydration/hydration mechanisms and kinetics. Many models have been developed to account for the dehydration kinetics of the crystalline hydrates [78]. Nucleation is the most significant phenomenon in determining the transformation kinetics, that is, the rate of formation of a new phase [8]. The dehydration kinetics to some extent will also depend upon the class of the hydrate system to which the drug belongs, particle size and morphology. The practical applications of understanding the dehydration kinetics, as indicated by Morris [9], are mainly the determination of the conditions for allowable exposure of bulk drug substances during development and processing, proper packaging, allowable temperature ranges for shipping, storage, and labeling of the final product, and the initial selection of a form for development.

3.4. Prediction of the formation of hydrates and solvates

Predicting the formation of solvates or hydrates of a compound and the number of molecules of water or solvent incorporated into the crystal lattice of a compound is complex and difficult. Each solid compound responds uniquely to the possible formation of solvates or hydrates and hence generalizations cannot be made for a series of related compounds. Certain molecular shapes and features favor the formation of crystals without solvent; these compounds tend to be stabilized by efficient packing of molecules in the crystal lattice, whereas other crystal forms are more stable in the presence of water and/or solvents. There may be too many possibilities so that no computer programs are currently available

for predicting the crystal structures of hydrates and solvates.

3.5. Characterization of hydrates and solvates

The common methods for the characterization of hydrates and solvates are polarized light microscopy and hot stage microscopy, DSC, TGA, Karl Fischer titrimetry, single-crystal X-ray diffractometry, powder X-ray diffractometry, and infrared spectroscopy. These methods have been reviewed in detail [21] and will also be discussed in detail in later chapters.

Pressure DSC is gaining increasing popularity in the study of solvates and hydrates where dehydration reactions occur above or near the boiling point of water. Using conventional DSC, it is very difficult to measure the heats of dehydration and heat of vaporization separately, but if one conducts DSC experiments at elevated pressures, the two processes may be completely separated. The advantage of using pressure DSC is that the pressure can be precisely controlled and the solids can be subjected to a controlled temperature program while under substantially elevated temperatures. The influence of elevated pressures on the solid-state behavior of carbamazepine dihydrate was studied by Han and Suryanarayanan [79]. In Fig. 12 it is shown that pressure DSC can separate the dehydration and vaporization endotherms of carbamazepine dihydrate during its conversion to the anhydrate form. Also the technique permitted the water liberated on dehydration to remain in intimate contact with the anhydrous phase formed which could significantly influence its solid-state properties.

The combined physical analytical techniques of thermogravimetry and infrared spectroscopy (TG/IR) can permit identification of the solvent incorporated into the crystal lattice. This combined technique has been used to study formulated products, such as capsules and tablets [80].

4. Current challenges and future directions

4.1. Origins of the challenges

A series of flow charts and decision trees have been presented and discussed [11,22] that can be

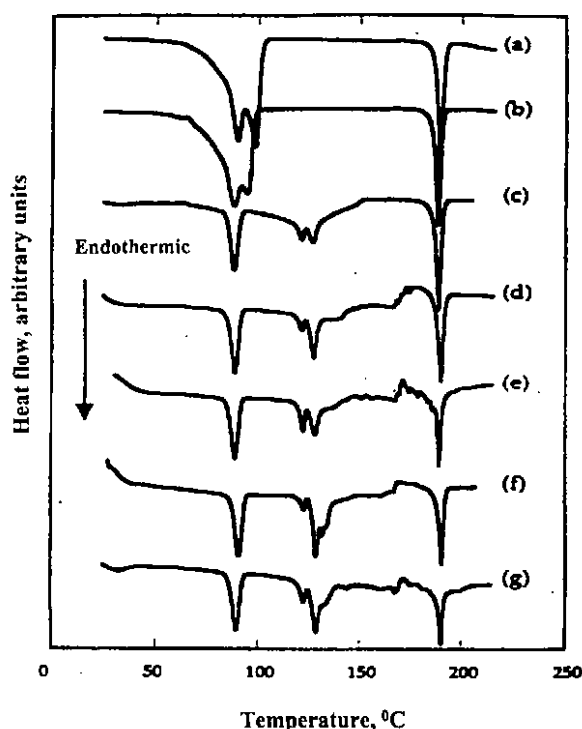


Fig. 12. Differential scanning calorimetry (DSC) curves of carbamazepine dihydrate at different pressures: (a) at atmospheric pressure in a conventional DSC cell, (b) at atmospheric pressure in a pressure DSC (PDSC) cell, (c) 100 p.s.i., (d) 200 p.s.i., (e) 300 p.s.i., (f) 400 p.s.i., and (g) 600 p.s.i. (1 p.s.i. = 6.9 kPa) ([79], reproduced with the permission of Elsevier Science).

used by investigators to characterize the polymorphs and solvates of compounds under development or for registration with regulatory authorities. Due to the complex and nonconventional behavior of various organic drug molecules, there are many opportunities for research and development in the area of characterization of polymorphs and solvates. Some of the problems which are commonly encountered during characterization of crystalline solids and which need to be addressed are: disorder in the crystal lattice due to pharmaceutical processing leading to conversion of a crystalline phase to an amorphous material or phase conversion from one form to the other; quantitating the amount of single polymorph in a mixture of polymorphs; identifying the solid form of the active ingredient in the formulated product, particularly when the drug is a minor component in the presence of numerous other materials (excipi-

ents); and the issues of disappearing polymorphs and the appearance of new polymorphs. In the following sections we will address some of these issues and some of the studies that have addressed these problems.

4.2. Phase transformations during processing

The effects of pharmaceutical processing on the crystalline state of drug polymorphs and solvates have been discussed recently by Brittain and Fiese [16]. Exposure to changes in temperature, pressure, relative humidity and comminution are encountered during processes such as drying, granulation, milling and compression. The stresses applied to crystals during pharmaceutical processing can cause defects in their crystal lattices, and contribute to lattice disorder, thus affecting the physical properties of the resulting powder [81]. This problem has been discussed in detail by Byrn et al. [10]. Arising from different degrees of crystalline disorder, the difficulty in reproducing materials with the same properties is a major concern in the pharmaceutical industry.

Milling, the last processing step in the production of bulk drug substance to reduce particle size, is often accompanied by a decrease in crystallinity due to the creation of lattice defects, beginning at the surface. The defects created by mechanical activation of the solid on the surface can migrate, transform, and change their number and nature. If the defects in the mechanically activated crystal heal to produce a crystal lattice different from the initial lattice, then a polymorphic transformation has taken place. Milling-induced polymorphic changes have been observed for many small drug molecules, such as fostedil, chloramphenicol palmitate, indomethacin and phenylbutazone [16]. Polymorphic transformation of the dipeptide sweetener, aspartame hemihydrate, can occur during milling [82]. Polymorph II of aspartame hemihydrate was found to transform to form I during ball-milling or on heating for 30 min at 160°C in the presence of steam as shown in the X-ray diffraction pattern (Fig. 13). The susceptibility of form II of aspartame hemihydrate to transform to form I has been attributed to the less symmetric crystal structure of form II compared to that of form I as studied by spectroscopic methods.

Some authors, such as Hüttenrauch [81] have

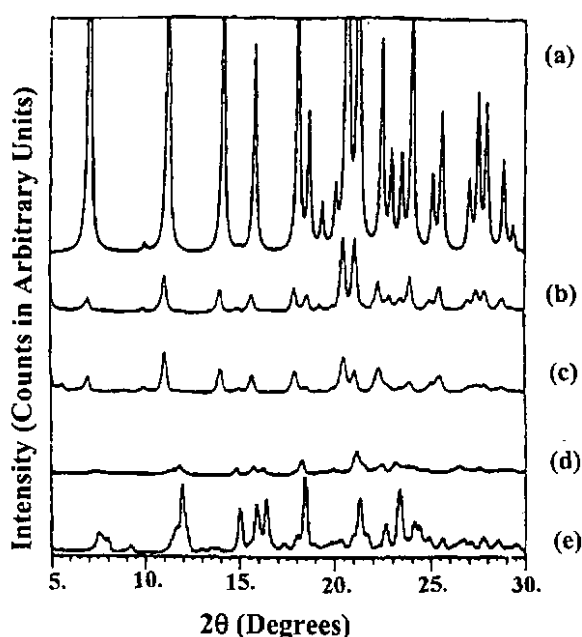


Fig. 13. Powder X-ray diffraction patterns of aspartame hemihydrate: (a) theoretical powder pattern calculated from the crystal structure of aspartame hemihydrate (form I) determined by Hatada et al., (1985) [99], (b) experimental powder pattern of the ball-milled aspartame hemihydrate (form I), (c) experimental powder pattern of aspartame hemihydrate that had been heated for 30 min at 160°C in the presence of steam (form I), (d) experimental powder pattern of aspartame hemihydrate after compression at 250 MPa for 1 min (form II), and (e) aspartame hemihydrate as received (form II) ([82], reproduced with the permission of the American Pharmaceutical Association).

suggested that the trauma to crystals during grinding may lead to a decrease in crystallinity, which should improve the compression capacity and dissolution rate of the drug molecules. This hypothesis was tested by studying the morphology, crystalline state, compression capacity and dissolution properties of native and ground crystals of aspirin and lactose monohydrate [83]. No significant increase in compression capacity was observed when native and ground crystals were compared. Only a slight increase in the dissolution rate was observed for ground aspirin crystals which was attributed to surface defects due to grinding resulting in improved crystal wetting.

The effect of roller compaction on lattice defects and phase change has been examined for aspirin [84]. The water vapor sorption isotherm obtained for aspirin on roller compaction indicated much more

water uptake than had been reported previously for other crystalline samples of aspirin under similar conditions. Various possibilities were suggested for the unusual water uptake of aspirin on roller compaction, such as formation of a polymorphic form of aspirin with a much greater affinity for water, formation of a crystalline hydrate in the aspirin sample, or significant reduction in particle size of the aspirin particles thereby providing an increased specific surface area for water vapor adsorption. It is also possible that roller compaction disrupted the crystalline order of some part of the aspirin crystals forming amorphous regions, which then take up relatively large quantities of water into their bulk structure. This example clearly indicates that processes, such as roller compaction, can introduce considerable disorder in the surface of crystals leading to a marked increase in the tendency to sorb water vapor.

Thermal activation, like mechanical activation during processing, also results in a high-energy state of crystals that may reorganize into a different lattice arrangement resulting in a phase change. The thermal stability of drug substances is important, because formulations are often dried at elevated temperatures after wet granulations so that the tablets may contain small regions of high temperature (hot spots) during compression. Various examples have shown that a change of temperature may influence the stability of drug molecules [16]. The effect of low temperatures, such as during freeze-drying, on the crystalline form of the drug has also been studied. The formation of a new mannitol hydrate during freeze-drying has been reported [85]. The formation of a crystalline hydrate by an excipient during freeze-drying may have several practical consequences, such that the difficulty of removing bound water from the crystal lattice can significantly limit the drying rate, while the residual water that is not removed by freeze-drying may be a potential threat to product stability if it is released during storage. The mannitol hydrate formed during freeze-drying survived the typical drying cycle and converted to the anhydrous polymorph of mannitol upon heating.

Spray drying has also been shown to lead to loss of crystallinity in materials, by a combination of processes involving rapid solidification of dissolved material and solid-state transitions due to milling effects in the atomiser. Spray drying leads to conver-

sion of a crystalline phase to an amorphous state and, because the amorphous state is metastable with respect to the crystalline form, phase transformations are likely to occur within the shelf life of the pharmaceutical product, resulting in loss of quality and potency in the product [86].

In view of the significant effects that the state of disorder in crystalline solids caused by pharmaceutical processing can have on the properties of pharmaceutical solids, it is important to be able to assess the extent of disorder in a solid quantitatively down to very low levels. Various methods have been used to measure the percent disorder, such as using predetermined mixtures, measurements of X-ray powder diffraction, density and heats of crystallization which revealed limits of detectability down to about 10%. Using water vapor sorption measurements under very carefully controlled conditions, it was possible to detect disorder as low as 1% in milled samples of sucrose [87]. A comparison of the four methods mentioned above for estimating the percent disorder of milled samples of sucrose gave very consistent results, once the underlying factors that make these techniques sensitive to the concentration of amorphous content were recognized and taken into account.

4.3. Degree of crystallinity

The previous section has emphasized that many pharmaceutical processes lead to a decrease in crystallinity of drug phases. Various studies have concluded that the formation of amorphous material during processing is highly undesirable. The amorphous material, being in a thermodynamically metastable state, is susceptible to reconversion to the crystalline state, affecting many physico-chemical characteristics of the drug. A later chapter provides detailed coverage of amorphous materials. An estimation of the degree of crystallinity of a sample before and after processing poses one of the larger challenges facing the pharmaceutical field. Powder X-ray diffractometry is still the commonly used method for determining the degree of crystallinity, though this method suffers from some limitations due to peak broadening, amorphous halo, and preferred orientation which make interpretation and quantitation difficult. DSC may not be a sensitive method for measuring crystallinity due to crystalliza-

tion of the amorphous content at elevated temperatures and the effects of differences in heat capacity. Solution calorimetry has been proposed as an accurate method for analysis of percent crystallinity [11,88,89]. A decrease in the endothermic enthalpy of solution indicates a decrease in the crystallinity of the sample. However, differences in surface area produced by grinding or by other processing techniques can also result in changes in the heat of wetting of a sample. Judicious choice of solvent can be employed to reduce such surface effects, which themselves contribute to the observed crystallinity of the sample.

Near infrared (NIR) spectroscopy is another technique being used to measure the degree of crystallinity, and has also proved useful in studies of the polymorphism and water content of sugars. The NIR spectrum of a sample contains both physical and chemical information. Being noninvasive, nondestructive and operable at room temperature, the method is a valuable tool with which to assess changes in the amorphous and crystalline state of lactose [90]. NIR has been used to follow the changes in the amorphous state, the onset of crystallization, and the changes between α - and β -lactose, which accompany the onset of crystallization. In another study, the nucleation and crystallization kinetics of amorphous lactose was investigated by gravimetry in an automated vacuum moisture balance. The combination of isothermal and nonisothermal activation energies allowed the investigation of both crystal growth and nucleation mechanisms and led to the separation of activation energies for nucleation and growth [91].

4.4. Characterization of mixtures of polymorphs

Another common problem encountered during drug development is quantitative control of the proportion of polymorphic forms present in a mixture. According to the US FDA regulations, the method of analysis for the proportion of forms must be validated, and also the proportion of forms must remain within stated limits through the retest date of the drug substance and potentially throughout the shelf life of the product. This is a very onerous requirement, especially if the forms have a tendency to interconvert. Byrn et al. [11] suggested that the best way to deal with this problem is probably by

developing methods to prepare only one crystal form and maintaining this form throughout processing. Powder X-ray diffractometry is often a useful method to determine the percentages of polymorphs in a mixture. However, the detection limit is variable from case to case, and is sometimes as high as 15%. It is therefore important to develop sensitive analytical methods with a lower limit of detection.

Attenuated total reflectance (ATR) FTIR spectroscopy has been shown to be valuable for the quantitative analysis of the polymorphic content of bulk pharmaceutical materials. The feasibility of using ATR-FTIR for the qualitative and quantitative analysis of mixtures of pharmaceutical polymorphs has been studied using three known polymorphs of ganciclovir as a model compound [92]. Definitive identification and quantitation of all three polymorphs could be achieved using ATR-FTIR spectroscopy in conjunction with partial least-squares modeling. This technique has many advantages, such as speed, nondestructiveness, relative ease of use, and most important, no sample pretreatment before measurement.

Raman spectroscopy is another technique that is being widely used to quantitatively estimate the percentage of one polymorph in a mixture of polymorphs. FT-Raman spectrometry offers many advantages, the most prominent being, minimal sample preparation, sensitivity to polymorphism, and noninterference from water. Two polymorphs of fluconazole were characterized using FT-Raman spectroscopy and principal components regression using cross validation provided quantitative analysis of the percentage of one polymorphic form in the mixture of other forms [93]. A novel sample holder was developed whereby the sample is held in an NMR tube which is rotated around its axis and at the same time moved up and down. This method of sample presentation leads to a large increase in the volume studied and is important for inhomogeneous samples for which sub-sampling is a problem. Possible degradation of the sample through heating by the laser can also be avoided [94].

X-Ray powder diffractometry is still the common method for the quantitative estimation of polymorphs in a mixture of polymorphs. This method requires that at least one high-intensity peak unique to each form is available for intensity measurements and that

the plot of the peak intensity ratio as a function of the weight ratio of the components should result in a straight line. Modern computer controlled X-ray powder diffractometers now permit quantitative analysis of multicomponent mixtures using the complete powder diffraction profile rather than a limited amount of low-angle integrated intensity data. Artificial neural networks (ANNs) in quantitative X-ray powder diffractometry were used successfully to identify and quantify the two known modifications of ranitidine hydrochloride even when the weight fraction of one polymorph in the mixture was as low as 0.01 [95]. ANNs have been used mostly in problems of pattern recognition and modeling, and is therefore useful in deciphering the pattern in diffraction data from polymorphic mixtures. The ANNs model predicted concentration precisely, accurately, and with minimal bias through a wide range of ratios of the two known ranitidine hydrochloride polymorphic forms in a mixture (Fig. 14). This method minimizes the problems associated with preferred orientation and overlapping X-ray lines. The same group of researchers has shown the potential of ANNs in combination with DRIFT spectroscopy to analyze the polymorphic purity of crystalline ranitidine hydrochloride as a bulk drug and as an active ingredient of

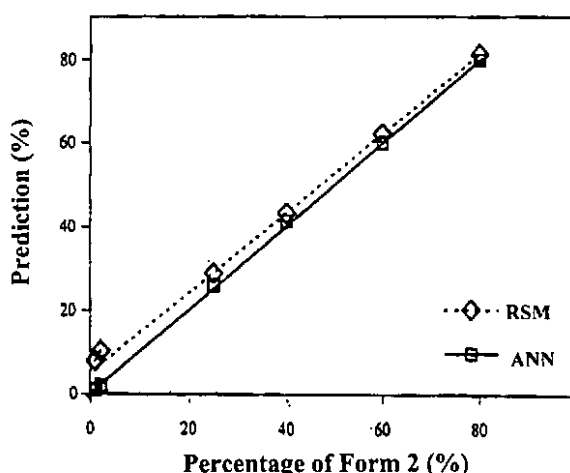


Fig. 14. Predicted concentrations of the two polymorphic forms (form 1 and 2) of ranitidine hydrochloride by the response surface methodology (RSM), a statistical modeling method, and by the artificial neural networks (ANNs) method, plotted against measured percentage of form 2 ([95], reproduced with the permission of Elsevier Science).

a tablet formulation. Simultaneous identification and quantification of all the ingredients in the tablet formulation was possible. This study has shown that the complex problem of quantifying a drug in mixtures containing two or more components with overlapping spectra can be solved by the DRIFT-ANNs technique [96].

Another technique, which is gaining popularity in the quantitative analysis of mixtures of polymorphs, is solid-state ^{13}C -CP-MAS NMR spectroscopy. This method was preferred for quantitative analysis of polymorphic mixtures of the herbicide, pendimethalin, which exists as two polymorphs with different colors and crystal habits [27]. ^{13}C -NMR provided the most sensitive and definitive evidence of the transition from the yellow to the orange form. This method enabled as little as 2% of orange pendimethalin to be determined in a sample consisting mostly of the yellow polymorph. It is the least invasive of the instrumental methods and can be used to detect the ratio of the two polymorphs in solid formulations.

A related challenge faced by the pharmaceutical industry is the determination of the polymorphic nature of the drug in the presence of excipients in a dosage form especially when the active drug is present as a low percentage of the overall formulation [97]. This problem can be addressed by developing sensitive techniques with lower limits of detection or by using a combination of techniques.

5. Conclusions

In order to save time and cost it is very important to choose the most suitable form of the crystalline drug in the initial stages of drug development. In recent years a good deal of research has been directed towards achieving this goal. Systematic isolation and early characterization of the largest number of possible forms of a drug reduces the chances of surprises at the late production stage due to identification of a new crystalline form or phase change. With the development of more sophisticated computational tools, the main focus of many investigators is to be able to predict all the possible forms of a drug from its molecular structure. Understanding the origins of the multiple solid forms of a drug

molecule, either due to differences in packing arrangement or conformation of the molecules, becomes the first step in prediction. Single crystal X-ray diffractometry and solid-state NMR spectroscopy are two techniques that are gaining increased application in determining the various crystal structures and the origins of polymorphism and pseudopolymorphism of a particular drug. When crystal structures can be calculated with certainty, it will be possible to predict the various polymorphs of a compound and this information could be used to guide experimental studies. This goal may be difficult to achieve owing to the complex molecular structures of new organic molecules and the presence of several molecules in each asymmetric unit, but the future development of improved force fields and increased computational speeds, may make it achievable.

Improved experimental methods leading to more accurate and detailed phase diagrams are also finding increased use in determining the stability of various polymorphs. It is important to make every effort to prepare and to identify the most stable polymorph in order to guide the selection of the optimal form for development. The emergence of sensitive methods and the use of combination techniques, facilitate the identification and the more accurate characterization of the various polymorphs of a drug molecule. One of the main analytical challenges faced by the pharmaceutical analyst is the development of better quantitative methods for identifying a single polymorph in a mixture of polymorphs and for determining the percentages of amorphous or crystalline content of the drug. More and more sensitive methods are being developed to address this problem.

An increased understanding of the phenomenon of polymorphism should enable pharmaceutical scientists to gain control over the crystallization process in order to selectively obtain the desired polymorph or suppress the growth of an undesired one. Phase changes during processing and scale-up are a problem, which may be avoided by carefully designed initial small-scale studies. The availability of detailed structural data, combined with strategic design of substrates and additives, has led to significant advances in the control over the polymorphs obtained in a particular crystallization [98]. With all the information available from these initial studies, it

should be possible to design and to select processing conditions which would give a desired polymorph and maintain the desired form throughout the various stages of drug processing and manufacture.

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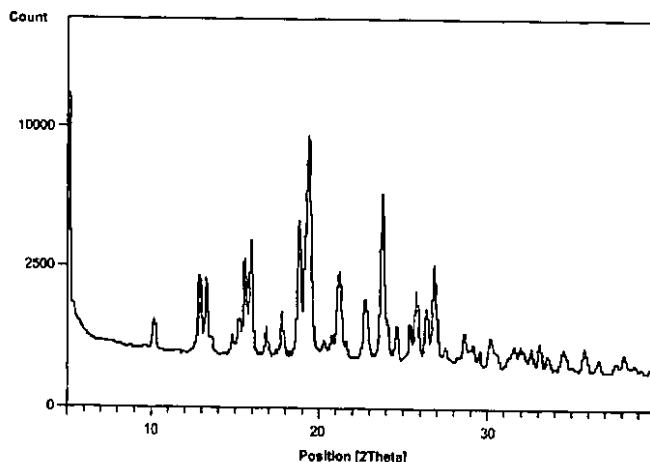


FIG. 1

(57) Abstract: Provided are certain solvates of 3-methoxy-N-[3-(2-methyl-2H-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide or a pharmaceutically acceptable salt thereof. Also provided are pharmaceutical compositions comprising such solvates and methods for their use.

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**CRYSTALLINE FORMS OF CERTAIN 3-PHENYL-PYRAZOLE DERIVATIVES AS
MODULATORS OF THE 5-HT_{2A} SEROTONIN RECEPTOR USEFUL FOR THE
TREATMENT OF DISORDERS RELATED THERETO**

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[0001] This application claims priority to U.S. Provisional Application No. 61/288,130, filed December 18, 2009, which is incorporated herein by reference.

[0002] Receptors for serotonin (5-hydroxytryptamine, 5-HT) are an important class of G protein coupled receptors. Serotonin receptors are divided into seven subfamilies, referred to as 5-HT₁ through 5-HT₇, inclusive. These subfamilies are further divided into subtypes. For example, the 5-HT₂ subfamily is divided into three receptor subtypes: 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}. Certain phenyl-pyrazoles are modulators of 5-HT_{2A} serotonin receptor activity useful in the treatment of platelet aggregation, coronary artery disease, myocardial infarction, transient ischemic attack, angina, stroke, atrial fibrillation, blood clot formation, asthma or symptoms thereof, agitation or a symptom thereof, behavioral disorders, drug induced psychosis, excitative psychosis, Gilles de la Tourette's syndrome, manic disorder, organic or NOS psychosis, psychotic disorder, psychosis, acute schizophrenia, chronic schizophrenia, NOS schizophrenia and related disorders, sleep disorders, diabetic-related disorders, progressive multifocal leukoencephalopathy, and the like.

[0003] Provided is 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-ylethoxy)-phenyl]-benzamide hydrate.

[0004] Also provided is (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate.

[0005] Also provided are pharmaceutical compositions comprising one or more pharmaceutically acceptable diluents and a therapeutically effective amount of at least one solvate described herein.

[0006] Also provided are processes for preparing the solvates described herein as well as solvates prepared by the processes.

[0007] Also provided are pharmaceutical compositions prepared by formulating a therapeutically effective amount of at least one solvate described herein with one or more pharmaceutically acceptable carrier to provide the pharmaceutical composition.

[0008] Also provided are methods for modulating the activity of a 5-HT_{2A} serotonin receptor in an individual by administering a pharmaceutical compositions prepared by formulating a therapeutically effective amount of at least one solvate described herein with one or more pharmaceutically acceptable carrier to provide the pharmaceutical composition.

[0009] Also provided are methods for treating a 5-HT_{2A} mediated disorder in an individual by administering a pharmaceutical compositions prepared by formulating a therapeutically effective amount of at least one solvate described herein with one or more pharmaceutically acceptable carrier to provide the pharmaceutical composition.

5

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Figure 1 shows powder X-ray diffraction pattern of 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate.

[0011] Figure 2 shows differential scanning calorimetry (DSC) and thermogravimetric analyses (TGA) plots for 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate.

[0012] Figure 3 shows representative DSC and TGA plots for (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate.

[0013] Figure 4 shows a proton NMR of (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate.

[0014] For clarity and consistency, the following definitions will be used throughout this document.

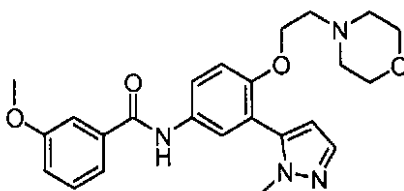
[0015] "Polymorphism" is defined as in the International Conference on Harmonization (ICH) Guideline Q6A Guideline: Specifications for New Drug Substances and Products: Chemical Substances, October 1999 and refers to the occurrence of different solid forms of the same drug substance. Polymorphs can be unsolvated or solvated, such as hydrated, crystal forms. Unsolvated crystal forms are crystals that do not have solvent incorporated within the crystal structure and include anhydrous crystal forms or anhydrites. Solvated crystal forms, or solvates, are crystalline solid adducts containing either stoichiometric or nonstoichiometric amounts of solvent molecules incorporated within the crystal structure. If the incorporated solvent is water, the solvates are also commonly known as hydrates. Amorphous solids consist of disordered arrangements of molecules and do not possess a distinguishable crystal lattice.

[0016] "Pharmaceutically acceptable salt" refers to a pharmaceutically acceptable organic or inorganic acid or base salt. Representative pharmaceutically acceptable salts include, e.g., alkali metal salts, alkali earth salts, ammonium salts, water-soluble and water-insoluble salts, such as the acetate, amsonate (4,4-diaminostilbene-2,2-disulfonate), benzenesulfonate, benzonate, bicarbonate, bisulfate, bitartrate, borate, bromide, butyrate, calcium, calcium edetate, camsylate, carbonate, chloride, citrate, clavulinate, dihydrochloride, edetate, edisylate, estolate, esylate, funarate, gluceptate, gluconate, glutamate, glycolylarsanilate, hexafluorophosphate, hexylresorcinate,

hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, N-methylglucamine ammonium salt, 3-hydroxy-2-naphthoate, oleate, oxalate, palmitate, pamoate (1,1-methene-bis-2-hydroxy-3-naphthoate, 5 einbonate), pantothenate, phosphate/diphosphate, picrate, polygalacturonate, propionate, p-toluenesulfonate, salicylate, stearate, subacetate, succinate, sulfate, sulfosalicylate, suramate, tannate, tartrate, teoclate, tosylate, triethiodide, and valerate salts.

[0017] In addition, the free base can be obtained by basifying a solution of the acid salt. Conversely, if the product is a free base, an addition salt, particularly a pharmaceutically acceptable 10 addition salt, may be produced by dissolving the free base in a suitable organic solvent and treating the solution with an acid, in accordance with conventional procedures for preparing acid addition salts from base compounds. Those skilled in the art will recognize various synthetic methodologies that may be used to prepare non-toxic pharmaceutically acceptable addition salts.

[0018] The term "3-methoxy-N-[3-(2-methyl-2H-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide" refers to a chemical entity with the following structure:



[0019] As used herein, the term "treating" refers to, for example, preventing, inhibiting, as well as ameliorating a disease, condition or disorder in an individual.

[0020] As used herein, the term "individual" or "patient," used interchangeably, refers to 20 any animal, including mammals, preferably mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, or primates, and most preferably humans.

[0021] As used herein, the phrase "therapeutically effective amount" refers to the amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue, system, animal, individual or human that is being sought by a researcher, veterinarian, 25 medical doctor or other clinician, which includes one or more of the following:

(1) preventing the disease; for example, preventing a disease, condition or disorder in an individual that may be predisposed to the disease, condition or disorder but does not yet experience or display the pathology or symptomatology of the disease;

(2) inhibiting the disease; for example, inhibiting a disease, condition or disorder in an 30 individual that is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., arresting further development of the pathology and/or symptomatology) such as stabilizing viral load in the case of a viral infection; and

(3) ameliorating the disease; for example, ameliorating a disease, condition or disorder in an individual that is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., reversing the pathology and/or symptomatology) such as lowering viral load in the case of a viral infection.

5 [0022] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination. subcombination. All combinations of the embodiments pertaining to
10 hydrates, as well as all combinations of uses and medical indications described herein, are specifically embraced by the present invention just as if each and every combination was individually explicitly recited. In addition, all subcombinations of the hydrates listed in the embodiments are also specifically embraced by the present invention just as if each and every subcombination of hydrates and subcombination of uses and medical indications was individually
15 and explicitly recited herein.

[0023] Provided is 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate. In some embodiments, the 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate contains water ranging from about 3.5% to about 4.2%, such as about 3.8%, by weight, as measured by TGA.

20 [0024] Provided is (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate. In some embodiments, the (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate contains ethanol ranging from about 5.0% to about 6.0%, such as about 5.3%, by weight, as measured by TGA.

25 [0025] The solvates described herein may be identified by one or more solid state analytical methods. For example, the solvates may be characterized according to one or more of, e.g., X-ray diffraction, unit cell constants, Fourier transform infrared spectroscopy, differential scanning calorimetry curve data, solid state nuclear magnetic resonance spectroscopy, and Raman spectroscopy.

30 [0026] A sample is considered to be a solvate if it is characterized as such by at least one of the methods described herein, regardless of any inconsistent or contradictory results obtained by any of the other methods described above. In addition, a sample is considered to be a solvate if it is characterized as such by at least one of the above methods under a particular set of experimental conditions, regardless of any inconsistent or contradictory results obtained by the
35 same method under a different set of experimental conditions.

- [0027] In some embodiments, the solvates described herein may be characterized according to differential scanning calorimetry curve data (DSC). For DSC, it is known that the temperatures observed will depend upon the rate of temperature change as well as sample preparation technique and the particular instrument employed. Thus, the values reported herein relating to DSC thermograms can vary by plus or minus about 4 °C.
- [0028] In some embodiments, the solvates may be characterized according to melting point. For example, also provided is 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate having a melting endotherm with an onset of about 105±4°C, as measured by differential scanning calorimetry.
- 10 [0029] Also provided is an embodiment of (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate having a melting endotherm with an onset of about 170±4°C, as measured by differential scanning calorimetry.
- 15 [0030] In some embodiments, the thermogravimetric analysis profile of the 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate is substantially as shown in Figure 2 wherein by "substantially" is meant that the reported TGA features can vary by about ±5°C.
- 20 [0031] In some embodiments, the thermogravimetric analysis profile of the (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate is substantially as shown in Figure 3 wherein by "substantially" is meant that the reported TGA features can vary by about ±5°C.
- 25 [0032] In some embodiments, the solvates described herein may be characterized according to X-ray powder diffraction. The intensity in the X-ray powder diffraction diagram of different batches of the solvates described herein may vary, because of preferred orientation or even variable hydration. Furthermore, in the X-ray powder diffraction diagram of the solvates described herein, there can be differences in the measured peaks, due to the difference of the measuring instruments and testing conditions during the X-ray diffraction measurement. But notwithstanding experimental and machine errors, and principles such as preferred orientation, one skilled in the art can find sufficient information in the XRPD data provided herein to identify
- 30 a specific crystalline form. In other words, not all of the data are necessary to identify the crystalline form.
- [0033] Owing to natural deviations in the samples or in the measuring method, the 2θ values of the peaks can be stated with an accuracy of +/- 0.2000 degrees 2θ. However, it is common to see some measurement variations in reported data due to, for example, instrumental variations and environmental disturbances, such as preferred orientation, sample surface and inter-
- 35 apparatus variability, and thus even the same forms of a compound may not exhibit the same exact

XRPD data (in terms of D-spacing and peak intensity) all the time. Thus, even when the specific numerical values are not identical in every measurement, if the overall pattern is reproduced and the peak locations and relative peak intensities are sufficiently similar, one of skill in the art, using known and accepted techniques for such evaluation, can conclude that all of the obtained data

5 demonstrate a single crystalline form.

[0034] For example, also provided is an embodiment of 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate with an XRPD, measured with CuK α radiation, having peaks chosen from those having about the following values: 5.0998, 18.7064, 19.1157, 19.3029, and 23.6567. Again, those multiple peaks are sufficient but not

10 necessary to identify that specific crystalline embodiment.

[0035] Also provided is an embodiment of 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate with an XRPD, measured with CuK α radiation, having peaks chosen from those having about the following values: 5.0998, 12.7991, 15.4771, 15.8201, 18.7064, 19.1157, 19.3029, 21.1196, 23.6567, and 26.7255. Again, those

15 multiple peaks are sufficient but not necessary to identify that specific crystalline embodiment.

[0036] Also provided is an embodiment of 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate with an XRPD, measured with CuK α radiation, having one or more peaks chosen from those set forth in Table 1 below. Again, those multiple peaks are sufficient but not necessary to identify that specific crystalline embodiment.

Table 1. PXRD Peaks

Pos. [°2 θ .]	Height [cts]	FWHM [°2 θ .]	d-spacing [Å]	Rel. Int. [%]
5.0998	16685.21	0.1338	17.32837	100.00
10.1034	502.62	0.1171	8.75519	3.01
12.7991	1743.46	0.1506	6.91662	10.45
13.2049	1636.11	0.1338	6.70497	9.81
13.4980	294.04	0.1004	6.56007	1.76
14.7576	235.93	0.1004	6.00284	1.41
15.0902	586.25	0.0836	5.87129	3.51
15.4771	2317.73	0.1338	5.72535	13.89
15.8201	3116.02	0.1673	5.60201	18.68
16.7468	417.97	0.1171	5.29402	2.51
17.6943	789.80	0.1338	5.01264	4.73
18.7064	3916.41	0.1506	4.74364	23.47
19.1157	4737.16	0.0669	4.64298	28.39
19.3029	8968.85	0.1506	4.59837	53.75

Table 1. PXRD Peaks

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
20.2058	232.91	0.1673	4.39490	1.40
20.6138	326.86	0.1171	4.30881	1.96
21.1196	1942.76	0.2676	4.20677	11.64
22.6440	1152.76	0.1506	3.92689	6.91
23.6567	5478.93	0.2007	3.76103	32.84
24.4655	505.78	0.1338	3.63850	3.03
25.2482	568.85	0.1171	3.52745	3.41
25.6649	1398.90	0.1840	3.47111	8.38
26.1913	838.97	0.1840	3.40253	5.03
26.7255	2159.11	0.1506	3.33573	12.94
27.3900	169.35	0.1338	3.25628	1.01
28.4861	412.85	0.1673	3.13344	2.47
29.0404	243.67	0.1673	3.07488	1.46
29.4217	135.96	0.1338	3.03588	0.81
30.0303	374.65	0.2342	2.97573	2.25
30.4984	153.30	0.1004	2.93112	0.92
31.4985	232.22	0.2007	2.84030	1.39
31.8583	235.44	0.1338	2.80904	1.41
32.4897	198.24	0.1338	2.75588	1.19
32.9338	269.98	0.1171	2.71973	1.62
33.4436	128.77	0.1673	2.67942	0.77
34.3265	212.68	0.2342	2.61250	1.27
35.7053	208.51	0.2676	2.51472	1.25
36.5207	92.02	0.2007	2.46042	0.55
37.9565	182.89	0.2342	2.37059	1.10

[0037] In some embodiments, the 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate contains no more than about 50% of any other polymorphic forms. In some embodiments, the 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate contains no more than about 10% of any other polymorphic forms. In some embodiments, the 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate contains no more than about 5% of any other polymorphic forms. In some embodiments, the 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-

3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate contains no more than about 1% of any other polymorphic forms.

[0038] In some embodiments, the 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate has a chemical purity of greater than about 95%. In some embodiments, the 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate has a chemical purity of greater than about 98%. In some embodiments, the 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate has a chemical purity of greater than about 99%.

[0039] In some embodiments, the (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate contains no more than about 50% of any other polymorphic forms. In some embodiments, the (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate contains no more than about 10% of any other polymorphic forms. In some embodiments, the (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate contains no more than about 5% of any other polymorphic forms. In some embodiments, the (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate contains no more than about 1% of any other polymorphic forms.

[0040] In some embodiments, the (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate has a chemical purity of greater than about 95%. In some embodiments, the (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate has a chemical purity of greater than about 98%. In some embodiments, the (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate has a chemical purity of greater than about 99%.

[0041] Also provided is a method for the preparation of 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate or a pharmaceutically acceptable salt thereof.

[0042] 3-Methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate can be prepared by any of the suitable procedures in the art for preparing crystalline polymorphs. In some embodiments, 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate can be prepared by a process comprising:

adding aqueous base to a solution of 4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium chloride; and

collecting the 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate.

[0043] In some embodiments, the aqueous base comprises aqueous sodium hydroxide. In some embodiments, the aqueous base comprises 1 N sodium hydroxide. In some embodiments, aqueous base is added until the mixture has a pH of ~8. In some embodiments, the 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate is collected by filtration.

[0044] In some embodiments, the method for the preparation of (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate comprises:

adding ethanol and oxalic acid to 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide; and

collecting the (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate.

[0045] In some embodiments, the ethanol comprises 95% aqueous ethanol. In some embodiments, less than an equivalent, such as about 0.5 equivalent, of oxalic acid is used. In some embodiments, the (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate is collected by filtration.

[0046] Also provided is a product of any of the methods of preparation described herein.

[0047] In some embodiments, the solvates described herein further comprises a detectable amount of at least one organic solvent. In some embodiments, the at least one organic solvent corresponds to a solvent used during the preparation of 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide or a pharmaceutically acceptable salt thereof.

[0048] Provided are compositions comprising at least one solvate described herein. In some embodiments, the compositions described herein comprise at least about 1, about 5, about 10, about 20, about 30, or about 40% by weight of at least one solvate described herein. In some embodiments, the compositions described herein comprise at least about 1, about 5, about 10, about 20, about 30, or about 40% by weight of 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate. In some embodiments, the compositions described herein comprise at least about 1, about 5, about 10, about 20, about 30, or about 40% by weight of a hydrate of (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate.

[0049] In some embodiments, the solvates described herein have activity as 5-HT_{2A} receptor modulators. Accordingly, the solvates described herein may be used in methods of

modulating the 5-HT_{2A} receptor by contacting the receptor with at least one solvate described herein. In some embodiments, at least one solvate described herein can be used to modulate 5-HT_{2A} receptors in an individual in need of such modulation by administering a therapeutically effective amount of at least one solvate described herein or a pharmaceutical composition thereof.

5 **[0050]** Also provided are methods of treating diseases associated with the 5-HT_{2A} receptor in an individual (e.g., patient) by administering to the individual in need of such treatment a therapeutically effective amount or dose of at least one solvate described herein or a pharmaceutical composition thereof. Example diseases can include any disease, disorder or condition that is directly or indirectly linked to expression or activity of the 5-HT_{2A} receptor.

10 **[0051]** Example diseases include platelet aggregation, coronary artery disease, myocardial infarction, transient ischemic attack, angina, stroke, atrial fibrillation, blood clot formation, asthma or symptoms thereof, agitation or a symptom thereof, behavioral disorders, drug induced psychosis, excitative psychosis, Gilles de la Tourette's syndrome, manic disorder, organic or NOS psychosis, psychotic disorder, psychosis, acute schizophrenia, chronic
15 schizophrenia, NOS schizophrenia and related disorders, sleep disorders, diabetic-related disorders, progressive multifocal leukoencephalopathy, and the like.

[0052] Also provided are methods of treating coronary artery disease, myocardial infarction, transient ischemic attack, angina, stroke, and atrial fibrillation comprising administering to a patient in need thereof a therapeutically effective amount of at least one solvate
20 described herein or a pharmaceutical composition thereof.

[0053] Also provided are methods of treating a condition associated with platelet aggregation comprising administering to a patient in need thereof a therapeutically effective amount of at least one solvate described herein or a pharmaceutical composition thereof.

[0054] Also provided are methods of reducing the risk of blood clot formation in an angioplasty or coronary bypass surgery individual comprising administering to a patient in need
25 thereof a therapeutically effective amount of at least one solvate described herein or a pharmaceutical composition thereof.

[0055] Also provided are methods of reducing the risk of blood clot formation in an individual suffering from atrial fibrillation comprising administering to a patient a therapeutically
30 effective amount of at least one solvate described herein or a pharmaceutical composition thereof.

[0056] Also provided are methods of treating a sleep disorder comprising administering to a patient a therapeutically effective amount of at least one solvate described herein or a pharmaceutical composition thereof.

[0057] Also provided are methods of treating a dyssomnia comprising administering to a patient a therapeutically effective amount of at least one solvate described herein or a pharmaceutical composition thereof.

5 [0058] Also provided are methods of treating a parasomnia comprising administering to a patient a therapeutically effective amount of at least one solvate described herein or a pharmaceutical composition thereof.

[0059] Also provided are methods of treating a diabetic-related disorder comprising administering to a patient a therapeutically effective amount of at least one solvate described herein or a pharmaceutical composition thereof.

10 [0060] Also provided are methods of treating progressive multifocal leukoencephalopathy comprising administering to a patient a therapeutically effective amount of at least one solvate described herein or a pharmaceutical composition thereof.

[0061] Also provided are methods of treating hypertension comprising administering to a patient a therapeutically effective amount of at least one solvate described herein or a
15 pharmaceutical composition thereof.

[0062] Also provided are methods of treating pain comprising administering to a patient a therapeutically effective amount of at least one solvate described herein or a pharmaceutical composition thereof.

[0063] In some embodiments, the above methods further comprise the step of identifying
20 a patient, where the patient is in need of treatment for the particular disease being treated, wherein the identifying step is performed prior to administration to the patient of the therapeutically effective amount of at least one solvate described herein or a pharmaceutical composition thereof.

[0064] Also provided is at least one solvate described herein or a pharmaceutical composition thereof for use in a method of treatment of the human or animal body by therapy.

25 [0065] Also provided is a pharmaceutical composition comprising at least one solvate described herein and at least one pharmaceutically acceptable carrier for use in a method of treatment of the human or animal body by therapy.

[0066] Also provided is at least one solvate described herein or a pharmaceutical composition thereof for use in a method of treatment of a 5HT_{2A}-related disorder.

30 [0067] Also provided is at least one solvate described herein or a pharmaceutical composition thereof in a method of treatment of platelet aggregation, coronary artery disease, myocardial infarction, transient ischemic attack, angina, stroke, atrial fibrillation, blood clot formation, asthma or symptoms thereof, agitation or a symptom thereof, behavioral disorders, drug induced psychosis, excitative psychosis, Gilles de la Tourette's syndrome, manic disorder,
35 organic or NOS psychosis, psychotic disorder, psychosis, acute schizophrenia, chronic

schizophrenia, NOS schizophrenia and related disorders, sleep disorders, diabetic-related disorders or progressive multifocal leukoencephalopathy.

5 [0068] Also provided is at least one solvate described herein or a pharmaceutical composition thereof for use in a method of treatment of coronary artery disease, myocardial infarction, transient ischemic attack, angina, stroke or atrial fibrillation.

[0069] Also provided is at least one solvate described herein or a pharmaceutical composition thereof for use in a method for treating a condition associated with platelet aggregation.

10 [0070] Also provided is at least one solvate described herein or a pharmaceutical composition thereof for use in a method for reducing the risk of blood clot formation in an angioplasty or coronary bypass surgery.

[0071] Also provided is at least one solvate described herein or a pharmaceutical composition thereof for use in a method for reducing the risk of blood clot formation in an individual suffering from atrial fibrillation.

15 [0072] Also provided is at least one solvate described herein or a pharmaceutical composition thereof for use in a method for treating a sleep disorder.

[0073] Also provided is at least one solvate described herein or a pharmaceutical composition thereof for use in a method for treating a parasomnia.

20 [0074] Also provided is at least one solvate described herein or a pharmaceutical composition thereof for use in a method for treating a dyssomnia.

[0075] Also provided is at least one solvate described herein or a pharmaceutical composition thereof for use in a method for treating a diabetic-related disorder.

[0076] Also provided is at least one solvate described herein or a pharmaceutical composition thereof for use in a method for treating progressive multifocal.

25 [0077] Also provided is at least one solvate described herein or a pharmaceutical composition thereof for use in a method for treating hypertension.

[0078] Also provided is at least one solvate described herein or a pharmaceutical composition thereof for use in a method for treating pain.

30 [0079] Also provided is at least one solvate described herein for the manufacture of a medicament for treating a 5HT_{2A}-related disorder.

[0080] Also provided is at least one solvate described herein for the manufacture of a medicament for treating platelet aggregation, coronary artery disease, myocardial infarction, transient ischemic attack, angina, stroke, atrial fibrillation, blood clot formation, asthma or symptoms thereof, agitation or a symptom thereof, behavioral disorders, drug induced psychosis, 35 excitative psychosis, Gilles de la Tourette's syndrome, manic disorder, organic or NOS psychosis,

psychotic disorder, psychosis, acute schizophrenia, chronic schizophrenia, NOS schizophrenia and related disorders, sleep disorders, diabetic-related disorders, progressive multifocal leukoencephalopathy.

5 [0081] Also provided is at least one solvate described herein for the manufacture of a medicament for treating coronary artery disease, myocardial infarction, transient ischemic attack, angina, stroke, and atrial fibrillation.

[0082] Also provided is at least one solvate described herein for the manufacture of a medicament for treating condition associated with platelet aggregation.

10 [0083] Also provided is at least one solvate described herein for the manufacture of a medicament for reducing the risk of blood clot formation in an angioplasty or coronary bypass surgery individual.

[0084] Also provided is at least one solvate described herein for the manufacture of a medicament for reducing the risk of blood clot formation in an individual suffering from atrial fibrillation.

15 [0085] Also provided is at least one solvate described herein for the manufacture of a medicament for treating a sleep disorder.

[0086] Also provided is at least one solvate described herein for the manufacture of a medicament for treating a dyssomnia.

20 [0087] Also provided is at least one solvate described herein for the manufacture of a medicament for treating a parasomnia.

[0088] Also provided is at least one solvate described herein for the manufacture of a medicament for treating a diabetic-related disorder.

[0089] Also provided is at least one solvate described herein for the manufacture of a medicament for treating progressive multifocal leukoencephalopathy.

25 [0090] Also provided is at least one solvate described herein for the manufacture of a medicament for treating hypertension.

[0091] Also provided is at least one solvate described herein for the manufacture of a medicament for treating pain.

30 [0092] In addition to the foregoing beneficial uses for the modulators of 5-HT_{2A} receptor activity disclosed herein, the solvates disclosed herein are believed to be useful in the treatment of several additional diseases and disorders, and in the amelioration of symptoms thereof. Without limitation, these include the following:

Antiplatelet Therapies (Conditions related to platelet aggregation):

[0093] Antiplatelet agents (antiplatelets) are prescribed for a variety of conditions. For example, in coronary artery disease they are used to help prevent myocardial infarction or stroke in patients who are at risk of developing obstructive blood clots (e.g., coronary thrombosis).

5 [0094] In a myocardial infarction (heart attack), the heart muscle does not receive enough oxygen-rich blood as a result of a blockage in the coronary blood vessels. If taken while an attack is in progress or immediately afterward (preferably within 30 minutes), antiplatelets can reduce the damage to the heart.

[0095] A transient ischemic attack ("TIA" or "mini-stroke") is a brief interruption of oxygen flow to the brain due to decreased blood flow through arteries, usually due to an obstructing blood clot. Antiplatelet drugs have been found to be effective in preventing TIAs.

[0096] Angina is a temporary and often recurring chest pain, pressure or discomfort caused by inadequate oxygen-rich blood flow (ischemia) to some parts of the heart. In patients with angina, antiplatelet therapy can reduce the effects of angina and the risk of myocardial infarction.

15 [0097] Stroke is an event in which the brain does not receive enough oxygen-rich blood, usually due to blockage of a cerebral blood vessel by a blood clot. In high-risk patients, taking antiplatelets regularly has been found to prevent the formation of blood clots that cause first or second strokes.

[0098] Angioplasty is a catheter based technique used to open arteries obstructed by a blood clot. Whether or not stenting is performed immediately after this procedure to keep the artery open, antiplatelets can reduce the risk of forming additional blood clots following the procedure(s).

[0099] Coronary bypass surgery is a surgical procedure in which an artery or vein is taken from elsewhere in the body and grafted to a blocked coronary artery, rerouting blood around the blockage and through the newly attached vessel. After the procedure, antiplatelets can reduce the risk of secondary blood clots.

25 [00100] Atrial fibrillation is the most common type of sustained irregular heart rhythm (arrhythmia). Atrial fibrillation affects about two million Americans every year. In atrial fibrillation, the atria (the heart's upper chambers) rapidly fire electrical signals that cause them to quiver rather than contract normally. The result is an abnormally fast and highly irregular heartbeat. When given after an episode of atrial fibrillation, antiplatelets can reduce the risk of blood clots forming in the heart and traveling to the brain (embolism).

30 [00101] 5-HT_{2A} receptors are expressed on smooth muscle of blood vessels and 5-HT secreted by activated platelets causes vasoconstriction as well as activation of additional platelets during clotting. There is evidence that a 5-HT_{2A} inverse agonist will inhibit platelet aggregation and thus be a potential treatment as an antiplatelet therapy (see Satimura, K, et al., Clin Cardiol 2002 Jan. 25 (1):28-32; and Wilson, H.C et al., Thromb Haemost 1991 Sep 2;66(3):355-60).

[00102] 5-HT_{2A} inverse agonists can be used to treat, for example, claudication or peripheral artery disease as well as cardiovascular complications (see Br. Med. J. 298: 424 – 430, 1989), Arterial thrombosis (see, Pawlak, D. et al. Thrombosis Research 90: 259 – 270, 1998), atherosclerosis (see, Hayashi, T. et al. Atherosclerosis 168: 23 – 31, 2003), vasoconstriction, caused by serotonin (see, Fujiwara, T. and Chiba, S. Journal of Cardiovascular Pharmacology 26: 503 – 510, 1995), restenosis of arteries following angioplasty or stent placement (see, Fujita, M. et al. Am Heart J. 145:e16 2003). It can also be used alone or in combination with thrombolytic therapy, for example, tPA (see, Yamashita, T. et al. Haemostasis 30:321 – 332, 2000), to provide cardioprotection following MI or postischemic myocardial dysfunction (see, Muto, T. et al. Mol. Cell. Biochem. 272: 119-132, 2005) or protection from ischemic injury during percutaneous coronary intervention (see, Horibe, E. Circulation Research 68: 68 – 72, 2004), and the like, including complications resulting therefrom.

[00103] 5-HT_{2A} inverse antagonists can increase circulating adiponectin in patients, suggesting that they would also be useful in protecting patients against indications that are linked to adiponectin, for example, myocardial ischemia reperfusion injury and arteriosclerosis (see Nomura, Shosaku, et al. Blood Coagulation and Fibrinolysis 2005, 16, 423-428).

[00104] The solvates disclosed herein may provide beneficial improvement in microcirculation to patients in need of antiplatelet therapy by antagonizing the vasoconstrictive products of the aggregating platelets in, for example and not limited to the indications described above. Accordingly, also provided are methods for reducing platelet aggregation in a patient in need thereof comprising administering to the patient at least one solvate disclosed herein or a pharmaceutical composition thereof. Also provided are methods for treating coronary artery disease, myocardial infarction, transient ischemic attack, angina, stroke, atrial fibrillation, or a symptom of any of the foregoing in a patient in need of the treatment, comprising administering to the patient at least one solvate described herein or a pharmaceutical composition thereof.

[00105] Also provided are methods for reducing risk of blood clot formation in an angioplasty or coronary bypass surgery patient, or a patient suffering from atrial fibrillation, comprising administering to the patient at least one solvate described herein or a pharmaceutical composition thereof at a time where such risk exists.

30 Asthma

[00106] 5-HT (5-hydroxytryptamine) has been linked to the pathophysiology of acute asthma (see Cazzola, M. and Matera, M.G., TIPS, 2000, 21, 13; and De Bie, J.J. et al., British J. Pharm., 1998, 124, 857-864). The solvates described herein may be useful in the treatment of asthma, and the treatment of the symptoms thereof. Accordingly, also provided are methods for treating asthma in a patient in need of the treatment, comprising administering to the patient at least one solvate described herein or a pharmaceutical composition thereof. Also provided are methods for treating a symptom

of asthma in a patient in need of the treatment, comprising administering to the patient at least one solvate described herein or a pharmaceutical composition thereof.

Agitation

- [00107] Agitation is a well-recognized behavioral syndrome with a range of symptoms, including hostility, extreme excitement, poor impulse control, tension and uncooperativeness (See Cohen-Mansfield J, and Billig, N., (1986), Agitated Behaviors in the Elderly. I. A Conceptual Review. *J Am Geriatr Soc* 34(10): 711-721).
- [00108] Agitation is a common occurrence in the elderly and often associated with dementia such as those caused by Alzheimer's disease, Lewy Body, Parkinson's, and Huntington's, which are degenerative diseases of the nervous system and by diseases that affect blood vessels, such as stroke, or multi-infarct dementia, which is caused by multiple strokes in the brain can also induce dementia. Alzheimer's disease accounts for approximately 50 to 70% of all dementias (See Koss E, et al., (1997), Assessing patterns of agitation in Alzheimer's disease patients with the Cohen-Mansfield Agitation Inventory. The Alzheimer's Disease Cooperative Study. *Alzheimer Dis Assoc Disord* 11(suppl 2):S45-S50).
- [00109] An estimated five percent of people aged 65 and older and up to 20 percent of those aged 80 and older are affected by dementia; of these sufferers, nearly half exhibit behavioral disturbances, such as agitation, wandering and violent outbursts.
- [00110] Agitated behaviors can also be manifested in cognitively intact elderly people and by those with psychiatric disorders other than dementia.
- [00111] Agitation is often treated with antipsychotic medications such as haloperidol in nursing home and other assisted care settings. There is emerging evidence that agents acting at the 5-HT_{2A} receptors in the brain have the effects of reducing agitation in patients, including Alzheimer's dementia (See Katz, I.R., et al., *J Clin Psychiatry* 1999 Feb., 60(2):107-115; and Street, J.S., et al., *Arch Gen Psychiatry* 2000 Oct., 57(10):968-976).
- [00112] The solvates described herein may be useful for treating agitation and symptoms thereof. Thus, provided are methods for treating agitation in a patient in need of such treatment comprising administering to the patient at least one solvate described herein or a pharmaceutical composition thereof. In some embodiments, the agitation is due to a psychiatric disorder other than dementia. Also provided are methods for treatment of agitation or a symptom thereof in a patient suffering from dementia comprising administering to the patient at least one solvate described herein or a pharmaceutical composition thereof. In some embodiments of such methods, the dementia is due to a degenerative disease of the nervous system, for example and without limitation, Alzheimers disease, Lewy Body, Parkinson's disease, and Huntington's disease, or dementia due to diseases that affect blood vessels, including, without limitation, stroke and multi-infarct dementia. Also provided are methods for treating agitation or a symptom thereof in a patient in need of such treatment, where

the patient is a cognitively intact elderly patient, comprising administering to the patient at least one solvate described herein or a pharmaceutical composition thereof.

Add-On therapy to Haloperidol in the treatment of schizophrenia and other disorders:

[00113] Schizophrenia is a psychopathic disorder of unknown origin, which usually appears
5 for the first time in early adulthood and is marked by a number of characteristics, psychotic
symptoms, progression, phasic development and deterioration in social behavior and professional
capability in the region below the highest level ever attained. Characteristic psychotic symptoms are
disorders of thought content (multiple, fragmentary, incoherent, implausible or simply delusional
contents or ideas of persecution) and of mentality (loss of association, flight of imagination,
10 incoherence up to incomprehensibility), as well as disorders of perceptibility (hallucinations), of
emotions (superficial or inadequate emotions), of self-perception, of intentions and impulses, of
interhuman relationships, and finally psychomotoric disorders (such as catatonia). Other symptoms
are also associated with this disorder. (See, American Statistical and Diagnostic Handbook).

[00114] Haloperidol (Haldol) is a potent dopamine D₂ receptor antagonist. It is widely
15 prescribed for acute schizophrenic symptoms, and is very effective for the positive symptoms of
schizophrenia. However, Haldol is not effective for the negative symptoms of schizophrenia and
may actually induce negative symptoms as well as cognitive dysfunction. In some embodiments,
adding a 5-HT_{2A} inverse agonist concomitantly with Haldol may provide benefits including the
ability to use a lower dose of Haldol without losing its effects on positive symptoms, while reducing
20 or eliminating its inductive effects on negative symptoms, and prolonging relapse to the patient's next
schizophrenic event.

[00115] Haloperidol is used for treatment of a variety of behavioral disorders, drug induced
psychosis, excitative psychosis, Gilles de la Tourette's syndrome, manic disorders, psychosis (organic
and NOS), psychotic disorder, psychosis, schizophrenia (acute, chronic and NOS). Further uses
25 include in the treatment of infantile autism, huntington's chorea, and nausea and vomiting from
chemotherapy and chemotherapeutic antibodies. Administration of 5-HT_{2A} inverse agonists disclosed
herein with haloperidol also may provide benefits in these indications.

[00116] Also provided are methods for treating a behavioral disorder, drug induced
psychosis, excitative psychosis, Gilles de la Tourette's syndrome, manic disorders, psychosis (organic
30 and NOS), psychotic disorder, psychosis, schizophrenia (acute, chronic and NOS) comprising
administering to the patient a dopamine D₂ receptor antagonist and at least one solvate described
herein or a pharmaceutical composition thereof.

[00117] Also provided are methods for treating a behavioral disorder, drug induced
psychosis, excitative psychosis, Gilles de la Tourette's syndrome, manic disorders, psychosis (organic
35 and NOS), psychotic disorder, psychosis, schizophrenia (acute, chronic and NOS) comprising

administering to the patient haloperidol and at least one solvate described herein or a pharmaceutical composition thereof.

[00118] Also provided are methods for treating infantile autism, huntington's chorea, or nausea and vomiting from chemotherapy or chemotherapeutic antibodies comprising administering to the patient a dopamine D₂ receptor antagonist and at least one solvate described herein or a pharmaceutical composition thereof.

[00119] Also provided are methods for treating infantile autism, huntington's chorea, or nausea and vomiting from chemotherapy or chemotherapeutic antibodies comprising administering to the patient haloperidol and at least one solvate described herein or a pharmaceutical composition thereof.

[00120] Also provided are methods for treating schizophrenia in a patient in need of the treatment comprising administering to the patient a dopamine D₂ receptor antagonist and at least one solvate described herein or a pharmaceutical composition thereof. Preferably, the dopamine D₂ receptor antagonist is haloperidol.

[00121] The administration of the dopamine D₂ receptor antagonist can be concomitant with administration of the at least one solvate described herein or a pharmaceutical composition thereof, or they can be administered at different times. Those of skill in the art will easily be able to determine appropriate dosing regimes for the most efficacious reduction or elimination of deleterious haloperidol effects. In some embodiments, haloperidol and the at least one solvate described herein or a pharmaceutical composition thereof are administered in a single dosage form, and in other embodiments, they are administered in separate dosage forms.

[00122] Also provided are methods of alleviating negative symptoms of schizophrenia induced by the administration of haloperidol to a patient suffering from schizophrenia, comprising administering to the patient at least one solvate described herein or a pharmaceutical composition thereof.

Sleep disorders

[00123] It is reported in the National Sleep Foundation's 2002 Sleep In America Poll, more than one-half of the adults surveyed (58%) report having experienced one or more symptoms of insomnia at least a few nights a week in the past year. Additionally, about three in ten (35%) say they have experienced insomnia-like symptoms every night or almost every night.

[00124] The normal sleep cycle and sleep architecture can be disrupted by a variety of organic causes as well as environmental influences. According to the International Classification of Sleep Disorders, there are over 80 recognized sleep disorders. Of these, at least one solvate described herein or a pharmaceutical composition thereof may be effective, for example, in any one or more of the following sleep disorders (ICSD – International Classification of Sleep Disorders: Diagnostic and

Coding Manual. *Diagnostic Classification Steering Committee*, American Sleep Disorders Association, 1990):

DYSSOMNIAS

Intrinsic Sleep Disorders:

- 5 [00125] Psychophysiological insomnia, Sleep state misperception, Idiopathic insomnia, Obstructive sleep apnea syndrome, Central sleep apnea syndrome, Central alveolar hypoventilation syndrome, Periodic limb movement disorder, Restless leg syndrome and Intrinsic sleep disorder NOS.

Extrinsic Sleep Disorders:

- 10 [00126] Inadequate sleep hygiene, Environmental sleep disorder, Altitude insomnia, Adjustment sleep disorder, Insufficient sleep syndrome, Limit-setting sleep disorder, SleepOnset association disorder, Nocturnal eating (drinking) syndrome, Hypnotic dependent sleep disorder, Stimulant-dependent sleep disorder, Alcohol-dependent sleep disorder, Toxin-induced sleep disorder and Extrinsic sleep disorder NOS.

15 Circadian Rhythm Sleep Disorders:

- [00127] Time zone change (jet lag) syndrome, Shift work sleep disorder, Irregular sleep-wake pattern, Delayed sleep phase syndrome, Advanced sleep phase syndrome, Non-24-hour sleep-wake disorder and Circadian rhythm sleep disorder NOS.

PARASOMNIAS

20 Arousal Disorders:

Confusional arousals, Sleepwalking and Sleep terrors.

Sleep-Wake Transition Disorders:

Rhythmic movement disorder, Sleep starts, Sleep talking and Nocturnal leg cramps.

SLEEP DISORDERS ASSOCIATED WITH MEDICAL/PSYCHIATRIC DISORDERS

25 Associated with Mental Disorders:

Psychoses, Mood disorders, Anxiety disorders, Panic disorders and Alcoholism.

Associated with Neurological Disorders:

Cerebral degenerative disorders, Dementia, Parkinsonism, Fatal familial insomnia, Sleep-related epilepsy, Electrical status epilepticus of sleep and Sleep-related headaches.

30 Associated with Other Medical Disorders:

Sleeping sickness, Nocturnal cardiac ischemia, Chronic obstructive pulmonary disease, Sleep-related asthma, Sleep-related gastroesophageal reflux, Peptic ulcer disease, Fibrositis syndrome, Osteoarthritis, Rheumatoid arthritis, Fibromyalgia and Post-surgical.

- [00128] The effects of sleep deprivation are more than excessive daytime sleepiness.

- 35 Chronic insomniacs report elevated levels of stress, anxiety, depression and medical illnesses (National Institutes of Health, National Heart, Lung, and Blood Institute, *Insomnia Facts Sheet*, Oct.

1995). Preliminary evidence suggests that having a sleep disorder that causes significant loss of sleep may contribute to increased susceptibility to infections due to immunosuppression, cardiovascular complications such as hypertension, cardiac arrhythmias, stroke, and myocardial infarction, compromised glucose tolerance, increased obesity and metabolic syndrome. The solvates described
5 herein may be useful to prevent or alleviate these complications by improving sleep quality.

[00129] The most common class of medications for the majority of sleep disorders are the benzodiazepines, but the adverse effect profile of benzodiazepines include daytime sedation, diminished motor coordination, and cognitive impairments. Furthermore, the National Institutes of Health Consensus conference on Sleeping Pills and Insomnia in 1984 have developed guidelines
10 discouraging the use of such sedative-hypnotics beyond 4-6 weeks because of concerns raised over drug misuse, dependency, withdrawal and rebound insomnia. Therefore, it is desirable to have a pharmacological agent for the treatment of insomnia, which is more effective and/or has fewer side effects than those currently used. In addition, benzodiazepines are used to induce sleep, but have little to no effect on the maintenance of sleep, sleep consolidation or slow wave sleep. Therefore,
15 sleep maintenance disorders are not currently well treated.

[00130] Clinical studies with agents of a similar mechanism of action as those described herein have demonstrated significant improvements on objective and subjective sleep parameters in normal, healthy volunteers as well as patients with sleep disorders and mood disorders [Sharpley AL, et al. Slow Wave Sleep in Humans: Role of 5-HT_{2A} and 5HT_{2C} Receptors. *Neuropharmacology*,
20 1994, Vol. 33(3/4):467-71; Winokur A, et al. Acute Effects of Mirtazapine on Sleep Continuity and Sleep Architecture in Depressed Patients: A Pilot Study. *Soc of Biol Psych*, 2000, Vol. 48:75-78; and Landolt HP, et al. Serotonin-2 Receptors and Human Sleep: Effect of Selective Antagonist on EEG Power Spectra. *Neuropsychopharmacology*, 1999, Vol. 21(3):455-66].

[00131] Some sleep disorders are sometimes found in conjunction with other conditions and accordingly those conditions may be treatable by at least one solvate described herein or a
25 pharmaceutical composition thereof. For example, but not limited to, patients suffering from mood disorders typically suffer from a sleep disorder that may be treatable by at least one solvate described herein or a pharmaceutical composition thereof. Having one pharmacological agent which treats two or more existing or potential conditions may be more cost effective, may lead to better
30 compliance and may have fewer side effects than taking two or more agents.

[00132] Also provided are methods for treating Sleep Disorders. Also provided are pharmaceutical agent, which may be useful in treating two or more conditions wherein one of the conditions is a sleep disorder. The solvates described herein may be used alone or in combination with a mild sleep inducer (i.e. antihistamine).

35 **Sleep Architecture:**

[00133] Sleep comprises two physiological states: Non rapid eye movement (NREM) and rapid eye movement (REM) sleep. NREM sleep consists of four stages, each of which is characterized by progressively slower brain wave patterns, with the slower patterns indicating deeper sleep. So called delta sleep, stages 3 and 4 of NREM sleep, is the deepest and most refreshing type of sleep. Many patients with sleep disorders are unable to adequately achieve the restorative sleep of stages 3 and 4. In clinical terms, patients' sleep patterns are described as fragmented, meaning the patient spends a lot of time alternating between stages 1 and 2 (semi-wakefulness) and being awake and very little time in deep sleep. As used herein, the term "fragmented sleep architecture" means an individual, such as a sleep disorder patient, spends the majority of their sleep time in NREM sleep stages 1 and 2, lighter periods of sleep from which the individual can be easily aroused to a Waking state by limited external stimuli. As a result, the individual cycles through frequent bouts of light sleep interrupted by frequent awakenings throughout the sleep period. Many sleep disorders are characterized by a fragmented sleep architecture. For example, many elderly patients with sleep complaints have difficulty achieving long bouts of deep refreshing sleep (NREM stages 3 and 4) and instead spend the majority of their sleep time in NREM sleep stages 1 and 2.

[00134] In contrast to fragmented sleep architecture, as used herein the term "sleep consolidation" means a state in which the number of NREM sleep bouts, particularly Stages 3 and 4, and the length of those sleep bouts are increased, while the number and length of waking bouts are decreased. In essence, the architecture of the sleep disorder patient is consolidated to a sleeping state with increased periods of sleep and fewer awakenings during the night and more time is spent in slow wave sleep (Stages 3 and 4) with fewer oscillation Stage 1 and 2 sleep. The solvates described herein may be effective in consolidating sleep patterns so that the patient with previously fragmented sleep can now achieve restorative, delta-wave sleep for longer, more consistent periods of time.

[00135] As sleep moves from stage 1 into later stages, heart rate and blood pressure drop, metabolic rate and glucose consumption fall, and muscles relax. In normal sleep architecture, NREM sleep makes up about 75% of total sleep time; stage 1 accounting for 5-10% of total sleep time, stage 2 for about 45-50%, stage 3 approximately 12%, and stage 4 13-15%. About 90 minutes after sleep onset, NREM sleep gives way to the first REM sleep episode of the night. REM makes up approximately 25% of total sleep time. In contrast to NREM sleep, REM sleep is characterized by high pulse, respiration, and blood pressure, as well as other physiological patterns similar to those seen in the active waking stage. Hence, REM sleep is also known as "paradoxical sleep." Sleep onset occurs during NREM sleep and takes 10-20 minutes in healthy young adults. The four stages of NREM sleep together with a REM phase form one complete sleep cycle that is repeated throughout the duration of sleep, usually four or five times. The cyclical nature of sleep is regular and reliable; a REM period occurs about every 90 minutes during the night. However, the first REM period tends to be the shortest, often lasting less than 10 minutes, whereas the later REM periods may last up to 40

minutes. With aging, the time between retiring and sleep onset increases and the total amount of night-time sleep decreases because of changes in sleep architecture that impair sleep maintenance as well as sleep quality. Both NREM (particularly stages 3 and 4) and REM sleep are reduced. However, stage 1 NREM sleep, which is the lightest sleep, increases with age.

- 5 [00136] As used herein, the term "delta power" means a measure of the duration of EEG activity in the 0.5 to 3.5 Hz range during NREM sleep and is thought to be a measure of deeper, more refreshing sleep. Delta power is hypothesized to be a measure of a theoretical process called Process S and is thought to be inversely related to the amount of sleep an individual experiences during a given sleep period. Sleep is controlled by homeostatic mechanisms; therefore, the less one sleeps
- 10 the greater the drive to sleep. It is believed that Process S builds throughout the wake period and is discharged most efficiently during delta power sleep. Delta power is a measure of the magnitude of Process S prior to the sleep period. The longer one stays awake, the greater Process S or drive to sleep and thus the greater the delta power during NREM sleep. However, individuals with sleep disorders have difficulty achieving and maintaining delta wave sleep, and thus have a
- 15 large build-up of Process S with limited ability to discharge this buildup during sleep. 5-HT_{2A} agonists tested preclinically and clinically mimic the effect of sleep deprivation on delta power, suggesting that subjects with sleep disorders treated with a 5-HT_{2A} inverse agonist or antagonist will be able to achieve deeper more refreshing sleep. These same effects have not been observed with currently marketed pharmacotherapies. In addition, currently marketed pharmacotherapies
- 20 for sleep have side effects such as hangover effects or addiction that are associated with the GABA receptor. 5-HT_{2A} inverse agonists do not target the GABA receptor and so these side effects are not a concern.

Subjective and objective determinations of sleep disorders:

- [00137] There are a number of ways to determine whether the onset, duration or quality of
- 25 sleep (e.g. non-restorative or restorative sleep) is impaired or improved. One method is a subjective determination of the patient, e.g., do they feel drowsy or rested upon waking. Other methods involve the observation of the patient by another during sleep, e.g., how long it takes the patient to fall asleep, how many times does the patient wake up during the night, how restless is the patient during sleep, etc. Another method is to objectively measure the stages of sleep using polysomnography.
- 30 [00138] Polysomnography is the monitoring of multiple electrophysiological parameters during sleep and generally includes measurement of EEG activity, electroculographic activity and electromyographic activity, as well as other measurements. These results, along with observations, can measure not only sleep latency (the amount of time required to fall asleep), but also sleep continuity (overall balance of sleep and wakefulness) and sleep consolidation (percent of sleeping
- 35 time spent in delta-wave or restorative sleep) which may be an indication of the quality of sleep.

[00139] There are five distinct sleep stages, which can be measured by polysomnography: rapid eye movement (REM) sleep and four stages of non-rapid eye movement (NREM) sleep (stages 1, 2, 3 and 4). Stage 1 NREM sleep is a transition from wakefulness to sleep and occupies about 5% of time spent asleep in healthy adults. Stage 2 NREM sleep, which is characterized by specific EEG waveforms (sleep spindles and K complexes), occupies about 50% of time spent asleep. Stages 3 and 4 NREM sleep (also known collectively as slow-wave sleep and delta-wave sleep) are the deepest levels of sleep and occupy about 10-20% of sleep time. REM sleep, during which the majority of vivid dreams occur, occupies about 20-25% of total sleep.

[00140] These sleep stages have a characteristic temporal organization across the night. NREM stages 3 and 4 tend to occur in the first one-third to one-half of the night and increase in duration in response to sleep deprivation. REM sleep occurs cyclically through the night. Alternating with NREM sleep about every 80-100 minutes. REM sleep periods increase in duration toward the morning. Human sleep also varies characteristically across the life span. After relative stability with large amounts of slow-wave sleep in childhood and early adolescence, sleep continuity and depth deteriorate across the adult age range. This deterioration is reflected by increased wakefulness and stage 1 sleep and decreased stages 3 and 4 sleep.

[00141] In addition, the solvates described herein may be useful for the treatment of the sleep disorders characterized by excessive daytime sleepiness such as narcolepsy. Inverse agonists at the serotonin 5-HT_{2A} receptor improve the quality of sleep at nighttime which can decrease excessive daytime sleepiness.

[00142] Also provided is the therapeutic use of at least one solvate described herein or a pharmaceutical composition thereof for the treatment of Sleep Disorders. The solvates described herein are potent inverse agonists at the serotonin 5-HT_{2A} receptor and may be effective in the treatment of Sleep Disorders by promoting one or more of the following: reducing the sleep onset latency period (measure of sleep induction), reducing the number of nighttime awakenings, and prolonging the amount of time in delta-wave sleep (measure of sleep quality enhancement and sleep consolidation) without effecting REM sleep. In addition, the solvates described herein can be effective either as a monotherapy or in combination with sleep inducing agents, for example but not limited to, antihistamines.

30 **Diabetic-Related Pathologies:**

[00143] Although hyperglycemia is the major cause for the pathogenesis of diabetic complications such as diabetic peripheral neuropathy (DPN), diabetic nephropathy (DN) and diabetic retinopathy (DR), increased plasma serotonin concentration in diabetic patients has also been implicated to play a role in disease progression (Pietraszek, M.H., et al. *Thrombosis Res.* 1992, 66(6), 765-74; and Andrzejewska-Buczko J, et al., *Klin Oczna.* 1996; 98(2), 101-4).

Serotonin is believed to play a role in vasospasm and increased platelet aggregability. Improving microvascular blood flow is able to benefit diabetic complications.

[00144] A recent study by Cameron and Cotter in *Naunyn Schmiedeberg's Arch Pharmacol.* 2003 Jun; 367(6):607-14, used a 5-HT_{2A} antagonist experimental drug AT-1015, and other non-specific 5-HT_{2A} antagonists including ritanserin and sarpogrelate. These studies found that all three drugs were able to produce a marked correction (82.6-99.7%) of a 19.8% sciatic motor conduction deficit in diabetic rats. Similarly, 44.7% and 14.9% reductions in sciatic endoneurial blood flow and saphenous sensory conduction velocity were completely reversed.

[00145] In a separate patient study, sarogrelate was evaluated for the prevention of the development or progression of diabetic nephropathy (Takahashi, T., et al., *Diabetes Res Clin Pract.* 2002 Nov; 58(2):123-9). In the trial of 24 months of treatment, sarpogrelate significantly reduced urinary albumin excretion level.

Glaucoma

[00146] Topical ocular administration of 5-HT₂ receptor antagonists result in a decrease in intra ocular pressure (IOP) in monkeys (Chang et al., *J. Ocul Pharmacol* 1:137-147 (1985)) and humans (Mastropasqua et al., *Acta Ophthalmol Scand Suppl* 224:24-25 (1997)) indicating utility for similar compounds such as 5-HT_{2A} inverse agonists in the treatment of ocular hypertension associated with glaucoma. The 5-HT₂ receptor antagonist ketanserin (Mastropasqua supra) and sarpogrelate (Takcnaka et al., *Investig Ophthalmol Vis Sci* 36:S734 (1995)) have been shown to significantly lower IOP in glaucoma patients.

Progressive Multifocal Leukoencephalopathy

[00147] Progressive multifocal leukoencephalopathy (PML) is a lethal demyelinating disease caused by an opportunistic viral infection of oligodendrocytes in immunocompromised patients. The causative agent is JC virus, a ubiquitous papovavirus that infects the majority of the population before adulthood and establishes a latent infection in the kidney. In immunocompromised hosts, the virus can reactivate and productively infect oligodendrocytes. This previously rare condition, until 1984 reported primarily in persons with underlying lymphoproliferative disorders, is now more common because it occurs in 4% of patients with AIDS. Patients usually present with relentlessly progressive focal neurologic defects, such as hemiparesis or visual field deficits, or with alterations in mental status. On brain MRI, one or more white matter lesions are present; they are hyperintense on T2-weighted images and hypointense on T1-weighted images. There is no mass effect, and contrast enhancement is rare. Diagnosis can be confirmed by brain biopsy, with demonstration of virus by *in situ* hybridization or immunocytochemistry. Polymerase chain reaction amplification of JC virus sequences from the CSF can confirm diagnosis without the need for biopsy [see, e.g., Antinori et al., *Neurology* (1997) 48:687-694; Berger and Major, *Seminars in Neurology* (1999) 19:193-200; and

Portegies, et al., *Eur. J. Neurol.* (2004) 11:297-304]. Currently, there is no effective therapy. Survival after diagnosis is about 3 to 5 months in AIDS patients.

5 [00148] JC virus enters cells by receptor-mediated clathrin-dependent endocytosis. Binding of JC virus to human glial cells (e.g., oligodendrocytes) induces an intracellular signal that is critical for entry and infection by a ligand-inducible clathrin-dependent mechanism [Querbes et al., *J Virology* (2004) 78:250-256]. Recently, 5-HT_{2A} was shown to be the receptor on human glial cells mediating infectious entry of JC virus by clathrin-dependent endocytosis [Elphick et al., *Science* (2004) 306:1380-1383]. 5-HT_{2A} antagonists, including ketanserin and ritanserin, inhibited JC virus infection of human glial cells. Ketanserin and ritanserin have inverse agonist activity at 5-HT_{2A}.

10 [00149] 5-HT_{2A} antagonists including inverse agonists have been contemplated to be useful in the treatment of PML [Elphick et al., *Science* (2004) 306:1380-1383]. Prophylactic treatment of HIV-infected patients with 5-HT_{2A} antagonists is envisioned to prevent the spread of JC virus to the central nervous system and the development of PML. Aggressive therapeutic treatment of patients with PML is envisioned to reduce viral spread within the central nervous system and prevent additional episodes of demyelination.

15 [00150] Also provided are methods for treating progressive multifocal leukoencephalopathy in a patient in need of such treatment, comprising administering to the patient at least one solvate described herein or a pharmaceutical composition thereof.

20 Hypertension

[00151] Serotonin has been observed to play an important role in the regulation of vascular tone, vasoconstriction, and pulmonary hypertension (see, Deuchar, G. et al. *Pulm. Pharmacol. Ther.* 18(1):23-31, 2005; and Marcos, E. et al. *Circ. Res.* 94(9):1263-70 2004). Ketanserin, a 5-HT_{2A} inverse agonist, have been demonstrated to protect against circulatory shocks, intracranial hypertension, and cerebral ischemia during heatstroke (see, Chang, C. et al. *Shock* 24(4): 336-340 2005); and to stabilize blood pressure in spontaneously hypertensive rats (see, Miao, C. *Clin. Exp. Pharmacol. Physiol.* 30(3): 189-193). Mainserin, a 5-HT_{2A} inverse agonist, has been shown to prevent DOCA-salt induced hypertension in rats (see, Silva, A. *Eur. J. Pharmacol.* 518(2-3): 152-7 2005).

30 Pain

[00152] 5-HT_{2A} inverse agonists are also effective for the treatment of pain. Sarpogrelate has been observed to provide a significant analgesic effect both on thermal induced pain in rats after intraperitoneal administration and on inflammatory pain in rats after either intrathecal or intraperitoneal administration (see, Nishiyama, T. *Eur. J. Pharmacol.* 516:18-22 2005). This same 35 5-HT_{2A} inverse agonist in humans has been shown to be an effective treatment for lower back

pain, leg pain and numbness associated with sciatica brought on by lumbar disc herniation (see, Kanayama, M. et al. J. Neurosurg: Spine 2:441-446 2005).

[00153] Also provided are pharmaceutical compositions comprising at least one solvate described herein and one or more pharmaceutically acceptable carriers.

5 [00154] In some embodiments, the at least one solvate is administered as a raw or pure chemical.

[00155] Also provided are pharmaceutical compositions comprising 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate and one or more pharmaceutically acceptable carriers.

10 [00156] Also provided are pharmaceutical compositions comprising (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate and one or more pharmaceutically acceptable carriers.

[00157] Also provided is a method of producing a pharmaceutical composition comprising admixing at least one solvate described herein and a pharmaceutically acceptable carrier.

15 [00158] Formulations may be prepared by any suitable method, typically by uniformly mixing the active compound(s) with liquids or finely divided solid carriers, or both, in the required proportions, and then, if necessary, forming the resulting mixture into a desired shape.

[00159] Conventional excipients, such as binding agents, fillers, acceptable wetting agents, tableting lubricants, and disintegrants may be used in tablets and capsules for oral
20 administration. Liquid preparations for oral administration may be in the form of solutions, emulsions, aqueous or oily suspensions, and syrups. Alternatively, the oral preparations may be in the form of dry powder that can be reconstituted with water or another suitable liquid vehicle before use. Additional additives such as suspending or emulsifying agents, non-aqueous vehicles (including edible oils), preservatives, and flavorings and colorants may be added to the liquid
25 preparations. Parenteral dosage forms may be prepared by dissolving at least one solvate described herein in a suitable liquid vehicle and filter sterilizing the solution before filling and sealing an appropriate vial or ampoule. These are just a few examples of the many appropriate methods well known in the art for preparing dosage forms.

[00160] Pharmaceutical compositions may be formulated using techniques well known to
30 those in the art. Suitable pharmaceutically-acceptable carriers, outside those mentioned herein, are known in the art; for example, see Remington, *The Science and Practice of Pharmacy*, 20th Ed., 2000, Lippincott Williams & Wilkins, (Editors: Gennaro, A. R., et al.). The carrier(s) is be "acceptable" in the sense of being compatible with the other ingredients of the for compositions and not overly deleterious to the recipient thereof.

- [00161] Pharmaceutical compositions include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration or in a form suitable for administration by inhalation, insufflation or by a transdermal patch. Transdermal patches dispense a drug at a controlled rate by presenting the drug for absorption in an efficient manner with a minimum of degradation of the drug. Typically, transdermal patches comprise an impermeable backing layer, a single pressure sensitive adhesive and a removable protective layer with a release liner. One of ordinary skill in the art will understand and appreciate the techniques appropriate for manufacturing a desired efficacious transdermal patch based upon the needs of the artisan.
- 10 [00162] The solvates described herein, together with a conventional adjuvant, carrier, or diluent, may thus be placed into the form of pharmaceutical compositions and unit dosages thereof, and in such form may be employed as solids, such as tablets or filled capsules, or liquids such as solutions, suspensions, emulsions, elixirs, gels or capsules filled with the same, all for oral use, in the form of suppositories for rectal administration; or in the form of sterile injectable solutions for parenteral (including subcutaneous) use. Such pharmaceutical compositions and unit dosage forms thereof may comprise conventional ingredients in conventional proportions, with or without additional active compounds or principles, and such unit dosage forms may contain any suitable effective amount of the active ingredient commensurate with the intended daily dosage range to be employed.
- 20 [00163] For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet, capsule, suspension or liquid. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of the active ingredient. Examples of such dosage units are capsules, tablets, powders, granules or a suspension, with conventional additives such as lactose, mannitol, corn starch or potato starch; with binders such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators such as corn starch, potato starch or sodium carboxymethylcellulose; and with lubricants such as talc or magnesium stearate. The active ingredient may also be administered by injection as a composition wherein, for example, saline, dextrose or water may be used as a suitable pharmaceutically acceptable carrier.
- 25 [00164] The solvates described herein can be used as active ingredients in pharmaceutical compositions, specifically as 5-HT_{2A} receptor modulators. By the term "active ingredient" is defined in the context of a "pharmaceutical composition" and shall mean a component of a pharmaceutical composition that provides the primary pharmacological effect, as opposed to an "inactive ingredient" which would generally be recognized as providing no pharmaceutical benefit.
- 35

[00165] The dose when using the solvates described herein can vary within wide limits, as is customary and is known to the physician, it is to be tailored to the individual conditions in each individual case. It depends, for example, on the nature and severity of the illness to be treated, on the condition of the patient, on the compound employed or on whether an acute or chronic disease state is treated or prophylaxis is conducted or on whether further active compounds are administered in addition to the solvates described herein. Representative doses include, but are not limited to, about 0.001 mg to about 5000 mg, about 0.001 mg to about 2500 mg, about 0.001 mg to about 1000 mg, 0.001 mg to about 500 mg, 0.001 mg to about 250 mg, about 0.001 mg to 100 mg, about 0.001 mg to about 50 mg, and about 0.001 mg to about 25 mg. Multiple doses may be administered during the day, especially when relatively large amounts are deemed to be needed, for example 2, 3 or 4, doses. Depending on the individual and as deemed appropriate from the patient's physician or care-giver it may be necessary to deviate upward or downward from the doses described herein.

[00166] The amount of active ingredient required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will ultimately be at the discretion of the attendant physician or clinician. In general, one skilled in the art understands how to extrapolate *in vivo* data obtained in a model system, typically an animal model, to another, such as a human. In some circumstances, these extrapolations may merely be based on the weight of the animal model in comparison to another, such as a mammal, preferably a human, however, more often, these extrapolations are not simply based on weights, but rather incorporate a variety of factors. Representative factors include the type, age, weight, sex, diet and medical condition of the patient, the severity of the disease, the route of administration, pharmacological considerations such as the activity, efficacy, pharmacokinetic and toxicology profiles of the particular compound employed, whether a drug delivery system is utilized, or whether an acute or chronic disease state is being treated or prophylaxis is conducted or on whether further active compounds are administered in addition to the solvates described herein and as part of a drug combination. The dosage regimen for treating a disease condition with the solvates described herein is selected in accordance with a variety of factors as cited above. Thus, the actual dosage regimen employed may vary widely and therefore may deviate from a preferred dosage regimen and one skilled in the art will recognize that dosage and dosage regimen outside these typical ranges can be tested and, where appropriate, may be used in the methods described herein.

[00167] The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations. The daily dose can be divided, especially when relatively large amounts are

administered as deemed appropriate, into several, for example 2, 3 or 4, part administrations. If appropriate, depending on individual behavior, it may be necessary to deviate upward or downward from the daily dose indicated.

5 [00168] The solvates described herein can be administered in a wide variety of oral and parenteral dosage forms.

[00169] For preparing pharmaceutical compositions from the solvates described herein, the selection of a suitable pharmaceutically acceptable carrier can be either solid, liquid or a mixture of both. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances which may
10 also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material.

[00170] In powders, the carrier is a finely divided solid which is in a mixture with the finely divided active component.

15 [00171] In tablets, the active component is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted to the desired shape and size.

[00172] The powders and tablets may contain varying percentage amounts of the active compound. A representative amount in a powder or tablet may contain from 0.5 to about 90 percent of the active compound; however, an artisan would know when amounts outside of this range are necessary. Suitable carriers for powders and tablets are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like.
20 The term "preparation" is intended to include the formulation of the active compound with encapsulating material as carrier providing a capsule in which the active component, with or without carriers, is surrounded by a carrier, which is thus in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be used as
25 solid forms suitable for oral administration.

[00173] For preparing suppositories, a low melting wax, such as an admixture of fatty acid glycerides or cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogeneous mixture is then poured into convenient sized
30 molds, allowed to cool, and thereby to solidify.

[00174] Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

35 [00175] Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water-propylene glycol solutions. For example, parenteral injection liquid

preparations can be formulated as solutions in aqueous polyethylene glycol solution. Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents.

The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

[00176] The solvates described herein may thus be formulated for parenteral administration (e.g. by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The pharmaceutical compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

[00177] Aqueous formulations suitable for oral use can be prepared by dissolving or suspending the active component in water and adding suitable colorants, flavors, stabilizing and thickening agents, as desired.

[00178] Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, or other well known suspending agents.

[00179] Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

[00180] For topical administration to the epidermis the solvates described herein may be formulated as ointments, creams or lotions, or as a transdermal patch.

[00181] Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents.

[00182] Formulations suitable for topical administration in the mouth include lozenges comprising active agent in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

5 [00183] Solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The formulations may be provided in single or multi-dose form. In the latter case of a dropper or pipette, this may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray, this may be achieved for example by means of a metering atomizing spray pump.

10 [00184] Administration to the respiratory tract may also be achieved by means of an aerosol formulation in which the active ingredient is provided in a pressurized pack with a suitable propellant. If the solvates described herein or pharmaceutical compositions comprising them are administered as aerosols, for example as nasal aerosols or by inhalation, this can be carried out, for example, using a spray, a nebulizer, a pump nebulizer, an inhalation apparatus, a metered
15 inhaler or a dry powder inhaler. Pharmaceutical forms for administration of the solvates described herein as an aerosol can be prepared by processes well-known to the person skilled in the art. For their preparation, for example, solutions or dispersions of the solvates described herein in water, water/alcohol mixtures or suitable saline solutions can be employed using customary additives, for example benzyl alcohol or other suitable preservatives, absorption enhancers for increasing the
20 bioavailability, solubilizers, dispersants and others, and, if appropriate, customary propellants, for example include carbon dioxide, CFCs, such as, dichlorodifluoromethane, trichlorofluoromethane, or dichlorotetrafluoroethane; and the like. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by provision of a metered valve.

[00185] In formulations intended for administration to the respiratory tract, including
25 intranasal formulations, the solvates described herein will generally have a small particle size for example of the order of 10 microns or less. Such a particle size may be obtained by means known in the art, for example by micronization. When desired, formulations adapted to give sustained release of the active ingredient may be employed.

[00186] Alternatively the active ingredients may be provided in the form of a dry powder,
30 for example, a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethylcellulose and polyvinylpyrrolidone (PVP). Conveniently the powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form for example in capsules or cartridges of, e.g., gelatin, or blister packs from which the powder may be administered by means of an inhaler.

35 [00187] The pharmaceutical preparations are preferably in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active

component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules.

Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

5 [00188] In some embodiments, tablets or capsules for oral administration and liquids for intravenous administration are used.

[00189] While the solvates described herein can be administered as the sole active pharmaceutical agent (i.e., mono-therapy), they can also be used in combination with other pharmaceutical agents (i.e., combination-therapy) for the treatment of the
10 diseases/conditions/disorders described herein. Accordingly, also provided are methods of treatment of 5-HT_{2A} serotonin receptor mediated disorders diseases comprising administering to an individual in need of such treatment a therapeutically-effective amount of at least one solvate described herein in combination with one or more additional pharmaceutical agent as described herein.

15 [00190] Suitable pharmaceutical agents that can be used in combination with the solvates described herein include other antiplatelet, antithrombotic or anticoagulant drugs, anti-arrhythmic agents, Cholesteryl ester transfer protein (CETP) inhibitors, Niacin or niacin analogs, Adenosine or adenosine analogs, Nitroglycerin or nitrates, prothrombolytic agents, and the like. Other pharmaceutical agents, including the agents set forth *infra*, are well known or will be readily
20 apparent in light of the instant disclosure, to one of ordinary skill in the art.

[00191] The solvates described herein can also be used in combination with other antiplatelet, antithrombotic or anticoagulant drugs such as thrombin inhibitors, platelet aggregation inhibitors such as aspirin, clopidogrel (Plavix®), ticlopidine or CS-747 (i.e., acetic acid 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl
25 ester and its active metabolite R-99224, (Z)-2-[1-[2-cyclopropyl-1(*S*)-(2-fluorophenyl)-2-oxoethyl]-4(*R*)-sulfanylpiperidin-3-ylidene]acetic acid}, abciximab (ReoPro®), eptifibatide (Integrilin®), tirofiban (Aggrastat®), warfarin, low molecular weight heparins (such as LOVENOX), GPIIb/GPIIIa blockers, PAI-1 inhibitors such as XR-330 [i.e., (3*Z*,6*Z*)-3-Benzylidene-6-(4-methoxybenzylidene)-1-methylpiperazine-2,5-dione] and T-686 [i.e., 3(*E*)-
30 Benzylidene-4(*E*)-(3,4,5-trimethoxybenzylidene)pyrrolidine-2,5-dione], inhibitors of α -2-antiplasmin such as anti- α -2-antiplasmin antibody and thromboxane receptor antagonists (such as ifetroban), prostacyclin mimetics, phosphodiesterase (PDE) inhibitors, such as dipyridamole (Persantine®) or cilostazol, PDE inhibitors in combination with thromboxane receptor antagonists/thromboxane A synthetase inhibitors (such as picotamide), serotonin-2-receptor
35 antagonists (such as ketanserin), fibrinogen receptor antagonists, hypolipidemic agents, such as

HMG-CoA reductase inhibitors, e.g., pravastatin, simvastatin, atorvastatin, fluvastatin, cerivastatin, AZ4522, and itavastatin (Nissan/Kowa); microsomal triglyceride transport protein inhibitors (such as disclosed in U.S. Pat. Nos. 5,739,135, 5,712,279 and 5,760,246), antihypertensive agents such as angiotensin-converting enzyme inhibitors (e.g., captopril, lisinopril or fosinopril); angiotensin-II receptor antagonists (e.g., irbesartan, losartan or valsartan); and/or ACE/NEP inhibitors (e.g., omapatrilat and gemopatrilat); β -blockers (such as propranolol, nadolol and carvedilol), PDE inhibitors in combination with aspirin, ifetroban, picotamide, ketanserin, or clopidogrel (Plavix®) and the like.

[00192] The solvates described herein can also be used in combination with anti-arrhythmic agents such as for atrial fibrillation, for example, amiodarone or dofetilide.

[00193] The solvates described herein can also be used in combination with Cholesteryl ester transfer protein (CETP) inhibitors for dyslipidemia and atherosclerosis, Niacin or niacin analogs for dyslipidemia and atherosclerosis, Adenosine or adenosine analogs for vasodilation, Nitroglycerin or nitrates for vasodilation.

[00194] The solvates described herein can be used in combination with prothrombolytic agents, such as tissue plasminogen activator (natural or recombinant), streptokinase, reteplase, activase, lanoteplase, urokinase, prourokinase, anisolated streptokinase plasminogen activator complex (ASPAC), animal salivary gland plasminogen activators, and the like. The solvates described herein may also be used in combination with β -adrenergic agonists such as albuterol, terbutaline, formoterol, salmeterol, bitolterol, pirbuterol, or fenoterol; anticholinergics such as ipratropium bromide; anti-inflammatory corticosteroids such as beclomethasone, triamcinolone, budesonide, fluticasone, flunisolide or dexamethasone; and anti-inflammatory agents such as cromolyn, nedocromil, theophylline, zileuton, zafirlukast, montelukast and pranleukast.

[00195] Suitable pharmaceutical agents that can be used in combination with solvates described herein include antiretrovirals [see, e.g., Turpin, *Expert Rev Anti Infect Ther* (2003) 1:97-128]. Some embodiments include methods of treatment of progressive multifocal leukoencephalopathy as described herein comprising administering to an individual in need of such treatment a therapeutically effective amount or dose of at least one solvate described herein in combination with at least one pharmaceutical agent selected from nucleoside reverse transcriptase inhibitors (for example, Retrovir®, Epivir®, Combivir®, Hivid®, Videx®, Trizvir®, Zerit®, Ziagen®, Vireo®, Emtricitabine, DAPD, and the like), non-nucleoside reverse transcriptase inhibitors (for example, Viramune®, Rescriptor®, Sustiva®, GW687, DPC083, TMC 125, Emivirine, Capravirine, BMS 561390, UC-781 and other oxathiin carboxyanilides, SJ-3366, Alkenyldiarylmethane (ADAM), Tivirapine, Calanolide A, HBY097, Loviride, HEPT Family

Derivatives, TIBO Derivatives, and the like), protease inhibitors (for example, Fortovase[®], Invirase[®], Novir[®], Crixivan[®], Viracep[®], Agceberasc[®], Kaletra[®], Atazanavir, Tipranavir, DMP450, and the like), inhibitors of HIV-cell interaction (for example, soluble CD4, toxin-conjugated CD4, monoclonal antibodies to CD4 or gp120, PRO 542, dextran sulfate, Rersobene, FP-23199, 5 Cyanovirin-N, Zintevir (T30177, AR177), L-chicoric acid and derivatives, and the like), coreceptor inhibitors ligands (for example, R5, X4, modified ligands (R5), modified ligands (X4), and the like), coreceptor inhibitors X4 (for example, T22, T134, ALX40-4C, AMD3100, bicyclam derivatives, and the like), coreceptor inhibitors R5 (for example, TAK-779, SCH-C (SCH-351125), SCH-D (SCH-350634), NSC 651016, ONO Pharmaceutical, Merck, and the like), 10 fusion inhibitors (for example, Fuzeon[®] (T-20, DP 178, enfuvritide) trimeris, T-1249, TMC125, and the like), integrase inhibitors (for example, SCITEP, L731,988, L708,906, L-870,812, S-1360, and the like), NCp7 nucleocapsid Zn finger inhibitors (for example, NOBA, DIBA, dithianes, PD-161374, pyridinioalkanoyl thioesters (PATES), azodicarbonamide (ADA), cyclic 2,2 dithio bisbenzamide, and the like), RNase H inhibitors (for example, BBHN, CPHM PD-26388, and the 15 like), Tat inhibitors (for example, dominant negative mutants, Ro24-7429, Ro5-3335, and the like), Rev inhibitors (for example, dominant negative mutants, Leptomycin B, PKF050-638, and the like), transcriptional inhibitors (for example, Temacrazinc, K-12 and K-37, EM2487, and the like), inhibitors of HIV assembly/maturation (for example, CAP-1 and CAP-2, and the like), and pharmaceutical agents directed to cellular anti-HIV targets (for example, LB6-B275 and 20 HRM1275, Cdk9 inhibitors, and the like).

[00196] In a certain embodiment, a solvates described herein can be used in conjunction with highly active antiretroviral therapy (HAART). When antiretroviral drugs are used in combinations of three or four drugs, this treatment is called HAART [see, e.g., Portegies, et al., *Eur. J. Neurol.* (2004) 11:297-304].

25 [00197] In some embodiment, the combination of a solvate described herein and pharmaceutical agent can be prepared by mixing the respective active components either all together or independently with a pharmaceutically acceptable carrier, excipient, binder, diluent, etc. as described herein, and administering the mixture or mixtures either orally or non-orally as a pharmaceutical composition(s). When a solvate described herein is administered as a combination 30 therapy with another active compound each can be formulated as separate pharmaceutical compositions given at the same time or at different times. Alternatively, in some embodiments, pharmaceutical compositions comprise at least one solvate described herein and the pharmaceutical agent(s) as a single pharmaceutical composition.

[00198] In some embodiments, a method of producing a pharmaceutical composition for 35 "combination-therapy" comprises admixing at least one solvate described herein, together with at

least one known pharmaceutical agent as described herein and a pharmaceutically acceptable carrier.

[00199] It is noted that when the 5-HT_{2A} receptor modulators are utilized as active ingredients in a pharmaceutical composition, these are not intended for use only in humans, but in other non-human mammals as well. Indeed, recent advances in the area of animal health-care mandate that consideration be given for the use of active agents, such as 5-HT_{2A} receptor modulators, for the treatment of a 5-HT_{2A} mediated disease or disorder in domestic animals (e.g., cats and dogs) and in other livestock animals (e.g., such as cows, chickens, fish, etc.). Those of ordinary skill in the art are readily credited with understanding the utility of such solvates described herein in such settings.

[00200] For the avoidance of any doubt, it is understood that those embodiments which refer to methods of treatment or other uses of the solvates described herein further embrace pharmaceutical compositions comprising the solvates described herein, as well as combinations of other pharmaceutical agents with solvates and pharmaceutical compositions described herein.

[00201] The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results.

20

EXAMPLES

EXAMPLE 1

4-(2-(4-(3-Methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium chloride

[00202] Anhydrous 4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium chloride can be prepared as described in WO 2007/136689.

[00203] A slurry of anhydrous 4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium chloride in water was stirred at ambient/stir plate temperature (about 26-28° C) for ~3 days. The anhydrous form was recovered.

EXAMPLE 2

3-Methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate

[00204] 100 mg of 4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium chloride were added to 3 mL of H₂O in a 4 mL vial. The solution was stirred until it became clear. 225 µL of 1 N NaOH was added to the vial to reach a

pH of ~8. A white precipitate formed. The solution was stirred for 1 hour at room temperature. The crystalline materials formed on the side of the vial were scraped off, and the solution was filtered using centrifuge tube filters. The material was dried overnight in the hood.

[00205] The sample was characterized by an X'Pert PRO MPD powder diffractometer (PANalytical, Inc.; EQ0233) with a Cu source set at 45 kV and 40 mA, Cu(K α) radiation and an X'Celerator detector. Briefly, samples were added to a sample holder and smoothed flat with a spatula and weigh paper. While the samples were spinning, X-ray diffractograms were obtained by a 12-minute scan over the (2 θ) range 5-40°.

[00206] Figure 1 shows the PXRD pattern of 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate and Table 1 above shows a list of peaks of PXRD pattern of 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate collected by X'Pert HighScore software.

[00207] Differential scanning calorimetry (DSC) studies were conducted using a TA Instruments, Q2000 (EQ0090 or EQ1980) at heating rate 10°C/min from ~25° C to ~270° C. The instruments were calibrated by the vendor for temperature and energy using the melting point and enthalpy of fusion of an indium standard. Thermal events (desolvation, melting, etc.) were evaluated using Universal Analysis 2000 software, version 4.1D, Build 4.1.0.16.

[00208] Thermogravimetric analyses (TGA) were conducted using a TA Instruments TGA Q500 (EQ0089) or Q5000 (EQ1982) at heating rate 10°C/min. The instruments were calibrated by the vendor for temperature using Alumel and Nickel standards (Curie points) and for weight using a standard weight. Thermal events such as weight-loss are calculated using the Universal Analysis 2000 software, version 4.1D, Build 4.1.0.16.

[00209] The DSC and TGA plots of 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate are shown in Figure 2. The TGA thermogram shows that the onset of weight loss event occurred at ~41° C at 10° C/min. The weight loss of 3.8% as shown on the TGA thermogram suggests about one mole of H₂O was loss (theoretical value is 4.0%). The DSC thermogram shows a broad endotherm peak at between 50 ~ 90° C that is consistent with the loss of H₂O from dehydration. The DSC thermogram also shows an apparent recrystallization exotherm after the dehydration, and a melting endotherm with an onset of ~105° C.

EXAMPLE 3

(4-(2-(4-(3-Methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium Carboxyformate Ethanol Solvate

[00210] To a 1-liter jacketed reactor equipped with an overhead stirrer, nitrogen inlet and bottom drain valve was charged, N-(4-hydroxy-3-(1-methyl-1*H*-pyrazol-5-yl)phenyl-3-

methoxybenzamide (35.0 g, 108 mmol), ethyl hydroxymorpholine (29.8 g, 227 mmol) and triphenylphosphine (59.6 g, 227 mmol). The reaction was cooled to -8 °C (jacket) and diisopropyl azodicarboxylate (46.0 g, 227 mmol) was added at -6 to 5 °C, over 30 min. The reaction was then allowed to warm to 25 °C and stirred for approximately 3 days. The reaction was cooled again to -5 °C and ethyl hydroxymorpholine (0.1 eq.), triphenylphosphine (0.1 eq.) and diisopropyl azodicarboxylate (0.1 eq.) were added. After 25 min, the reaction was warmed to 25 °C. The reaction mixture was divided in seven aliquots, approximately equal by weight. One such aliquot was concentrated, the residue was taken up in ethanol (95%) and oxalic acid (1.46 g, 0.5 eq.) was added. The mixture was stirred overnight at 20-25 °C during which time a precipitate formed. The mixture was filtered at room temperature for 1.5 h and the solid was dried to give the title compound (5.49 g).

[00211] Representative DSC and TGA plots of (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate are shown in Figure 3. The TGA thermogram of (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate shows two primary weight loss events occurred prior to the degradation of the free base. The first weight loss event is consistent with the loss of approximately 0.59 moles of ethanol; the second weight loss event suggests about 0.5 moles of oxalic acid was lost.

[00212] A proton NMR of (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium carboxyformate ethanol solvate is shown in Figure 4.

[00213] Those skilled in the art will recognize that various modifications, additions, substitutions, and variations to the illustrative examples set forth herein can be made without departing from the spirit of the invention and are, therefore, considered within the scope of the invention.

What is claimed is:

1. A solvate of 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide or a pharmaceutically acceptable salt thereof that is 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide hydrate.
5
2. A solvate of 3-methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide or a pharmaceutically acceptable salt thereof that is (4-(2-(4-(3-methoxybenzamido)-2-(1-methyl-1*H*-pyrazol-5-yl)phenoxy)ethyl)morpholin-4-ium
10 carboxyformate ethanol solvate.
3. A pharmaceutical composition comprising at least one solvate of claim 1 and at least one pharmaceutically acceptable carrier.
- 15 4. A pharmaceutical composition comprising at least one solvate of claim 2 and at least one pharmaceutically acceptable carrier.
5. A method of treating a 5HT_{2A}-related disorder comprising administering to a patient in need thereof a therapeutically effective amount of at least one solvate of claim 1 or claim 2.
20
6. The method according to claim 5 wherein the 5HT_{2A}-related disorder is selected from platelet aggregation, coronary artery disease, myocardial infarction, transient ischemic attack, angina, stroke, atrial fibrillation, blood clot formation, asthma or symptoms thereof, agitation or a symptom thereof, behavioral disorders, drug induced psychosis, excitative psychosis, Gilles de la
25 Tourette's syndrome, manic disorder, organic or NOS psychosis, psychotic disorder, psychosis, acute schizophrenia, chronic schizophrenia, NOS schizophrenia and related disorders, sleep disorders, diabetic-related disorders and progressive multifocal leukoencephalopathy.
7. The method according to claim 6 wherein the 5HT_{2A}-related disorder is a condition
30 associated with platelet aggregation.
8. A method of treating a 5HT_{2A}-related disorder comprising administering to a patient in need thereof a therapeutically effective amount of a pharmaceutical composition of claim 3 or 4.

9. The method according to claim 8 wherein the 5HT_{2A}-related disorder is selected from platelet aggregation, coronary artery disease, myocardial infarction, transient ischemic attack, angina, stroke, atrial fibrillation, blood clot formation, asthma or symptoms thereof, agitation or a symptom thereof, behavioral disorders, drug induced psychosis, excitative psychosis, Gilles de la
5 Tourette's syndrome, manic disorder, organic or NOS psychosis, psychotic disorder, psychosis, acute schizophrenia, chronic schizophrenia, NOS schizophrenia and related disorders, sleep disorders, diabetic-related disorders and progressive multifocal leukoencephalopathy.
10. The method according to claim 9 wherein the 5HT_{2A}-related disorder is a condition
10 associated with platelet aggregation.
11. The solvate of claim 1 or 2 for use in a method of treatment of the human or animal body by therapy.
- 15 12. The pharmaceutical composition of claim 3 or 4 for use in a method of treatment of the human or animal body by therapy.
13. The solvate of claim 1 or 2 for use in a method of treatment of a 5HT_{2A}-related disorder.
- 20 14. The solvate of claim 1 or 2 for use in a method of treatment of platelet aggregation, coronary artery disease, myocardial infarction, transient ischemic attack, angina, stroke, atrial fibrillation, blood clot formation, asthma or symptoms thereof, agitation or a symptom thereof, behavioral disorders, drug induced psychosis, excitative psychosis, Gilles de la Tourette's syndrome, manic disorder, organic or NOS psychosis, psychotic disorder, psychosis, acute
25 schizophrenia, chronic schizophrenia, NOS schizophrenia and related disorders, sleep disorders, diabetic-related disorders or progressive multifocal leukoencephalopathy.
15. The solvate of claim 1 or 2 for use in a method for treating a condition associated with platelet aggregation.
30
16. The pharmaceutical composition of claim 3 or 4 for use in a method of treatment of a 5HT_{2A}-related disorder.
17. The pharmaceutical composition of claim 3 or 4 for use in a method of treatment of
35 platelet aggregation, coronary artery disease, myocardial infarction, transient ischemic attack,

angina, stroke, atrial fibrillation, blood clot formation, asthma or symptoms thereof, agitation or a symptom thereof, behavioral disorders, drug induced psychosis, excitative psychosis, Gilles de la Tourette's syndrome, manic disorder, organic or NOS psychosis, psychotic disorder, psychosis, acute schizophrenia, chronic schizophrenia, NOS schizophrenia and related disorders, sleep disorders, diabetic-related disorders or progressive multifocal leukoencephalopathy.

18. The pharmaceutical composition of claim 3 or 4 for use in a method for treating a condition associated with platelet aggregation.

19. Use of the solvate of claim 1 or 2 for the manufacture of a medicament for treating a 5HT_{2A}-related disorder.

20. The use according to claim 19, wherein said 5HT_{2A}-related disorder is selected from platelet aggregation, coronary artery disease, myocardial infarction, transient ischemic attack, angina, stroke, atrial fibrillation, blood clot formation, asthma or symptoms thereof, agitation or a symptom thereof, behavioral disorders, drug induced psychosis, excitative psychosis, Gilles de la Tourette's syndrome, manic disorder, organic or NOS psychosis, psychotic disorder, psychosis, acute schizophrenia, chronic schizophrenia, NOS schizophrenia and related disorders, sleep disorders, diabetic-related disorders and progressive multifocal leukoencephalopathy.

21. The use according to claim 20, wherein said 5HT_{2A}-related disorder is a condition associated with platelet aggregation.

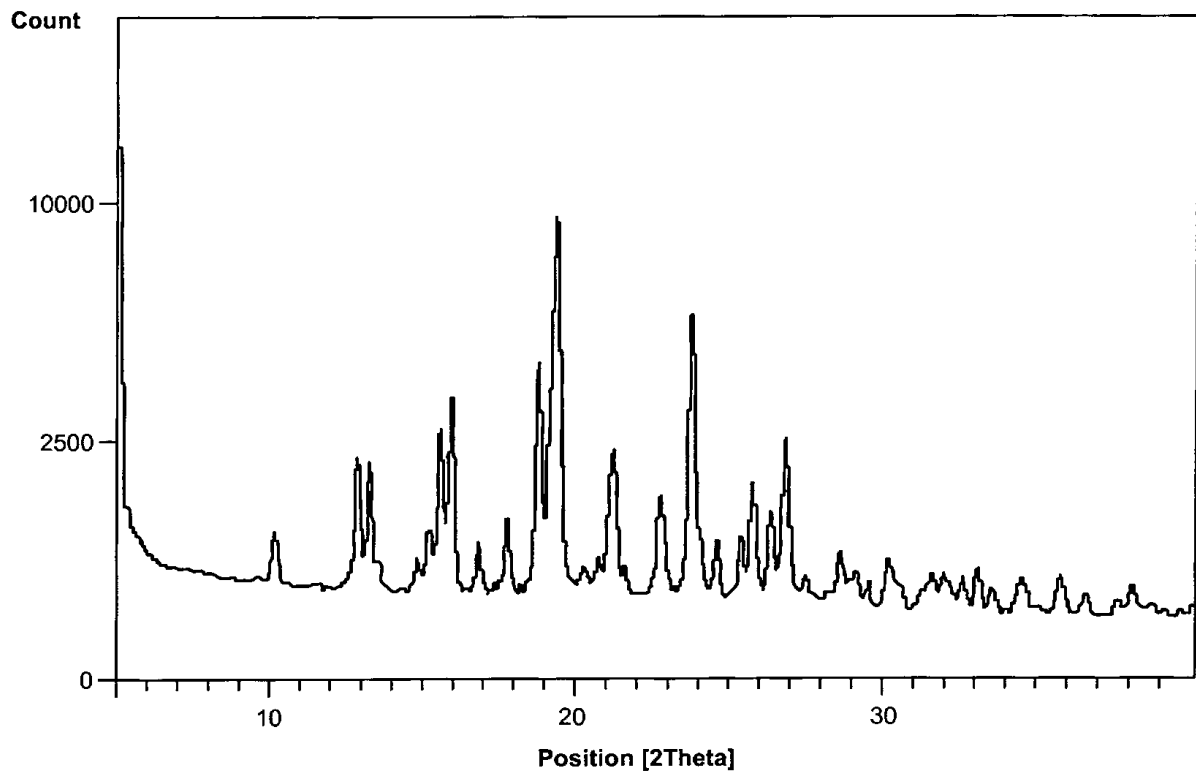


FIG. 1

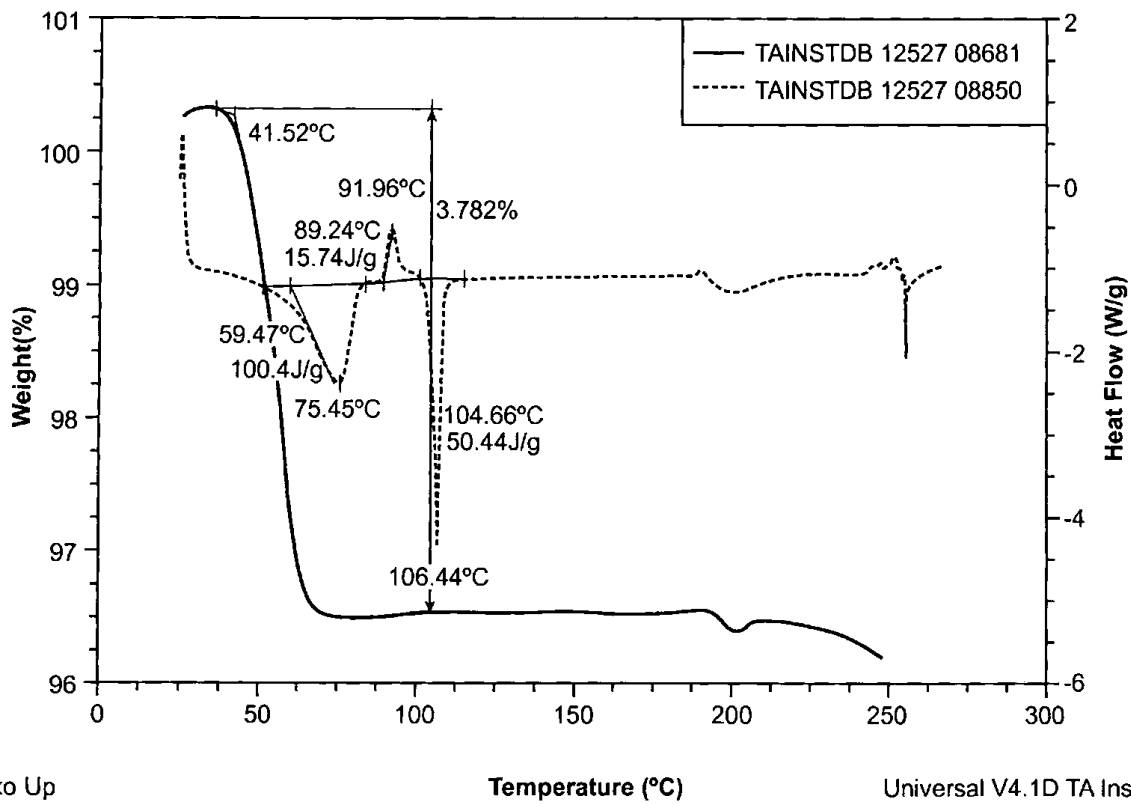
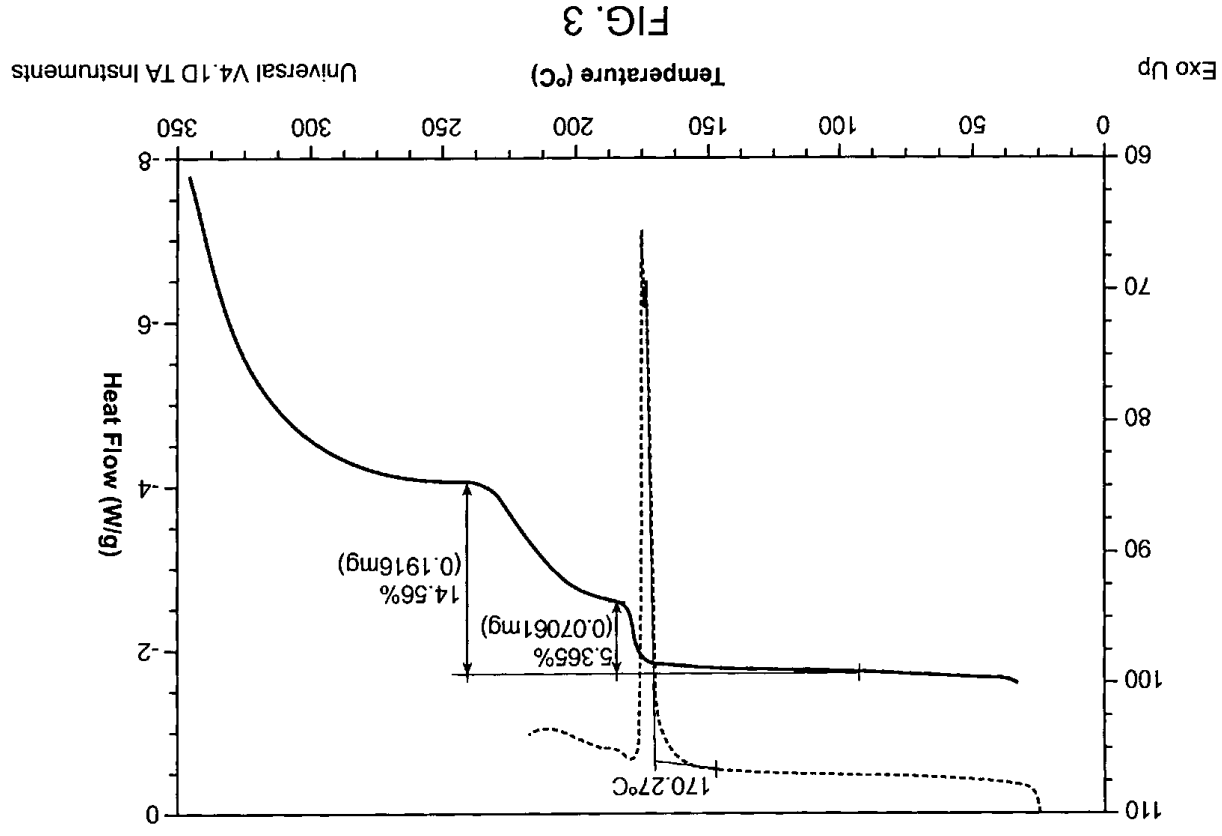


FIG. 2



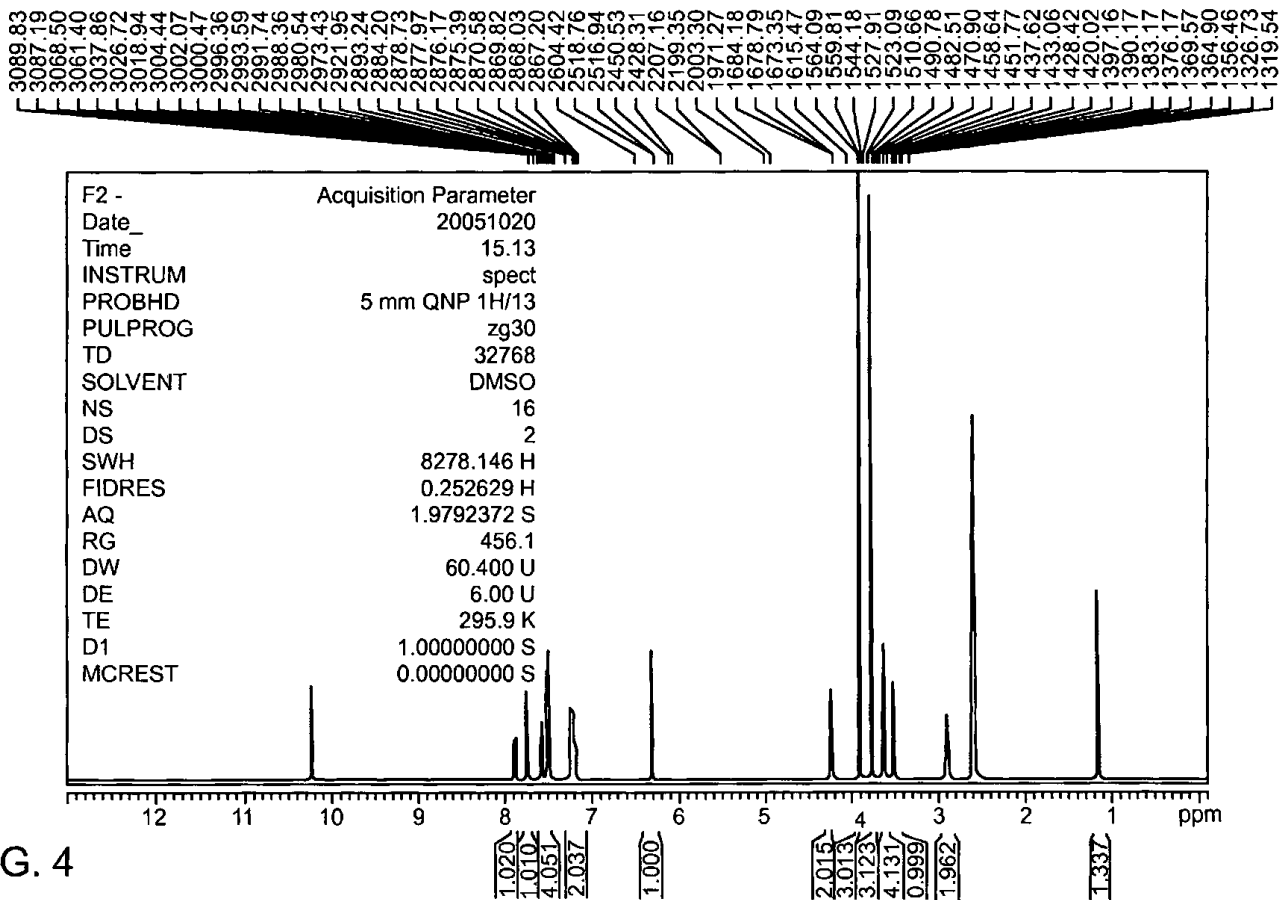


FIG. 4

Discovery and Structure–Activity Relationship of 3-Methoxy-*N*-(3-(1-methyl-1*H*-pyrazol-5-yl)-4-(2-morpholinoethoxy)phenyl)benzamide (APD791): A Highly Selective 5-Hydroxytryptamine_{2A} Receptor Inverse Agonist for the Treatment of Arterial Thrombosis

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Serotonin, which is stored in platelets and is released during thrombosis, activates platelets via the 5-HT_{2A} receptor. 5-HT_{2A} receptor inverse agonists thus represent a potential new class of antithrombotic agents. Our medicinal program began with known compounds that displayed binding affinity for the recombinant 5-HT_{2A} receptor, but which had poor activity when tested in human plasma platelet inhibition assays. We herein describe a series of phenyl pyrazole inverse agonists optimized for selectivity, aqueous solubility, antiplatelet activity, low hERG activity, and good pharmacokinetic properties, resulting in the discovery of **10k** (APD791). **10k** inhibited serotonin-amplified human platelet aggregation with an IC₅₀ = 8.7 nM and had negligible binding affinity for the closely related 5-HT_{2B} and 5-HT_{2C} receptors. **10k** was orally bioavailable in rats, dogs, and monkeys and had an acceptable safety profile. As a result, **10k** was selected for further evaluation and advanced into clinical development as a potential treatment for arterial thrombosis.

Introduction

Arterial thrombosis is the formation of a blood clot or thrombus inside an artery or arteriole that restricts or blocks the flow of blood and, depending upon location, can result in acute coronary syndrome or stroke. The formation of a thrombus is usually initiated by blood vessel injury, which triggers platelet aggregation and adhesion of platelets to the vessel wall. Treatments aimed at inhibiting platelet aggregation have demonstrated clear clinical benefits in the setting of acute coronary syndrome and stroke. Current antiplatelet therapies include aspirin, which irreversibly inhibits cyclooxygenase (COX) and results in reduced thromboxane production, clopidogrel and prasugrel, which inhibit platelet adenosine diphosphate (ADP) P₂Y₁₂ receptors, and platelet glycoprotein IIb/IIIa receptor antagonists. Another class of antiplatelet drugs, protease-activated thrombin receptor (PAR-1) antagonists, are also being evaluated in the clinic for the treatment of acute coronary syndrome.^{1a} The most advanced candidate in this class, *N*-[(1*R*,3*aR*,4*aR*,6*R*,8*aR*,9*S*,9*aS*)-9-{2-[5-(3-fluorophenyl)pyridin-2-yl]vinyl}-1-methyl-3-oxoperhydro-naphtho-[2,3-*c*]furan-6-yl]-carbamate ethyl ester (SCH-530348), is currently in phase 3 trials for the prevention of arterial thrombosis.^{1b,c}

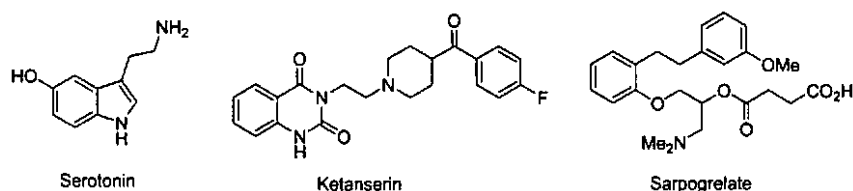
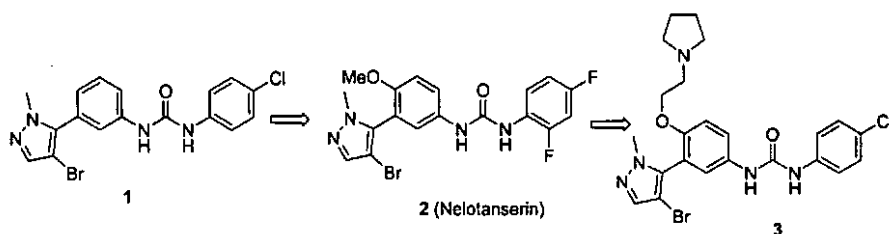
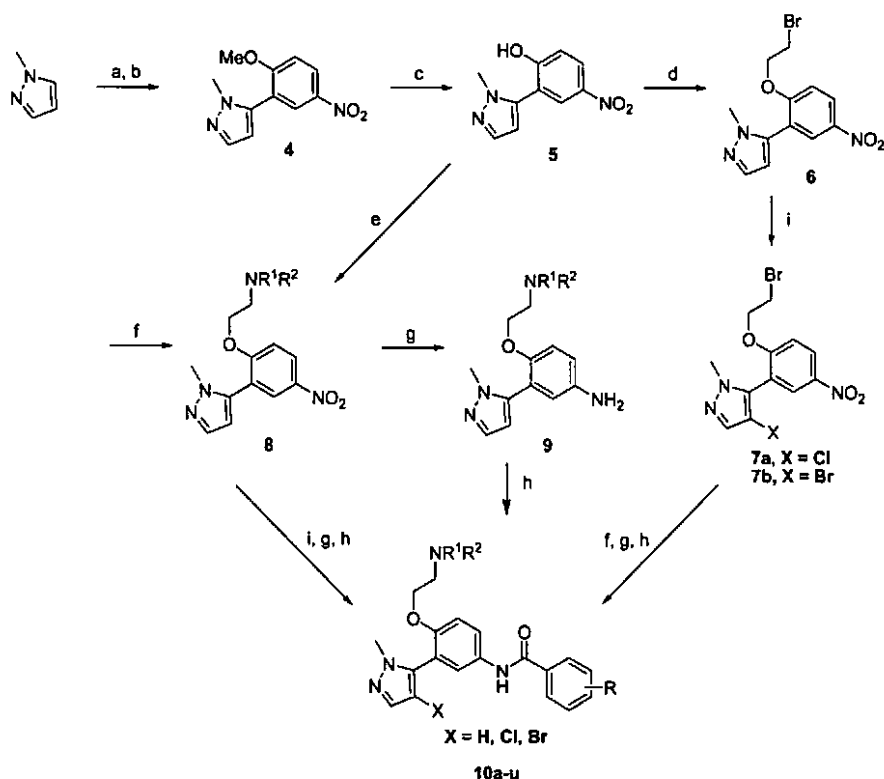
The 5-HT_{2A} receptor is one of 15 different serotonin receptor subtypes.² In the cardiovascular system, modulation of 5-HT_{2A} receptors on vascular smooth muscle cells and platelets is thought to play an important role in the regulation of cardiovascular function.^{3,4} Platelets are activated by a variety of agonists such as ADP, thrombin, thromboxane, serotonin, epinephrine, and collagen. Upon platelet activation at the site of blood vessel injury, a number of factors including serotonin (5-HT) are released. Although by itself serotonin is a weak activator of platelet aggregation, in vitro it can amplify aggregation induced by other agonists as mentioned above.^{5,6} Therefore, serotonin released from activated platelets may induce further platelet aggregation and enhance thrombosis.

The 5-HT_{2A} receptor antagonist ketanserin (Figure 1) was shown in clinical studies to reduce early restenosis⁷ and decrease myocardial ischemia during coronary balloon angioplasty.⁸ However, in another study, ketanserin did not significantly improve clinical outcomes, and the rate of adverse events was higher than that observed in the control group.⁹ Some of the adverse events reported in the latter study could be specific to ketanserin and resulted from its lack of 5-HT_{2A} receptor selectivity. Other 5-HT_{2A} antagonists with improved selectivity profiles have shown promise in clinical studies. For example, sarpgrelate (Figure 1) was shown to inhibit restenosis following coronary stenting.¹⁰

Because the 5-HT_{2A} receptor is expressed both in peripheral tissues and in the central nervous system (CNS), compounds with limited CNS partitioning would be preferred to maximize cardiovascular and blood platelet pharmacological activity while minimizing CNS effects. In addition, because 5-HT_{2A}

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^aAbbreviations: COX, cyclooxygenase; ADP, adenosine diphosphate; SAR, structure–activity relationship; hERG, human ether-a-go-go-related gene; CNS, central nervous system; 5-HT, serotonin; AUC, area under the plasma concentration time curve, iv, intravenous; IP, inositol phosphate.

Figure 1. Serotonin and known 5-HT_{2A} receptor antagonists.Figure 2. Phenyl-pyrazole urea based 5-HT_{2A} receptor inverse agonists.Scheme 1. Synthesis of Phenyl-Pyrazole Amide Derivatives^a

^a Reagents and conditions: (a) *n*-BuLi, THF at -78°C , then ZnCl_2 ; (b) 2-iodo-4-nitroanisole, $\text{Pd}(\text{PPh}_3)_4$; (c) AlCl_3 , 1,2-dichloroethane; (d) 1,2-dibromoethane, K_2CO_3 , DMSO; (e) $\text{HOCH}_2\text{CH}_2\text{NR}^1\text{R}^2$, Ph_3P , DIAD, THF; (f) HNR^1R^2 , K_2CO_3 , DMF; (g) zinc dust, NH_4Cl , THF; (h) $\text{R-PhCO}_2\text{H}$, HATU, triethylamine, DMF or R-Ph-COCl , triethylamine, THF; (i) NBS or NCS, MeOH or DMF.

receptor inverse agonists are thought to reduce thrombus formation via inhibition of serotonin-mediated amplification of platelet aggregation without inhibiting agonist driven aggregation per se, it is possible that this class of inhibitors will have an improved bleeding risk side effect profile compared to what has been observed with other classes of antithrombotic drugs.

We have previously identified a series of phenyl-pyrazole urea-based 5-HT_{2A} inverse agonists (e.g., compound 1, Figure 2).¹¹ This structure–activity relationship (SAR) effort led to the discovery of 2 (Nelotanserin, Figure 2), which was advanced into clinical trials for the treatment of insomnia.¹²

Although such ureas are potent 5-HT_{2A} inverse agonists, they were found to possess limited aqueous solubility, a property that we thought would be important for good antiplatelet activity. We have since reported on modifications to this urea series in which we incorporated amino moieties into the scaffold. The resulting series of phenyl-pyrazole ureas as exemplified by 3^{13–15} were significantly more soluble in aqueous solution. Moreover these compounds also showed improved inhibition of platelet aggregation.¹⁴ Unfortunately, many of these solubilized ureas had suboptimal selectivity profiles and poor pharmacokinetic properties. To further explore the scope of the soluble phenyl-pyrazole scaffold, with a particular goal

of improving the pharmacokinetic and pharmaceutical characteristics, the SAR was expanded from ureas to sulfonamides, carbamates, and amides. Herein, we describe SAR efforts that led to the identification of **10k** (APD791), a highly selective 5-HT_{2A} inverse agonist with high receptor binding affinity, potent activity on human platelets, a clean safety profile, and outstanding pharmaceutical properties.

Chemistry

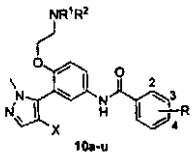
The syntheses of the 1-*N*-alkyl-5-phenyl-pyrazole scaffold **4**, its subsequent modification to introduce a basic amine moiety to provide intermediates **8**, and formation of final amides **10** are outlined in Scheme 1. Starting with commercially available *N*-methyl pyrazole, lithiation with *n*-BuLi followed by a Negishi coupling with 2-iodo-4-nitroanisole furnished intermediate **4**.¹³ Demethylation with aluminum trichloride afforded phenol **5**. Reaction with 1,2-dibromoethane gave the bromo-tethered compound **6**. Alkylation of **6** with a series of amines introduced basic functionalities into the molecules providing **8**. Alternatively, **8** could also be prepared via Mitsunobu reaction from phenol **5** and the appropriate amino-ethanol derivatives. Reduction of the nitro group with zinc dust afforded anilines **9**, which were then coupled with acids or acid chlorides to furnish the desired amides **10**.¹³ Halogenation of the pyrazole ring, where appropriate, could be effected at different stages. Bromo-tethered analogue **6** was treated with either *N*-chlorosuccinimide (NCS) or *N*-bromosuccinimide (NBS) to form chloro or bromo pyrazoles **7a** and **7b**, respectively. Amination, reduction, and amidation of **7** led to the final products **10**. Alternatively, halogens could be introduced after the amine moiety was incorporated from **8**, and thus halogenation, subsequent reduction, and amidation of **8** provided an alternative approach to final products **10**.

Results and Discussion

In vitro competitive binding assays with radiolabeled 2,5-dimethoxy-4-iodoamphetamine (¹²⁵I-DOI) were used to determine *K_i* values for all compounds against recombinant h5-HT_{2A} and h5-HT_{2C} receptors (Table 1). Compounds were also tested for inhibition of serotonin-induced amplification of ADP-stimulated platelet aggregation in human platelet rich plasma, and in selected cases, for human ether-a-go-go-related gene (hERG) inhibition in a patch clamp assay (Table 1). As previously observed in the phenyl-pyrazole urea series, the phenyl-pyrazole amides showed excellent affinity for the 5-HT_{2A} receptor. In addition, and in contrast to the urea series,¹⁴ the amides generally showed a very high degree of selectivity for the 5-HT_{2A} receptor over the 5-HT_{2C} receptor. In all the examples tested, the selectivity over the 5-HT_{2B} receptor was uniformly high and thus affinity for this receptor was not examined on a routine basis.

A wide range of monosubstitutions on the phenyl ring was evaluated, all well tolerated regardless of the electron withdrawing or donating groups. The *meta*-substituted analogues were found to be higher affinity ligands for the 5-HT_{2A} receptor than the *para*-substituted analogues. For example, the *meta*-F analogue **10e** and *meta*-CF₃ analogue **10i** had slightly higher affinity than the corresponding *para*-F analogue **10f** and *para*-CF₃ analogue **10h**. Furthermore, the *meta*-substituted analogues were more selective for the 5-HT_{2A} receptor than the *para*-substituted analogues. For instance, **10b** (*meta*-CF₃) had lower affinity at the 5-HT_{2C} receptor and slightly higher affinity at the 5-HT_{2A} receptor than **10a** (*para*-CF₃),

Table 1. Binding Affinities of Compounds **10a–u** at Human 5-HT_{2A} and 5-HT_{2C} Receptors, Activity of Inhibition of Serotonin Mediated Amplification of ADP-Stimulated Human Platelet Aggregation and Inhibition of hERG Channel



Comp.	NR ¹ R ²	X	R	5-HT _{2A} <i>K_i</i> ^a (nM)	5-HT _{2C} <i>K_i</i> ^a (nM)	Inhibition of Platelet Aggregation IC ₅₀ (nM) (n)	hERG ^b (% Inh.) (3 μM)
10a		H	4-CF ₃	1.9	225	2.0 (3)	66 ^c
10b		H	3-CF ₃	0.6	1140	3.2 (1)	80 ^c
10c		H	3-CF ₃	12	9250	189 (1)	50
10d		H	2,4-di-F	8.6	>10000	27 (4)	16 ^{c,e}
10e		H	3-F	5.1	>10000	86 (4)	43
10f		H	4-F	18	>10000	100 (1)	<i>d</i>
10g		Cl	3-F	0.6	3380	22 (2)	46
10h		H	4-CF ₃	2.4	5900	61 (8)	33 ^c
10i		H	3-CF ₃	0.7	>10000	18 (6)	50 ^{d,f}
10j		H	3,4-di-F	4.1	>10000	29 (3)	56
10k		H	3-OMe	4.9	>10000	8.7 (3)	32 ^d
10l		H	3-CP ₃	287	>10000	<i>d</i>	<i>d</i>
10m		Cl	3-CF ₃	5.3	>10000	127 (1)	53
10n		H	3-CF ₃	4.3	5614	145 (1)	75
10o		H	3-F	15	>10000	40 (5)	41 ^b
10p		Cl	3-F	2.2	8600	70 (1)	43
10q		H	3-OMe	4.5	>10000	77 (5)	24
10r		H	3-OMe	5.1	5890	57 (1)	17
10s		H	3-OMe	28	>10000	<i>d</i>	23
10t		H	3-OMe	1.8	5874	5.8 (3)	36
10u		H	3-OMe	2.2	2347	6.9 (3)	22

^a *K_i* values are the mean of at least three experiments performed in triplicate, determined from 10 concentrations, all *K_i* values were calculated from IC₅₀ values using the method of Cheng and Prusoff²² with standard deviation < 0.4 log units. ^b hERG patch clamp assays were performed at Aviva Biosciences, San Diego, CA (<http://www.avivabio.com>). ^c Measured at 1 μM concentration. ^d Not determined. ^e IC₅₀ = 4.1 μM. ^f IC₅₀ = 0.9 μM. ^g IC₅₀ = 11 μM. ^h IC₅₀ = 4.1 μM.

making **10b** much more selective at the 5-HT_{2A} receptor (1900-fold) than **10a** (118-fold). Therefore, during lead optimization at other positions in the molecules (pyrazole halogenations and incorporation of amino functionalities), we focused more heavily on the *meta*- than *para*-substituted analogues. Among the different *meta*-substituents, lipophilic

CF₃ groups significantly enhanced the 5-HT_{2A} receptor affinity. For example, **10i** (*meta*-CF₃) had 6- to 7-fold higher affinity than **10k** (*meta*-OMe) or **10e** (*meta*-F) analogues.

The effect of pyrazole halogenation on receptor affinity was also examined. In general, bromination or chlorination of the pyrazole ring led to increased affinity for the 5-HT_{2A} receptor when compared directly to the nonhalogenated pyrazoles. For example, chlorination of **10l** provided **10m**, which showed a more than 50-fold increase in the 5-HT_{2A} receptor affinity. In another example, addition of a chlorine to **10e** resulted in **10g**, which showed a 8-fold improvement in 5-HT_{2A} receptor affinity.

A wide range of amino substituents was also evaluated. As shown in Table 1, almost all amino groups provided compounds with good binding affinity at the 5-HT_{2A} receptor with the exception of the oxo-piperazinyl group (**10l**), which was 20- to 480-fold weaker compared to other amino groups with the same phenyl ring and pyrazole ring substitutions. As discussed earlier, binding affinity with this amino group was improved by introducing a chlorine atom onto the pyrazole (**10m**). Whereas all amino groups showed good (e.g., 118-fold, **10a**) to excellent (e.g., 2040-fold, **10k**) selectivity for the 5-HT_{2A} receptor vs the 5-HT_{2C} receptor, a morpholino group appeared to be most beneficial, showing very little affinity for the 5-HT_{2C} receptor even with halogen substitutions on the pyrazole.

On the basis of their structural similarity to our previous series of 5-HT_{2A} receptor ligands (including **2**), which were all functional inverse agonists,¹² we expected the compounds in these current series to have the same functional response in vitro. In this current investigation, we confirmed this expectation for selected compounds using inhibition of total inositol phosphate (IP) accumulation as a functional assay to determine the inverse agonist potency.¹⁹ For example, **10b** had an IC₅₀ of 23 nM in the IP assay, and **10k** inhibited IP accumulation with an IC₅₀ of 5.2 nM.

After this initial examination of their receptor binding affinities and selectivity profile, compounds were then evaluated in a second functional (antagonist) assay for their ability to inhibit serotonin-mediated amplification of ADP-stimulated human platelet aggregation. As shown in Table 1, IC₅₀ values for inhibition of platelet aggregation ranged from 2 to 189 nM. In general, platelet activity did not correlate well with binding affinity, but compounds were usually somewhat less potent in the platelet inhibition assay than in the binding assay. The degree of difference between the *K_i* value in binding and the IC₅₀ value in the platelet assay varied from 1- to 2-fold (**10a** and **10k**) to 30-fold (**10n**) and depended on compound structure. Interestingly, removal of serum proteins from platelet rich plasma resulted in a left shift in the IC₅₀ for inhibition of platelet aggregation, suggesting that protein binding may play a significant role in the observed differences between platelet aggregation activity and receptor affinity. Hence, the platelet assay served as a useful guide to receptor activity in the presence of serum proteins, and only compounds with a modest shift between the values for the two assays were considered for further evaluation.

In general, compounds containing pyrrolidino and morpholino moieties had smaller shifts compared to compounds containing other basic amines. For example, **10j** and **10k** had same receptor affinity as **10m** and **10n**, but **10j** and **10k**, which had a morpholino moiety, are much more potent than **10m** or **10n** in the platelet assay. Halogen substitutions of the pyrazole usually increased binding affinities but not necessarily platelet

activities, as exemplified by **10p** and **10o**. Although, in another example, pyrazole chlorination was more successful, **10g** (chloro-pyrazole) being superior to **10e** in binding affinity as well as platelet activity. We reasoned that as compounds became more lipophilic after introduction of a halogen, they would likely be more protein bound, which in turn would result in a significantly greater shift between *K_i* in the binding assay and IC₅₀ values in the platelet assay. In contrast, we could discern no clear trend on how the substitutions on the phenyl ring affected the platelet activity.

After introducing basic amines into the scaffold, both aqueous solubility and platelet activity were improved, but one of the liabilities we observed was the introduction of a significant interaction with the hERG channel. As a result, compounds of interest were screened in a patch clamp electrophysiology assay at a single concentration of 3 or 1 μM (Table 1).

Several previously well-explored approaches were employed to attenuate hERG activity.^{16,17} The first was to reduce the p*K_a* of the basic nitrogen, which proved to be quite effective in reducing hERG activity while still maintaining the receptor affinity and platelet activity. As discussed earlier, both morpholino- and pyrrolidino-containing compounds trended toward improved potency in the platelet aggregation assay. However, due to the high p*K_a* of the pyrrolidine nitrogen, many of the pyrrolidino-containing compounds had very high activity in the hERG channel assay and were therefore dropped from further evaluation. In contrast, compounds with a morpholino-substituent had somewhat reduced activity at the hERG channel. For example, **10b** containing a pyrrolidine (p*K_a* 9.56)¹⁸ showed 80% inhibition at 1 μM while the analogous **10i**, which incorporated a morpholino-substituent (p*K_a* 6.38), showed only 50% inhibition at the same concentration. The p*K_a* of the basic nitrogen could also be reduced by introducing electron withdrawing groups adjacent to the amine moiety, again resulting in reduced hERG channel blockade. Compound **10e**, which contained a *gem*-difluoropyrrolidine (p*K_a* 4.91), had a further attenuation of hERG activity to 50% inhibition at 3 μM. Increasing the polarity of molecules (lowering clog*P*) is another well explored method of decreasing hERG activity. Compounds **10q**, **10r**, and **10s** all contained polar groups attached to the amine moiety and each had significantly lower hERG inhibition, typically less than 30% at 3 μM. In addition, **10q** (*meta*-OMe on phenyl ring, 24% inhibition at 3 μM) had lower hERG activity than **10n** (*meta*-CF₃ on phenyl ring, 75% inhibition at 3 μM), showing that making changes to other areas of the molecule to reduce clog*P* (in this case from 3.16 to 2.22) was also able to assist in decreasing hERG activity. This strategy of optimization of receptor affinity and selectivity and the subsequent management of platelet activity and hERG activity led us to the initial identification of our lead compound. **10k** was a selective, high affinity 5-HT_{2A} receptor inverse agonist with excellent activity in the platelet aggregation assay. In addition, **10k** showed only moderate hERG inhibition of 32% at 3 μM in the screening assay, and this translated into an IC₅₀ of 11 μM when tested in the same patch clamp assay in full concentration-response mode.

Another challenge encountered after introducing the basic amine functionality was the poor microsomal stability of compounds in rodents, which resulted in poor in vivo rodent pharmacokinetic parameters. Pharmacokinetic profiles of selected compounds in rat are shown in Table 2. In general, as expected with poor microsomal stability, most compounds

Table 2. Rat Pharmacokinetic Parameters of Selected Compounds after 10 mg/kg Oral Dose^a

compd	C _{max} (ng/mL)	AUC _{last} (h·ng/mL)	T _{1/2} (h)	IV CL _{total} (L/h/kg)	V _{ss} (L/kg)	F (%) ^b
10d	56	171	1.5	14	11.7	22
10i	202	288	1.8	9.6	5.1	27
10a	453	3831	2.8	1.1	5.4	43

^a Values represent the mean of *n* = 3 animals. ^b % *F* calculated relative to a 2 mg/kg iv dosing.

Table 3. Pharmacokinetic Profile of 10k in Preclinical Species after 10 mg/kg Oral Dose^a

species	C _{max} (ng/mL)	AUC _{last} (h·ng/mL)	T _{1/2} (h)	IV CL _{total} (L/h/kg)	V _{ss} (L/kg)	F (%) ^b
rat	58	43	1.0	22	29	10
dog	2280	3544	0.9	1.2	1.3	45
monkey	1462	1922	2.1	0.9	0.6	16

^a Values represent the mean of *n* = 3 animals. ^b % *F* calculated relative to a 2 mg/kg i.v. dosing.

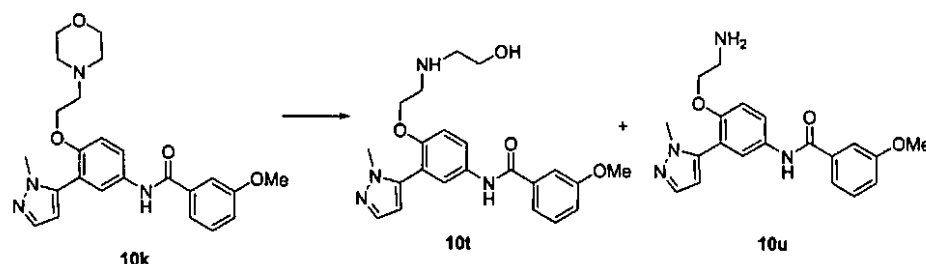


Figure 3. Compound 10k and its active metabolites 10t and 10u.

tested exhibited very high plasma clearance, low exposure, and poor oral bioavailability. Compound 10d was a typical example (Table 2). We observed that a lipophilic CF₃ group sometimes improved these pharmacokinetic parameters. For example, 10i, with a CF₃ group on the phenyl ring, had slightly lower clearance than 10g and improved C_{max} and AUC compared to 10d. However, the hERG activity of 10i prevented this compound from moving forward. The combination of a CF₃ group on the phenyl ring and a pyrrolidino group (10a) resulted in much lower clearance, very good exposure, and oral bioavailability. However, due to the high hERG activity and the significant 5-HT_{2C} receptor affinity, 10a was not suitable for further development despite its potent platelet activity and good pharmacokinetic profile.

The pharmacokinetic profile of 10k in preclinical species was initially somewhat unpromising (Table 3). Compound 10k had modest pharmacokinetic characteristics in rats, but in monkeys and dogs, 10k had quite acceptable pharmacokinetic properties. Plasma clearance was low, oral bioavailability was moderate to good, and overall exposure was high. In addition, two major active metabolites of 10k were identified (Figure 3). 10t and 10u were generated upon incubation of 10k with human, dog and rat liver microsomes and were also observed in vivo across all species tested. These two metabolites were essentially equipotent with 10k in receptor affinity, inhibition of platelet aggregation, and target selectivity (Table 1). Remarkably, after lengthy struggles to control hERG inhibition within the series, the metabolites of 10k also turned out to be only weak inhibitors of the hERG channel. Thus, there were no known off-target effects of these major metabolites that would preclude the further development of 10k, and indeed, it was envisioned that their generation in vivo could extend the duration of biological activity for 10k. Neither metabolite was suitable as a development candidate in its own right, however, as neither showed good exposure after oral administration, which we ascribed to lack of intestinal absorption.

The in vivo activity of 10k was tested in beagle dogs using inhibition of ex vivo serotonin induced platelet aggregation and inhibition of arterial thrombosis in the Folts model, a classic canine model of recurrent coronary thrombosis, considered to mimic many of the key pathophysiological features of unstable angina, as previously described.^{19,20} Levels of serotonin-mediated amplification of collagen-stimulated platelet aggregation were determined in whole blood following a single oral dose of 10 mg/kg of 10k. Complete inhibition of serotonin-mediated aggregation was observed 1 h after dosing of 10k and was maintained at the 6, 12, and 24 h time points with 86%, 57%, and 58% inhibition, respectively. Similar results were obtained using the ex vivo assay following 10 days of repeat dosing of 10k at a 1 mg/kg dose.¹⁹ In the Folts model, 10k attenuated recurrent thrombosis in anesthetized dogs either pretreated with 10k or treated with 10k after the onset of recurrent thrombosis, at a dose of 0.07 mg/kg iv bolus followed by continuous iv fusion of 1.16 µg/kg/min. Flow-time area averaged 58–59% following administration of 10k vs 21–28% in saline controls.²⁰ Overall, this profile, including the potential for an extended duration of action via the generation of active metabolites, was deemed sufficiently attractive for us to select 10k for further development.

Summary

10k is a novel 5-HT_{2A} inverse agonist with high receptor binding affinity (*K_i* = 4.9 nM) and functional inverse agonism of inositol phosphate accumulation (IC₅₀ = 5.2 nM).¹⁹ 10k had excellent selectivity for the 5-HT_{2A} receptor versus 5-HT_{2C} (*K_i* > 10000 nM) and 5-HT_{2B} (*K_i* > 10000 nM) receptors in competition binding assays (> 2000-fold selectivity on both receptors). 10k had limited CNS partitioning, with a brain to plasma ratio of 0.05 (based on C_{max} at 30 mg/kg dose) in rat. Further profiling against a panel of 70 human GPCRs, ion channels, and transporters (Cerep) showed that 10k had no appreciable activities on any other receptors or transporters tested. Patch clamp analysis suggested that 10k had

limited hERG channel inhibition potential, with an IC_{50} of 11 μ M. On the basis of CYP450 inhibition assays using human liver microsomes, **10k** appeared to have little potential for drug–drug interactions. **10k** exhibited potent inhibition of serotonin mediated amplification of ADP-stimulated human and dog platelet aggregation (IC_{50} = 8.7 and 23.1 nM, respectively). Oral administration of **10k** to beagle dogs resulted in acute (1 h) and subchronic (10 day) inhibition of serotonin mediated amplification of collagen-stimulated platelet aggregation in whole blood ex vivo. In the Folts model, **10k** improved coronary patency even after the onset of recurrent thrombosis. Taken together, these data suggest that **10k**, a high affinity, selective, orally bioavailable 5-HT_{2A} receptor inverse agonist, represents a promising compound for development as an antiplatelet agent.

Experimental Section

Chemistry. All reagents were commercially available and used without further purification unless stated otherwise. Microwave irradiations were performed using either a Smith Synthesizer or an Emrys Optimizer (Biotage). Column chromatography was carried out on prepacked silica gel columns using KP-Sil supplied by Biotage and on silica gel columns using Kieselgel 60, 0.063–0.200 mm (Merck). Proton nuclear magnetic resonance (¹H NMR) spectra were recorded on a Bruker Avance-400 equipped with a Quad Nucleus probe (QNP) or a Broad-band Inverse (BBI) probe and z-gradient. Chemical shifts (δ) are given in parts per million (ppm), with the residual solvent signal used as a reference. Coupling constants (J) are reported in Hz. NMR abbreviations are used as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, b = broad. Melting points were recorded on differential scanning calorimetry (DSC).

Analytical HPLC/MS was conducted on a PE Sciex API 150EX mass spectrometer with an electrospray source, using a Shimadzu Inc. LC-10A VP UV detector monitoring at 214 nm, Analyst 1.2 software, and either (a) a Gilson 215 autosampler and an Alltech Prevail C18 column (5 μ , 250 mm $\times$ 4.6 mm), using a gradient of 5% v/v CH₃CN (containing 1% v/v TFA) in H₂O (containing 1% v/v TFA) (t = 0.0 min) gradient to 95% v/v CH₃CN in H₂O (t = 6.0 min), 3.5 mL/min, or (b) a PE 200 autosampler and a Supelco Discovery C18 column (5 μ , 50 mm $\times$ 2.1 mm), using a gradient of 5% v/v CH₃CN (containing 1% v/v TFA) in H₂O (containing 1% v/v TFA) (t = 0.0 min) gradient to 95% v/v CH₃CN in H₂O (t = 5.0 min), 0.75 mL/min. Preparative HPLC was conducted on a Varian Prostar reverse phase HPLC using either (a) a Phenomenex Luna C18 column (10 μ , 250 mm $\times$ 21.2 mm), 5% (v/v) CH₃CN (containing 0.1% v/v TFA) in H₂O (containing 0.1% v/v TFA) gradient to 95% CH₃CN, 20 mL/min, λ = 220 nm, or (b) a Phenomenex Luna C18 column (10 μ , 250 mm $\times$ 50 mm), 5% (v/v) CH₃CN (containing 0.1% v/v TFA) in H₂O (containing 0.1% v/v TFA) gradient to 95% CH₃CN, 50 mL/min, λ = 220 nm. The purity of all tested compounds was $\geq$ 95% (peak area % at 214 nm) using the analytical methods described above unless stated otherwise.

5-(2-Methoxy-5-nitro-phenyl)-1-methyl-1H-pyrazole (4). A solution of *N*-methylpyrazole (5.12 g, 62.4 mmol) in THF (100 mL) was cooled to -78°C and 2.5 M *n*-butyllithium in hexane (27.5 mL, 68.8 mmol) was added. After stirring for 30 min at the same temperature, 0.5 M zinc chloride in THF (107 mL, 53.5 mmol) and 1 M zinc chloride in diethyl ether (15.3 mL, 15.2 mmol) were added. The mixture was allowed to warm up to room temperature and 2-iodo-4-nitroanisole (17.4 g, 62.4 mmol) followed by tetrakis(triphenylphosphine)palladium (3.6 g, 3.1 mmol) were added. The reaction mixture was heated to 60°C overnight. Once the reaction was complete, the solvents were removed under reduced pressure and the residue partitioned between ethyl acetate and brine. The

combined organic phases were dried over MgSO₄, filtered, and partly concentrated until a solid started to precipitate. Hexane (approximately 3 volumes) was added and the resultant precipitate collected by filtration and washed with a cold 3:1 mixture of hexane/ethyl acetate to afford **4** (8.6 g, 59%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ : 3.74 (s, 3H), 3.96 (s, 3H), 6.31 (d, J = 1.6 Hz, 1H), 7.08 (d, J = 9.2 Hz, 1H), 7.56 (d, J = 2.0 Hz, 1H), 8.19 (d, J = 2.8 Hz, 1H), 8.34 (dd, J = 2.8, 9.2 Hz, 1H). LCMS m/z = 234.1 [M + H]⁺.

2-(2-Methyl-2H-pyrazol-3-yl)-4-nitro-phenol (5). A mixture of **4** (3 g, 12.9 mmol) in 1,2-dichloroethane (50 mL) was cooled to 0°C with stirring. Aluminum trichloride (6.9 g, 51.6 mmol) was added. The resulting mixture was heated at 80°C for 3.5 h. The crude mixture was diluted with ethyl acetate and the solid removed by filtration. The resultant filtrate was washed with a 10% solution of potassium tartrate and the solvent removed by evaporation. The residue was purified by HPLC to give **5** as a yellow solid (1.08 g, 38%). ¹H NMR (400 MHz, DMSO-*d*₆) δ : 3.74 (s, 3H), 6.38 (d, J = 1.6 Hz, 1H), 7.16 (d, J = 9.2 Hz, 1H), 7.48 (d, J = 2.0 Hz, 1H), 8.05 (d, J = 2.8 Hz, 1H), 8.24 (dd, J = 2.8, 9.2 Hz, 1H), 12.0 (s, 1H). LCMS m/z = 220.1 [M + H]⁺.

5-[2-(2-Bromo-ethoxy)-5-nitro-phenyl]-1-methyl-1H-pyrazole (6). To a suspension of **5** (1.042 g, 4.76 mmol) in THF (50 mL) at rt was added triphenylphosphine (2.138 g, 8.15 mmol) and 2-bromoethanol (0.580 mL, 8.21 mmol). Diisopropyl azodicarboxylate (DIAD) (1.58 mL, 7.81 mmol) was added dropwise to the stirring mixture. On completion of the addition, the reaction mixture was stirred at rt for a further 2 h and then concentrated to yield an oil which was purified by SiO₂ column chromatography (eluent: 40% ethyl acetate/60% hexanes) to afford **6** (1.40 g, 90%) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ : 3.73 (s, 3H), 3.82–3.77 (m, 2H), 4.59–4.54 (m, 2H), 6.43 (d, J = 1.8 Hz, 1H), 7.42 (d, J = 9.2 Hz, 1H), 7.51 (d, J = 1.8 Hz, 1H), 8.12 (d, J = 2.9 Hz, 1H), 8.36 (dd, J = 9.2, 2.9 Hz, 1H). LCMS m/z = 326.1/328.1 [M + H]⁺.

5-[2-(2-Bromo-ethoxy)-5-nitro-phenyl]-4-chloro-1-methyl-1H-pyrazole (7a). A mixture of **6** (470 mg, 1.44 mmol) and *N*-chlorosuccinimide (192 mg, 1.44 mmol) in DMF (2 mL) was heated under microwave irradiation at 100°C for 20 min. The mixture was quenched with water, extracted with ethyl acetate, and the combined extracts dried in vacuo to give **7a** as a yellow solid (462 mg, 89%) which was used in subsequent reactions without purification. ¹H NMR (400 MHz, CDCl₃) δ : 3.55–3.61 (m, 2H), 3.78 (s, 3H), 4.44 (t, J = 5.6 Hz, 2H), 7.13 (d, J = 9.1 Hz, 1H), 7.57 (s, 1H), 8.23 (s, 1H), 8.40 (dd, J = 2.8, 9.1 Hz, 1H). LCMS m/z = 360.1/362.1 [M + H]⁺.

4-Bromo-5-[2-(2-bromo-ethoxy)-5-nitro-phenyl]-1-methyl-1H-pyrazole (7b). A mixture of **6** (200 mg, 0.61 mmol) and *N*-bromosuccinimide (109 mg, 0.61 mmol) in DMF (2 mL) was heated under microwave irradiation at 100°C for 10 min. The mixture was quenched with water, extracted with ethyl acetate, and the combined extracts dried in vacuo to give **7b** as a yellow solid (200 mg, 81%), which was used in subsequent reactions without purification. ¹H NMR (400 MHz, CDCl₃) δ : 3.53–3.64 (m, 2H), 3.78 (s, 3H), 4.38–4.49 (m, 2H), 7.11 (d, J = 9.1 Hz, 1H), 7.57 (s, 1H), 8.23 (s, 1H), 8.39 (dd, J = 2.8, 9.1 Hz, 1H). LCMS m/z = 404.1/406.1/407.9 [M + H]⁺.

General Procedure A, Preparation of Intermediates 8 via Alkylation: 1-Methyl-5-[5-nitro-2-[2-(pyrrolidin-1-yl)ethoxy]phenyl]-1H-pyrazole (8a). A mixture of **6** (390 mg, 1.20 mmol), pyrrolidine (104 mg, 1.46 mmol), and potassium carbonate (218 mg, 1.58 mmol) in DMF (4 mL) was heated under microwave irradiation at 100°C for 15 min. The resulting mixture was purified by HPLC to give the TFA salt of **8a** as a yellow solid (400 mg, 77%). ¹H NMR (400 MHz, CD₃CN) δ : 1.80–1.88 (m, 2H), 1.94–2.00 (m, 2H), 2.79–2.89 (m, 2H), 3.37–3.45 (m, 2H), 3.48–3.53 (m, 2H), 3.72 (s, 3H), 4.43–4.46 (m, 2H), 6.40 (d, J = 2.0 Hz, 1H), 7.25 (d, J = 9.2 Hz, 1H), 7.56 (d, J = 2.0 Hz, 1H), 8.20 (d, J = 2.9 Hz, 1H), 8.38 (dd, J = 2.9, 9.2 Hz, 1H). LCMS m/z = 317.2 [M + H]⁺.

5-[2-[2-(3,3-Difluoropyrrolidin-1-yl)ethoxy]-5-nitrophenyl]-1-methyl-1H-pyrazole (8c). 8c was prepared in a manner similar to that described for 8a, using 6 (50 mg, 0.15 mmol) and 3,3-difluoropyrrolidine hydrochloride (26 mg, 0.18 mmol) as starting materials, to give the TFA salt of 8c as a yellow solid (16 mg, 22%). ¹H NMR (400 MHz, CD₃CN) δ: 2.35–2.45 (m, 2H), 3.31–3.43 (m, 4H), 3.49–3.56 (m, 2H), 3.69 (s, 3H), 4.46–4.49 (m, 2H), 6.34–6.36 (m, 1H), 7.22 (d, *J* = 9.3 Hz, 1H), 7.49–7.50 (m, 1H), 8.18 (d, *J* = 3.0 Hz, 1H), 8.38 (dd, *J* = 2.9, 9.0 Hz, 1H). LCMS *m/z* = 353.3 [M + H]⁺.

4-[2-[2-(2-Methyl-2H-pyrazol-3-yl)-4-nitro-phenoxy]-ethyl]-morpholine (8d). A mixture of triphenylphosphine (7.5 g, 28.6 mmol) and diisopropyl azodicarboxylate (5.66 mL, 28.6 mmol) in THF (50 mL) was stirred at 0 °C for 10 min, then 5 (3.5 g, 15.9 mmol) and 2-morpholin-4-yl-ethanol (3.66 mL, 28.6 mmol) were added at 0 °C. The reaction mixture was allowed to warm to rt and was stirred for another hour. The solvent was removed in vacuo, and the crude mixture was purified by HPLC to provide 8g as a yellow solid (9.44 g, 89%). ¹H NMR (400 MHz, DMSO-*d*₆) δ: 2.92–3.58 (m, 6H), 3.69 (s, 3H), 3.75–3.85 (m, 2H), 4.54–4.56 (m, 2H), 4.77–4.85 (m, 2H), 6.39 (d, *J* = 1.8 Hz, 1H), 7.45 (d, *J* = 9.2 Hz, 1H), 7.52 (d, *J* = 1.8 Hz, 1H), 8.16 (d, *J* = 2.9 Hz, 1H), 8.43 (dd, *J* = 9.2, 2.9 Hz, 1H), 8.88 (s, 1H). LCMS *m/z* = 333.4 [M + H]⁺.

4-[2-[2-(1-Methyl-1H-pyrazol-5-yl)-4-nitrophenoxy]ethyl]-piperazin-2-one (8l). 8l was prepared in a manner similar to that described for 8a, using 6 (500 mg, 1.53 mmol) and piperazin-2-one (153 mg, 1.53 mmol) as starting materials. The TFA salt of 8l was isolated as a yellow solid (353 mg, 67%). ¹H NMR (400 MHz, DMSO-*d*₆) δ: 2.07 (s, 2H), 3.11–3.23 (m, 4H), 3.42–3.52 (m, 2H), 3.60–3.66 (m, 1H), 3.69 (s, 3H), 4.51–4.56 (m, 2H), 6.40 (d, *J* = 1.9 Hz, 1H), 7.45 (d, *J* = 9.3 Hz, 1H), 7.52 (d, *J* = 1.9 Hz, 1H), 8.16 (d, *J* = 2.9 Hz, 1H), 8.43 (dd, *J* = 9.2, 2.9 Hz, 1H). LCMS *m/z* = 346.1 [M + H]⁺.

1-[4-[2-(2-(1-Methyl-1H-pyrazol-5-yl)-4-nitrophenoxy)ethyl]-piperazin-1-yl]ethanone (8n). 8n was prepared in a manner similar to that described for 8a, using 6 (325 mg, 1.0 mmol) and 1-(piperazin-1-yl)ethanone (153 mg, 1.2 mmol) as starting materials, to give the TFA salt of 8n as a yellow solid (423 mg, 100%). ¹H NMR (400 MHz, DMSO-*d*₆) δ: 2.02 (s, 3H), 2.74–3.40 (m, 6H), 3.50–3.60 (m, 2H), 3.68 (s, 3H), 3.75–4.35 (m, 2H), 4.50–4.60 (m, 2H), 6.40 (d, *J* = 1.9 Hz, 1H), 7.45 (d, *J* = 9.3 Hz, 1H), 7.54 (d, *J* = 1.9 Hz, 1H), 8.16 (d, *J* = 2.9 Hz, 1H), 8.43 (dd, *J* = 9.2, 2.9 Hz, 1H). LCMS *m/z* = 374.3 [M + H]⁺.

General Procedure B, Reduction of Nitro Group to Intermediates 9: 3-(1-Methyl-1H-pyrazol-5-yl)-4-[2-(pyrrolidin-1-yl)ethoxy]aniline (9a). To a solution of 8a (2.52 g, 7.98 mmol) in saturated ammonium chloride (4 mL), water (4 mL), and THF (10 mL) was added zinc dust (2.81 g, 43 mmol) at 0 °C. The mixture was stirred at rt for 1 h and then filtered through a pad of celite to remove the solids. The filtrate was concentrated in vacuo to give 9a as a white solid in essentially quantitative yield (2.24 g). LCMS *m/z* = 287.2 [M + H]⁺. The product was used in the next step without purification.

4-[2-(3,3-Difluoropyrrolidin-1-yl)ethoxy]-3-(1-methyl-1H-pyrazol-5-yl)aniline (9c). 9c was prepared from 8c (258 mg, 0.73 mmol) in a manner similar to that described for 9a, providing 9c as a white solid in quantitative yield (230 mg). LCMS *m/z* = 323.2 [M + H]⁺. The product was used in the next step without further purification.

3-(1-Methyl-1H-pyrazol-5-yl)-4-(2-morpholinoethoxy)aniline (9d). 9d was prepared from 8d (1.91 g, 5.75 mmol) in a manner similar to that described for 9a, providing 9d as a white solid in quantitative yield (1.87 g). LCMS *m/z* = 303.4 [M + H]⁺. The product was used in the next step without further purification.

4-[2-[4-Amino-2-(1-methyl-1H-pyrazol-5-yl)phenoxy]ethyl]-piperazin-2-one (9l). 9l was prepared from 8l (353 mg, 1.02 mmol) in a manner similar to that described for 9a, providing 9l as a white solid (245 mg, 76%). LCMS *m/z* = 315.8 [M + H]⁺. The product was used in the next step without further purification.

1-[4-[2-(4-Amino-2-(1-methyl-1H-pyrazol-5-yl)phenoxy)ethyl]-piperazin-1-yl]ethanone (9n). 9n was prepared from 8n (373 mg, 0.67 mmol) in a manner similar to that described for 9a, providing 9n as a white solid (180 mg, 78%). LCMS *m/z* = 344.1 [M + H]⁺. The product was used in the next step without further purification.

General Procedure C, Preparation of Amides 10 via HATU Coupling: *N*-[3-(2-Methyl-2H-pyrazol-3-yl)-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-4-trifluoromethyl-benzamide (10a). A mixture of 9a (57 mg, 0.2 mmol), 4-trifluoromethyl-benzoic acid (42 mg, 0.22 mmol), *N,N,N',N'*-tetramethyl-*O*-(7-azabenzotriazol-1-yl)-uronium hexafluorophosphate (HATU, 91 mg, 0.24 mmol) and triethylamine (0.1 mL) in DMF (2 mL) was heated under microwave irradiation at 100 °C for 15 min. After removal of excess solvent, the crude product was purified by HPLC to give the TFA salt of 10a as a white solid (65 mg, 71%). ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 1.73–1.76 (m, 2H), 1.87–1.92 (m, 2H), 2.85–2.90 (m, 2H), 3.29–3.32 (m, 2H), 3.53–3.57 (m, 2H), 3.69 (s, 3H), 4.32 (t, *J* = 4.8 Hz, 2H), 6.32 (d, *J* = 2.0 Hz, 1H), 7.26 (d, *J* = 8.6 Hz, 1H), 7.50 (d, *J* = 1.8 Hz, 1H), 7.75 (d, *J* = 2.5 Hz, 1H), 7.88–7.94 (m, 3H), 8.15 (d, *J* = 8.8 Hz, 2H), 9.81 (s, 1H), 10.5 (s, 1H). LCMS *m/z* = 459.4 [M + H]⁺.

[3-(2-Methyl-2H-pyrazol-3-yl)-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-3-trifluoromethyl-benzamide (10b). 10b was prepared in a manner similar to that described for 10a, using 9a (57 mg, 0.2 mmol) and 3-trifluoromethyl-benzoic acid (57 mg, 0.3 mmol) to give the TFA salt of 10b as a white solid (42 mg, 46%). ¹H NMR (CD₃CN, 400 MHz) δ: 1.25–1.40 (m, 2H), 1.81–1.88 (m, 2H), 2.79–2.82 (bs, 2H), 3.36–3.46 (m, 4H), 3.71 (s, 3H), 4.32 (t, *J* = 4.8 Hz, 2H), 6.29 (d, *J* = 1.9 Hz, 1H), 7.13 (d, *J* = 8.9 Hz, 1H), 7.46 (d, *J* = 1.9 Hz, 1H), 7.64 (d, *J* = 2.8 Hz, 1H), 7.72 (t, *J* = 7.8 Hz, 1H), 7.80 (dd, *J* = 2.9, 8.9 Hz, 1H), 7.89 (d, *J* = 7.9 Hz, 1H), 8.17 (d, *J* = 7.9 Hz, 1H), 8.23 (s, 1H), 8.95 (s, 1H). LCMS *m/z* = 459.4 [M + H]⁺.

General Procedure D, Preparation of Amides 10 via Acid Chloride Coupling: *N*-[4-[2-(3,3-Difluoropyrrolidin-1-yl)ethoxy]-3-(1-methyl-1H-pyrazol-5-yl)phenyl]-3-(trifluoromethyl)-benzamide (10c). A mixture of 9c (64 mg, 0.2 mmol), 3-trifluoromethylbenzoyl chloride (62 mg, 0.3 mmol), and triethylamine (0.1 mL) in THF (2 mL) was stirred at room temperature for 20 min. The solvent was evaporated and the crude product was purified by HPLC to give the TFA salt of 10c as a white solid (33 mg, 33%). ¹H NMR (acetone-*d*₆, 400 MHz) δ: 2.43–2.52 (m, 2H), 3.38–3.46 (m, 4H), 3.56–3.61 (m, 2H), 3.75 (s, 3H), 4.45 (t, *J* = 4.5 Hz, 2H), 6.29 (s, 1H), 7.22 (d, *J* = 8.8 Hz, 1H), 7.44 (s, 1H), 7.79–7.81 (m, 2H), 7.91–7.95 (m, 2H), 8.30 (d, *J* = 8.3 Hz, 2H), 9.82 (s, 1H). LCMS *m/z* = 495.3 [M + H]⁺.

2,4-Difluoro-*N*-[3-(2-Methyl-2H-pyrazol-3-yl)-4-(2-morpholino-4-yl-ethoxy)-phenyl]-benzamide (10d). 10d was prepared in a manner similar to that described for 10c, using 9d (200 mg, 0.66 mmol) and 2,4-difluorobenzoyl chloride (140 mg, 0.79 mmol) to give the TFA salt of 10d as a white solid (99 mg, 34%); mp (HCl salt, recrystallized from iPrOH) 140–142 °C. ¹H NMR (acetone-*d*₆, 400 MHz) δ: 2.92–3.06 (m, 2H), 3.13–3.17 (m, 2H), 3.50–3.58 (m, 2H), 3.68 (s, 3H), 3.82–3.85 (m, 4H), 4.36 (t, 2H), 6.29 (s, 1H), 7.24 (d, *J* = 9.1 Hz, 2H), 7.44 (dt, *J* = 10.1, 2.8 Hz, 1H), 7.49 (s, 1H), 7.67 (s, 1H), 7.72–7.79 (m, 1H), 7.81 (dd, *J* = 9.1, 2.8 Hz, 1H), 9.43 (s, 1H). LCMS *m/z* = 443.5 [M + H]⁺.

3-Fluoro-*N*-[3-(2-methyl-2H-pyrazol-3-yl)-4-(2-morpholino-4-yl-ethoxy)-phenyl]-benzamide (10e). 10e was prepared in a manner similar to that described for 10c, using 9d (120 mg, 0.40 mmol) and 3-fluorobenzoyl chloride (76 mg, 0.47 mmol) to give the TFA salt of 10e as a white solid (102 mg, 61%). ¹H NMR (acetone-*d*₆, 400 MHz) δ: 2.99–3.25 (m, 2H), 3.25–3.49 (m, 2H), 3.69 (t, *J* = 4.5 Hz, 2H), 3.75 (s, 3H), 3.77–3.96 (m, 4H), 4.59 (t, *J* = 4.8 Hz, 2H), 6.30 (t, *J* = 1.5 Hz, 1H), 7.23 (d, *J* = 9.1 Hz, 1H), 7.36 (dt, *J* = 2.5, 8.6 Hz, 2H), 7.49 (t, *J* = 1.8 Hz, 1H), 7.54–7.61 (m, 1H), 7.74 (dt, *J* = 2.0, 9.8 Hz, 1H), 7.82 (t, *J* = 2.4 Hz, 1H), 7.84 (d, *J* = 7.8 Hz, 1H), 7.94 (dt, *J* = 2.4, 9.1 Hz, 1H), 9.64 (s, 1H). LCMS *m/z* = 425.4 [M + H]⁺.

4-Fluoro-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide (10f). 10f was prepared in a manner similar to that described for 10a, using 9d (60 mg, 0.20 mmol) and 4-fluorobenzoic acid (36 mg, 0.26 mmol) to give the TFA salt of 10f as a white solid (22 mg, 26%). ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 3.02 (m, 2H), 3.14–3.17 (m, 2H), 3.50–3.59 (m, 4H), 3.69 (s, 3H), 3.82–3.85 (m, 2H), 4.36 (t, *J* = 4.8 Hz, 2H), 6.29 (s, 1H), 7.24 (d, *J* = 9.1 Hz, 1H), 7.37 (t, *J* = 8.8 Hz, 2H), 7.49 (s, 1H), 7.72 (s, 1H), 7.88 (dd, *J* = 2.8, 9.1 Hz, 1H), 8.03 (dd, *J* = 5.6, 8.8 Hz, 2H), 9.86 (s, 1H), 10.3 (s, 1H). LCMS *m/z* = 425.4 [M + H]⁺.

***N*-[3-(4-Chloro-2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-3-fluoro-benzamide (10g).** 10g was prepared in a manner similar to that described for 10c and was obtained as the TFA salt as an off-white solid (44%). ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 3.10 (m, 4H), 3.59 (m, 4H), 3.67 (s, 3H), 3.87 (m, 2H), 4.34 (m, 1H), 7.31 (d, *J* = 9.1 Hz, 1H), 7.46 (dt, *J* = 2.5, 9.0 Hz, 1H), 7.59 (m, 1H), 7.67 (s, 1H), 7.73 (d, *J* = 2.6 Hz, 1H), 7.77 (m, 1H), 7.82 (m, 1H), 7.96 (dd, *J* = 2.6, 9.0 Hz, 1H), 10.3 (s, 1H). LCMS *m/z* = 459/461 [M + H]⁺.

***N*-[3-(2-Methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-4-trifluoromethyl-benzamide (10h).** 10h was prepared in a manner similar to that described for 10a, using 9d (60 mg, 0.20 mmol) and 4-trifluoromethylbenzoic acid (49 mg, 0.26 mmol) to give the TFA salt of 10h as a white solid (27 mg, 28%). ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 3.00–3.03 (m, 2H), 3.16–3.19 (m, 2H), 3.52–3.60 (m, 4H), 3.70 (s, 3H), 3.84–3.87 (m, 2H), 4.39 (t, *J* = 5.0 Hz, 2H), 6.31 (s, 1H), 7.28 (d, *J* = 9.0 Hz, 1H), 7.51 (s, 1H), 7.75 (s, 1H), 7.94 (d, *J* = 8.3 Hz, 2H), 8.16 (d, *J* = 8.8 Hz, 2H), 8.89 (s, 1H), 9.88 (s, 1H), 10.5 (s, 1H). LCMS *m/z* = 475.4 [M + H]⁺.

***N*-[3-(2-Methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-3-trifluoromethyl-benzamide (10i).** 10i was prepared in a manner similar to that described for 10c, using 9d (50 mg, 0.16 mmol) and 3-trifluoromethylbenzoyl chloride and was obtained as a white solid (72 mg, 92%). ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 2.34 (t, *J* = 4.0 Hz, 4H), 2.61 (t, *J* = 8.0 Hz, 2H), 3.51 (t, *J* = 4.0 Hz, 4H), 3.71 (s, 3H), 4.12 (t, *J* = 8.0 Hz, 2H), 6.26 (d, *J* = 1.8 Hz, 1H), 7.21 (d, *J* = 9.0 Hz, 1H), 7.45 (d, *J* = 1.8 Hz, 1H), 7.69 (d, *J* = 2.6 Hz, 1H), 7.81–7.77 (m, 1H), 7.85–7.82 (m, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 8.26 (d, *J* = 7.9 Hz, 1H), 8.29 (s, 1H), 10.4 (bs, 1H). LCMS *m/z* = 475 [M + H]⁺.

3,4-Difluoro-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide (10j). 10j was prepared in a manner similar to that described for 10c, using 9d (120 mg, 0.40 mmol) and 3,4-difluorobenzoyl chloride (84 mg, 0.48 mmol) to give the TFA salt of 10j as a white solid (110 mg, 63%). ¹H NMR (acetone-*d*₆, 400 MHz) δ: 3.11 (bs, 2H), 3.37 (s, 2H), 3.68 (t, *J* = 4.8 Hz, 2H), 3.75 (s, 3H), 3.86 (bs, 4H), 4.59 (t, *J* = 4.8 Hz, 2H), 6.30 (s, 1H), 7.23 (d, *J* = 8.8 Hz, 1H), 7.48 (m, 2H), 7.80 (s, 1H), 7.87–7.92 (m, 2H), 7.93–7.99 (m, 1H), 9.67 (s, 1H). LCMS *m/z* = 443.3 [M + H]⁺.

3-Methoxy-*N*-[3-(2-methyl-2*H*-pyrazol-3-yl)-4-(2-morpholin-4-yl-ethoxy)-phenyl]-benzamide (10k). 10k was prepared in a manner similar to that described for 10c, using 9d (120 mg, 0.40 mmol) and 3-methoxybenzoyl chloride (81 mg, 0.48 mmol) to give the TFA salt of 10k as a white solid (88 mg, 51%); mp (HCl salt, recrystallized from iPrOH) 214–216 °C. ¹H NMR (acetone-*d*₆, 400 MHz) δ: 2.99–3.21 (m, 2H), 3.22–3.45 (m, 2H), 3.66 (t, *J* = 4.8 Hz, 2H), 3.75 (s, 3H), 3.85 (s, 3H), 3.79–3.89 (m, 4H), 4.58 (t, *J* = 4.8 Hz, 2H), 6.29 (d, *J* = 2.0 Hz, 1H), 7.13 (dd, *J* = 2.5, 8.3 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 1H), 7.42 (t, *J* = 7.8 Hz, 1H), 7.47 (d, *J* = 1.7 Hz, 1H), 7.52 (t, *J* = 1.7 Hz, 1H), 7.56 (d, *J* = 7.0 Hz, 1H), 7.80–7.83 (m, 1H), 7.91–7.96 (m, 1H), 9.54 (s, 1H). LCMS *m/z* = 437.5 [M + H]⁺.

***N*-[3-(2-Methyl-2*H*-pyrazol-3-yl)-4-[2-(3-oxo-piperazin-1-yl)-ethoxy]-phenyl]-3-trifluoromethyl-benzamide (10l).** 10l was prepared in a manner similar to that described for 10c, using 9l (79 mg, 0.25 mmol) and 3-trifluoromethyl benzoyl chloride (63 mg, 0.30 mmol) to give the TFA salt of 10l as a white solid

(52 mg, 41%). ¹H NMR (CD₃CN, 400 MHz) δ: 3.16–3.21 (m, 2H), 3.30–3.36 (m, 2H), 3.48 (t, *J* = 4.8 Hz, 2H), 3.65 (s, 2H), 3.73 (s, 3H), 4.40 (t, *J* = 4.4 Hz, 2H), 6.33 (d, *J* = 1.8 Hz, 1H), 6.65 (s, 1H), 7.14 (d, *J* = 9.0 Hz, 1H), 7.51 (d, *J* = 1.7 Hz, 1H), 7.65 (d, *J* = 2.6 Hz, 1H), 7.72 (t, *J* = 7.8 Hz, 1H), 7.81 (dd, *J* = 2.6, 8.8 Hz, 1H), 7.89 (d, *J* = 7.8 Hz, 1H), 8.18 (d, *J* = 7.8 Hz, 1H), 8.23 (s, 1H), 8.93 (s, 1H). LCMS *m/z* = 488.2 [M + H]⁺.

***N*-[3-(4-Chloro-2-methyl-2*H*-pyrazol-3-yl)-4-[2-(3-oxo-piperazin-1-yl)-ethoxy]-phenyl]-3-trifluoromethyl-benzamide (10m).** 10m was prepared in a manner similar to that described for 10c, using 4-[2-[4-amino-2-(4-chloro-1-methyl-1*H*-pyrazol-5-yl)phenoxy]-ethyl]piperazin-2-one (88 mg, 0.25 mmol) and 3-trifluoromethyl benzoyl chloride (52 mg, 0.25 mmol) to give the TFA salt of 10m as a white solid (70 mg, 53%). ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 3.20–3.24 (m, 4H), 3.62–3.78 (m, 8H), 4.31–4.38 (m, 2H), 7.31 (d, *J* = 9.1 Hz, 1H), 7.65 (s, 1H), 7.71 (d, *J* = 2.8 Hz, 1H), 7.77–7.81 (m, 1H), 7.93–7.99 (m, 2H), 8.25–8.29 (m, 2H), 10.5 (s, 1H). LCMS *m/z* = 522.3/524.2 [M + H]⁺.

***N*-[4-[2-(4-Acetylpiperazin-1-yl)ethoxy]-3-(1-methyl-1*H*-pyrazol-5-yl)phenyl]-3-(trifluoromethyl)benzamide (10n).** 10n was prepared in a manner similar to that described for 10c, using 9n (60 mg, 0.18 mmol) and 3-trifluoromethylbenzoyl chloride (36 mg, 0.18 mmol) to give the TFA salt of 10n as a white solid (36 mg, 40%, 92% purity by LCMS). ¹H NMR (CD₃CN, 400 MHz) δ: 1.94 (s, 3H), 3.32 (t, *J* = 4.8 Hz, 2H), 3.50–3.85 (m, 8H), 3.62 (s, 3H), 4.29 (t, *J* = 4.8 Hz, 2H), 6.23 (s, 1H), 7.03 (d, *J* = 9.1 Hz, 1H), 7.43 (d, *J* = 1.8 Hz, 1H), 7.55–7.82 (m, 4H), 8.08–8.10 (m, 1H), 8.15 (s, 1H), 8.86 (s, 1H). LCMS *m/z* = 516.4 [M + H]⁺.

***N*-[4-[2-(4-Acetylpiperazin-1-yl)ethoxy]-3-(1-methyl-1*H*-pyrazol-5-yl)phenyl]-3-fluorobenzamide (10o).** 10o was prepared in a manner similar to that described for 10c, using 9n (100 mg, 0.29 mmol) and 3-fluorobenzoyl chloride (46 mg, 0.29 mmol) to give the TFA salt of 10o as a white solid (38 mg, 28%); mp (free base, recrystallized from iPrOH/heptane = 70/30) 139–140 °C. ¹H NMR (CD₃CN, 400 MHz) δ: 2.09 (s, 3H), 3.32–3.38 (m, 6H), 3.48 (t, *J* = 4.8 Hz, 4H), 3.79 (s, 3H), 4.43 (t, *J* = 4.8 Hz, 2H), 6.39 (d, *J* = 2.0 Hz, 1H), 7.18 (d, *J* = 9.1 Hz, 1H), 7.40 (dt, *J* = 7.4, 8.6 Hz, 1H), 7.57–7.63 (m, 2H), 7.70–7.74 (m, 2H), 7.80–7.82 (m, 1H), 7.85 (dd, *J* = 2.8, 9.1 Hz, 1H), 8.93 (s, 1H). LCMS *m/z* = 466.4 [M + H]⁺.

***N*-[4-[2-(4-Acetylpiperazin-1-yl)ethoxy]-3-(4-chloro-1-methyl-1*H*-pyrazol-5-yl)phenyl]-3-fluorobenzamide (10p).** 10p was prepared in a manner similar to that described for 10c, using 1-[4-[2-(4-amino-2-(4-chloro-1-methyl-1*H*-pyrazol-5-yl)phenoxy)-ethyl]piperazin-1-yl]ethanone (86 mg, 0.23 mmol) and 3-fluorobenzoyl chloride (43 mg, 0.27 mmol) to give the TFA salt of 10p as an off-white solid (33 mg, 29%). ¹H NMR (CD₃CN, 400 MHz) δ: 1.97 (s, 3H), 2.24–2.38 (m, 4H), 2.60–2.69 (m, 2H), 3.17 (d, *J* = 4.8 Hz, 2H), 3.31–3.36 (m, 3H), 3.67 (s, 3H), 4.07–4.13 (m, 2H), 7.25 (d, *J* = 9.0 Hz, 1H), 7.44 (t, *J* = 8.5 Hz, 1H), 7.56–7.62 (m, 1H), 7.63 (s, 1H), 7.71 (d, *J* = 2.5 Hz, 1H), 7.77 (d, *J* = 9.8 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.90 (dd, *J* = 9.0, 2.5 Hz, 1H), 10.3 (s, 1H). LCMS *m/z* = 500.7 [M + H]⁺.

***N*-[4-[2-(4-Acetylpiperazin-1-yl)ethoxy]-3-(1-methyl-1*H*-pyrazol-5-yl)phenyl]-3-methoxybenzamide (10q).** Step 1: Preparation of 4-(2-Bromo-ethoxy)-3-(2-methyl-2*H*-pyrazol-3-yl)-phenylamine. To a solution of *N*-[4-(2-bromo-ethoxy)-3-(2-methyl-2*H*-pyrazol-3-yl)-phenyl]-acetamide (3 g, 8.9 mmol) in methanol (6 mL) at 0 °C was added sulfuric acid (2.2 mL, 42 mmol). The reaction mixture was then heated to reflux for 1.5 h. The mixture was cooled, quenched with water, and cautiously neutralized with sodium hydroxide solution until the pH was around 7. The mixture was extracted twice with ethyl acetate, and the combined organic layers concentrated in vacuo to give the title compound as an off-white solid (2.6 g, 99%). LCMS *m/z* = 296.1/298.1 [M + H]⁺. The intermediate was used in the next step without further purification.

Step 2: Preparation of *N*-[4-(2-Bromo-ethoxy)-3-(2-methyl-2*H*-pyrazol-3-yl)-phenyl]-3-methoxybenzamide. A mixture of the intermediate obtained from step 1 (1.01 g, 3.42 mmol),

3-methoxy-benzoyl chloride (700 mg, 4.10 mmol), and triethylamine (1.0 mL) in THF (5 mL) was stirred at room temperature for 10 min. Excess solvent was evaporated and the crude product redissolved in acetonitrile. H₂O was added slowly to eventually provide an approximately 3/2 CH₃CN/H₂O v/v mixture, and the resulting precipitate was collected by filtration and dried to give the title compound as an off-white solid (957 mg, 65%). LCMS *m/z* = 430.3/432.3 [M + H]⁺. This was used in the next step without further purification.

Step 3: Preparation of *N*-(4-[2-(4-Acetylpiperazin-1-yl)ethoxy]-3-(1-methyl-1*H*-pyrazol-5-yl)phenyl)-3-methoxybenzamide (10q). A mixture of the intermediate obtained from step 2 (90 mg, 0.21 mmol), 1-piperazin-1-yl-ethanone (32 mg, 0.25 mmol), and K₂CO₃ (116 mg, 0.84 mmol) in DMF (2 mL) was heated under microwave irradiation at 120 °C for 20 min. The crude reaction mixture was purified by HPLC to give the TFA salt of 10q as a white solid (97 mg, 97%). ¹H NMR (acetone-*d*₆, 400 MHz) δ: 2.07 (s, 3H), 3.30 (bs, 4H), 3.71 (t, *J* = 4.8 Hz, 2H), 3.75 (s, 3H), 3.79 (bs, 2H), 3.87 (s, 3H), 4.60 (t, *J* = 4.8 Hz, 2H), 4.99 (bs, 2H), 6.32 (s, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 9.0 Hz, 1H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.53 (m, 2H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.82 (s, 1H), 7.94 (dd, *J* = 8.8, 2.5 Hz, 1H), 9.56 (s, 1H). LCMS *m/z* = 478.3 [M + H]⁺.

4-[2-[4-(3-Methoxy-benzoylamino)-2-(2-methyl-2*H*-pyrazol-3-yl)-phenoxy]-ethyl]-piperazine-1-carboxylic Acid Amide (10r). 10r was prepared in a manner similar to that described for 10q, using the intermediate from step 2 of 10q (90 mg, 0.21 mmol) and piperazine-1-carboxamide (32 mg, 0.25 mmol) to give the TFA salt of 10r as a white solid (52 mg, 52%). ¹H NMR (CD₃CN, 400 MHz) δ: 2.70–3.25 (m, 6H), 3.30–3.37 (m, 1H), 3.40 (t, *J* = 4.8 Hz, 2H), 3.71 (s, 3H), 3.86 (s, 3H), 4.36 (t, *J* = 4.8 Hz, 2H), 5.13 (bs, 2H), 6.30 (s, 1H), 7.11 (d, *J* = 9.0 Hz, 1H), 7.14 (d, *J* = 8.0 Hz, 1H), 7.43 (t, *J* = 7.8 Hz, 1H), 7.45 (s, 1H), 7.49 (d, *J* = 7.8 Hz, 1H), 7.51 (s, 1H), 7.63 (s, 1H), 7.80 (d, *J* = 9.0 Hz, 1H), 8.75 (s, 1H). LCMS *m/z* = 479.4 [M + H]⁺.

3-Methoxy-*N*-(3-(1-methyl-1*H*-pyrazol-5-yl)-4-[2-(4-(methylsulfonyl)piperazin-1-yl)ethoxy]phenyl)benzamide (10s). 10s was prepared in a manner similar to that described for 10q, using the intermediate from step 2 of 10q (90 mg, 0.21 mmol) and 1-(methylsulfonyl)-piperazine (41 mg, 0.25 mmol) to give the TFA salt of 10s as a white solid (65 mg, 61%). ¹H NMR (CD₃CN, 400 MHz) δ: 2.85 (s, 3H), 3.05–3.67 (m, 8H), 3.44 (t, *J* = 4.7 Hz, 2H), 3.72 (s, 3H), 3.86 (s, 3H), 4.37 (t, *J* = 4.7 Hz, 2H), 6.32 (d, *J* = 1.8 Hz, 1H), 7.10–7.16 (m, 2H), 7.41–7.55 (m, 4H), 7.65 (d, *J* = 2.4 Hz, 1H), 7.82 (dd, *J* = 8.8, 2.5 Hz, 1H), 8.76 (s, 1H). LCMS *m/z* = 514.5 [M + H]⁺.

***N*-(4-[2-(2-Hydroxyethylamino)ethoxy]-3-(1-methyl-1*H*-pyrazol-5-yl)phenyl)-3-methoxybenzamide (10t).** 10t was prepared in a manner similar to that described for 10q, using the intermediate from step 2 of 10q (200 mg, 0.46 mmol) and ethanolamine (43 mg, 0.70 mmol) to give the TFA salt of 10t as a white solid (150 mg, 78%). ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 2.82–2.90 (m, 2H), 3.30–3.36 (m, 2H), 3.50–3.55 (m, 2H), 3.69 (s, 3H), 3.83 (s, 3H), 4.24–4.28 (m, 2H), 6.32 (d, *J* = 1.8 Hz, 1H), 7.14–7.18 (m, 1H), 7.21 (d, *J* = 9.1 Hz, 1H), 7.42–7.55 (m, 4H), 7.61 (d, *J* = 2.4 Hz, 1H), 7.88 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.89–7.95 (br s, 2H), 10.2 (s, 1H). LCMS *m/z* = 411.4 [M + H]⁺.

***N*-(4-(2-Aminoethoxy)-3-(1-methyl-1*H*-pyrazol-5-yl)phenyl)-3-methoxybenzamide (10u).** 10u was prepared in a manner similar to that described for 10q, using the intermediate from step 2 of 10q (500 mg, 1.16 mmol) and ammonia in methanol (11.6 mmol) to give the TFA salt of 10u as a white solid (226 mg, 53%). ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 3.11–3.22 (m, 2H), 3.71 (s, 3H), 3.85 (s, 3H), 4.16 (t, *J* = 5.8 Hz, 2H), 6.36 (d, *J* = 1.8 Hz, 1H), 7.14–7.18 (m, 1H), 7.24 (d, *J* = 9.0 Hz, 1H), 7.42–7.55 (m, 4H), 7.65 (d, *J* = 2.4 Hz, 1H), 7.82 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.89–7.95 (br s, 3H), 10.2 (s, 1H). LCMS *m/z* = 367.5 [M + H]⁺.

Biological Assays. [¹²⁵I]DOI Binding to Recombinant Human 5-HT_{2A} and 5-HT_{2C} Receptors. Radioligand binding assays for

human 5-HT_{2A} and 5-HT_{2C} receptors were developed using crude plasma membranes prepared from HEK293 cells stably expressing these receptors.²¹ The 5-HT₂ agonist [¹²⁵I]DOI (0.5 nM) was used as radioligand and nonspecific radioligand binding was determined in the presence of 10 μM cold DOI. Competition experiments consisted of addition of 95 μL of assay buffer (20 mM HEPES, pH 7.4, 10 mM MgCl₂), 50 μL of membranes (5–25 μg protein), 50 μL of [¹²⁵I]DOI (0.5 nM final assay concentration), and 5 μL of test compound diluted in assay buffer (final concentrations ranging from 1 pM to 10 μM) to 96-well Perkin-Elmer GF/C microtiter plates, and incubations were performed for one hour at room temperature. Each radioligand competition study consisted of testing eight different concentrations in which triplicate determinations were performed for each test compound concentration. Assay incubations were terminated by rapid filtration of microtiter plates under vacuum pressure using a Brandell cell harvester, followed by washing filter plates several times with ice-cold wash buffer (50 mM Tris-HCl, pH 7.4). Plates were then dried at 45 °C in an oven for a minimum of two hours, 25 μL of BetaScint scintillation cocktail was added to each well, and microtiter plates were counted in a Packard TopCount scintillation counter.

Human Platelet Aggregation Assay. Platelet aggregation was measured turbidometrically at 37 °C. Platelet rich plasma was prepared from anticoagulated whole blood obtained from male and female donors. Platelet concentrations in the platelet rich plasma were adjusted to 250000 platelets/μL using platelet poor plasma, then 500 μL of platelet rich plasma was preincubated at 37 °C and stirred at 1200 rpm with inhibitors for 1 min before induction of aggregation by the simultaneous addition of 5-HT (final concentration 1 μM) and ADP (final concentration 1 μM). The maximal amplitude of aggregation response within 3 min was measured in triplicate using the Chronolog model 490 aggregometer. Percent inhibition of aggregation was calculated from the maximum decrease in optical density of the controls and of the samples containing inhibitor.

Rat Pharmacokinetics. Male Sprague-Dawley rats were dosed orally or intravenously at 10 and 2 mg/kg, respectively, in 80% PEG400 and 20% phosphate buffered saline. Animals were fasted overnight prior to oral dose administration. Whole blood samples were collected from the jugular vein over a 24 h period postdose. Plasma was prepared from sodium heparin treated whole blood and separated by centrifugation. Plasma samples were assayed using a selective HPLC/MS/MS method. The HPLC/MS/MS was operated in multiple reaction monitoring (MRM) mode under optimized conditions for detection of selected compounds and the internal standard using positive ions formed by electrospray ionization. Quantitation was determined using a weighted regression analysis of peak area ratios of analyte and internal standard. The method provided a lower limit of quantitation of 1 ng/mL and an upper limit of quantitation of 2000 ng/mL. Serial sampling (at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 15, 18 and 21 h post dosing) was used to define the plasma concentration vs time profile.

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