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SUPERINTENDENCIA DE INDUSTRIA Y COME
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Re. : Código 011-378-444
Expediente No. 06 - 23.144
Fase Nacional de Solicitud
de Patente PCT
CELGENE CORPORATION

En relación con el expediente de la referencia y dando cumplimiento a la última providencia recaída sobre este asunto por medio del presente escrito me permito manifestarles lo siguiente:

1. Con el único propósito de hacer más expedito el trámite de la presente solicitud y sin consentir a las objeciones presentadas en el Oficio de la SIC, adjunto sírvanse encontrar un nuevo pliego de reivindicaciones para reemplazar a las que se presentaron previamente. Las reivindicaciones propuestas están relacionadas, entre otras cosas, con formas cristalinas particulares de la lenalidomida, p.ej., formas cristalinas A, B y E y composiciones farmacéuticas que comprenden las formas cristalinas. En las reivindicaciones propuestas, el término "cerca de" que aparece en las anteriores reivindicaciones 39 - 42, y que corresponde a las reivindicaciones propuestas 19 - 22, ha sido eliminado. Además, las anteriores reivindicaciones 15 - 22 y 27 - 38 han sido canceladas sin la intención de perjudicar los derechos del Solicitante de proseguir el objeto cancelado en una o más solicitudes divisionales.
2. Con el propósito de reforzar los argumentos a favor de la patentabilidad de la materia aquí reclamada, me permito adjuntar también una Declaración del Profesor William Jones, PhD, de la Universidad de Cambridge, la cual se encuentra debidamente legalizada y oficialmente traducida. El Profesor Jones es Profesor del Departamento de Química de la Universidad de Cambridge y es un experto en el campo de la química farmacéutica del estado sólido. En tal sentido, la Declaración es altamente relevante para la evaluación de las formas cristalinas de la lenalidomida que se reivindican aquí. La sociedad que

represento solicita de manera respetuosa que la Superintendencia de Industria y Comercio considere la Declaración del Profesor Jones.

3. El Oficio de la SIC alega que “el estudio de polimorfismo es una tarea habitual en el campo de la farmacéutica en la preformulación de preparaciones farmacéuticas y que la variedad de formas cristalinas en un compuesto permite variar las propiedades de solubilidad, estabilidad y otras”. El Oficio también alega que “dentro de los estudios de preformulación, a menudo se incluye un trabajo riguroso para determinar la presencia de polimorfos en los medicamentos y que los parámetros investigados de forma habitual incluyen la cantidad de polimorfos presentes, el grado relativo de estabilidad de los diversos polimorfos, los intervalos de estabilidad térmica de cada polimorfo, las solubilidades, los métodos de preparación de cada forma, el efecto de la micronización o tableteo y la interacción con componentes de la formulación y una vez que se ha establecido que el compuesto presenta polimorfismo, existen procedimientos a disposición del preformulador para preparar las diferentes formas en cantidades más grandes para evaluación adicional y adaptación... para su incorporación a las formas fisiológicas”.
4. El Oficio de la SIC sostiene que “por tanto, resultaría obvio para la persona medianamente versada en la técnica diseñar una secuencia de cristalización o recristalización en diferentes solventes, o con solventes en diferentes proporciones, con el fin de obtener el polimorfo de interés, sobre todo si se conoce el fundamento teórico de la obtención de cristales por diferencias de solubilidad y cambiando parámetros adicionales como temperatura, velocidad de enfriamiento, agitación, presión, evaporación, etc., con el fin de obtener las formas cristalinas reivindicadas”.
5. Mi representada no se encuentra de acuerdo con las anteriores afirmaciones del Examinador porque el estado de la técnica no enseña ni sugiere formas cristalinas de la lenalidomida, mucho menos las formas cristalinas específicas citadas como parte de las reivindicaciones en cuestión, p.ej., formas cristalinas A, B y E. En el momento de la radicación de la presente solicitud, el estado relevante de la técnica revela, en el mejor de los casos, la estructura química de la lenalidomida. La mera revelación de una estructura química no sugiere ninguna forma cristalina del compuesto en particular. Por ejemplo, el mero conocimiento de la estructura química de la lenalidomida no sugiere si la lenalidomida puede ser precipitada como material cristalino, mucho menos las

formas cristalinas específicas citadas como parte de las reivindicaciones en cuestión. Las reivindicaciones pendientes de la presente solicitud se relacionan con nuevas formas cristalinas de la lenalidomida, lo cual no había sido ni revelado ni sugerido por el estado de la técnica cuando se radicó la presente solicitud. Como lo explica en detalle el Profesor Jones, debido a la complejidad molecular de la lenalidomida, en el momento en que se radicó la presente solicitud, una persona medianamente versada en el estado de la técnica no podría haber predicho las formas cristalinas de la lenalidomida de las reivindicaciones en cuestión. Ver la Declaración del Profesor Jones en las páginas 3 – 6 y 8 – 10.

6. En cuanto hace a la composición química, algunas de las formas cristalinas de la lenalidomida descritas en la presente solicitud contienen solvente (p.ej., agua) en el entramado cristalino. Por ejemplo, la Forma B de la lenalidomida es un hemihidrato y la Forma E de la lenalidomida es un dihidrato. El hemihidrato de la lenalidomida de las reivindicaciones en cuestión comprende cerca de 0,5 equivalentes molares de agua en forma de complejo en la estructura cristalina por mol de la lenalidomida. La fórmula empírica del hemihidrato, por tanto, puede ser descrita como $C_{13}H_{13}N_3O_3 \cdot (0,5 H_2O)$. De manera parecida, el dihidrato de la lenalidomida de las reivindicaciones en cuestión comprende cerca de dos equivalentes molares de agua en forma de complejo en la estructura cristalina por mol de la lenalidomida. Por tal razón, la fórmula empírica del dihidrato cristalino reivindicado puede ser descrita como $C_{13}H_{13}N_3O_3 \cdot (2 H_2O)$. Estas fórmulas empíricas no son descritas o sugeridas por ninguna de las referencias citadas. La persona medianamente versada en la técnica habría predicho que la lenalidomida puede existir como un hemihidrato cristalino o un dihidrato cristalino. Por esta razón adicional, las formas cristalinas reivindicadas no son obvias.
7. Una persona medianamente versada en el estado de la técnica no podría haber obtenido fácilmente las formas cristalinas de la lenalidomida de las reivindicaciones en cuestión. La aseveración en el Oficio de la SIC de que el proceso para obtener las formas cristalinas reivindicadas de la lenalidomida es “habitual” carece enteramente de respaldo y, contraría al estado de la técnica vigente en el momento en que la presente solicitud fue radicada.
8. La experimentación sobre la química del estado sólido de un compuesto farmacéutico puede ser bastante difícil e impredecible. Con base únicamente

en el conocimiento de la estructura química de una molécula farmacéutica, suele ser imposible predecir si la molécula va a formar, p.ej., un aceite, un sólido amorfo, un sólido cristalino, o una mezcla de los anteriores. En teoría, un compuesto puede existir en formas no cristalinas, una forma cristalina, o múltiple formas cristalinas. Como lo explica el Profesor Jones, quien es un experto en el campo de la química farmacéutica del estado sólido, la cantidad de experimentación requerida para verificar y entender la formación de las formas cristalinas de la lenalidomida es sustancial y extensa y el proceso dista de ser habitual. Ver la Declaración del Profesor Jones en las páginas 4 y 6 – 7.

9. Además, las formas cristalinas de la lenalidomida de las reivindicaciones en cuestión poseen propiedades superiores, como, p.ej., estabilidad física y vida útil prolongadas, lo cual no podría haber sido predicho por una persona medianamente versada en la técnica. Sírvanse anotar que el estado de la técnica no enseña o sugiere estas propiedades beneficiosas de las formas cristalinas de la lenalidomida citadas como parte de las reivindicaciones en cuestión, incluidas estabilidad térmica, ausencia de higroscopicidad, solubilidad, disolución, estabilidad física y estabilidad mecánica a largo plazo, todas las cuales pueden afectar la idoneidad de una forma cristalina en particular para usarla como un material farmacéutico. Ver la Declaración del Profesor Jones en las páginas 10 – 13. Algunas de las superiores propiedades de las formas cristalinas reivindicadas de la lenalidomida se resumen a continuación.

i. La presente solicitud revela datos sobre las propiedades beneficiosas e inesperadas de estabilidad térmica asociadas con ciertas formas sólidas reivindicadas de la lenalidomida. Estas propiedades no son enseñadas o sugeridas por los antecedentes citados del estado de la técnica. Por ejemplo:

- Con respecto a la forma cristalina A de la lenalidomida, las curvas diferenciales de calorimetría de barrido (DSC) (Fig. 4) y las curvas del análisis gravimétrico térmico (TGA) (Fig. 4) reveladas en la presente solicitud indican que la Forma A no sufre transiciones térmicas significativas por debajo de cerca de 270°C, la temperatura a la cual se funde. La ausencia de cualquier evento térmico significativo por debajo de cerca de 270°C indica que la Forma A posee estabilidad térmica inesperada y beneficiosa para muchos

procesos farmacéuticos, como secado, formulación y almacenamiento.

- Con respecto a la Forma cristalina B de la lenalidomida, las curvas diferenciales de calorimetría de barrido (DSC) (Figs. 9,42,43 y 44), las curvas del análisis gravimétrico térmico (TGA) (Figs. 9,37,38 y 39) y los datos de espectroscopia térmica gravimétrica infrarroja (TG-IR) (Fig. 10) reveladas en la presente solicitud indican que la Forma B no sufre transiciones térmicas significativas por debajo de cerca de 146°C, temperatura a la cual pierde el agua de hidratación. La ausencia de cualquier evento térmico significativo por debajo de cerca de 146°C indica que la Forma B posee estabilidad térmica inesperada y beneficiosa para muchos procesos farmacéuticos, como secado, formulación y almacenamiento.
- Con respecto a la Forma cristalina E de la lenalidomida, las curvas DSC (Figs. 24 y 45) y las curvas TGA (Figs. 24 y 40) para la Forma E indican que esta forma cristalina no sufre transiciones térmicas significativas por debajo de cerca de 90°C, temperatura a la cual pierde agua de hidratación. La ausencia de cualquier evento térmico significativo por debajo de cerca de 90°C indica que la Forma E posee estabilidad térmica inesperada y beneficiosa para muchos procesos farmacéuticos, como secado, formulación y almacenamiento.

La estabilidad térmica suele ser reconocida como una importante propiedad para el desarrollo exitoso de un compuesto farmacéutico. El hecho de que las Formas A, B y E son térmicamente estables ilustra un aspecto clave del nivel inventivo asociado con las presentes reivindicaciones.

- ii. La presente solicitud también revela datos sobre las propiedades inesperadas y beneficiosas de sorción/desorción de humedad asociadas con ciertas formas sólidas reivindicadas de la lenalidomida. Estas propiedades no son enseñadas o sugeridas por el estado de la técnica. Por ejemplo:

- En lo que respecta a la forma cristalina A de la lenalidomida, la isoterma de sorción/desorción de humedad (Fig. 5) revelada en la presente solicitud indica que la Forma A no exhibe una ganancia de

peso significativa (p.ej., menos de 1% por peso hasta cerca de 95% de humedad relativa). Según esto, la Forma A es sustancialmente no higroscópica.

- En lo que respecta a la Forma cristalina B de la lenalidomida, la isoterma de sorción/desorción de humedad (Fig. 11) reveladas en la presente solicitud indica que la Forma B no exhibe una ganancia de peso significativa (p.ej., menos de 1 % por peso hasta cerca de 95% de humedad relativa). Según esto, la Forma B es sustancialmente no higroscópica.
- En lo que respecta a la Forma cristalina E de la lenalidomida, la isoterma de sorción/desorción de humedad (Fig. 25) revelada en la presente solicitud indica que la Forma E no exhibe una ganancia de peso significativa (p.ej., menos de 1% por peso hasta cerca de 95% de humedad relativa). Según esto, la Forma E es sustancialmente no higroscópica.

La ausencia de higroscopicidad es una propiedad beneficiosa importante para el desarrollo exitoso de un producto farmacéutico, que puede ser crítica, p.ej., para la formulación, el procesamiento y las consideraciones de almacenamiento para un material farmacéutico. El hecho de que las Formas A, B y E sean no higroscópicas ilustra un aspecto clave del nivel inventivo asociado con las presentes reivindicaciones.

iii. La presente solicitud también revela datos concernientes a las propiedades de solubilidad y disolución asociadas con ciertas formas sólidas reivindicadas de la lenalidomida. Estas propiedades no son enseñadas o sugeridas por el estado de la técnica. Por ejemplo:

- En lo que respecta a la forma cristalina A de la lenalidomida, la presente solicitud revela datos de solubilidad en las páginas 27 – 28 y en la Figura 51. Estos datos indican que la Forma A tiene una solubilidad beneficiosa, hasta cerca de 6,2 mg/mL, en el medio de disolución seleccionado. Ver la Tabla 6 en la página 27. La presente solicitud también contiene datos de una tasa de disolución beneficiosa para la Forma A en las páginas 28 – 30 y en las Figuras 53 – 54.

- En lo que respecta a la Forma cristalina B de la lenalidomida, la presente solicitud revela datos de solubilidad en las páginas 27 – 28 y en la Figura 52. Estos datos indican que la Forma B tiene una solubilidad beneficiosa, hasta cerca de 5,8 mg/mL, en el medio de disolución seleccionado. Ver la Tabla 6 en la página 27. Además, este estudio indica que la Forma B también tiene buena estabilidad en agua. Ver la página 27, líneas 19-21 y la tabla 7 en la página 28. La presente solicitud también contiene datos de una tasa de disolución beneficiosa para la Forma B en las páginas 28-30 y en las Figuras 53-54.
- En lo que respecta a la Forma cristalina E de la lenalidomida, la presente solicitud revela datos de solubilidad en las páginas 27-28. Estos datos indican que la Forma E tiene una solubilidad beneficiosa, hasta cerca de 4,7 mg/mL, en el medio de disolución seleccionado. Ver la Tabla 6 en la página 27. La presente solicitud también contiene datos de una tasa de disolución beneficiosa para la Forma E en las páginas 28-30 y en las Figuras 53-54.

Los datos de una tasa beneficiosa de solubilidad y disolución, como los que se presentan para las Formas A, B y E en la presente solicitud, suponen un importante indicador de biodisponibilidad suficiente para una forma sólida de un compuesto farmacéutico. La biodisponibilidad, por su parte, es un factor clave en la eficacia terapéutica de una forma sólida de un compuesto farmacéutico. Los datos de una tasa beneficiosa de solubilidad y disolución de las Formas A, B y E ilustran un aspecto clave del nivel inventivo asociado con las presentes reivindicaciones.

iv. La presente solicitud también revela datos concernientes a las propiedades de estabilidad física a largo plazo asociadas con ciertas formas sólidas reivindicadas de la lenalidomida. Estas propiedades no son enseñadas o sugeridas por el estado de la técnica. Por ejemplo:

- En lo que respecta a la forma cristalina A de la lenalidomida, la presente solicitud revela datos de estabilidad física a largo plazo en condiciones particulares de estrés de temperatura/humedad en la página 8, líneas 29-31. Estos datos indican que la Forma A típicamente no se convierte en una forma sólida diferente bajo dos condiciones diferentes de estrés de temperatura/humedad relativa

(temperatura ambiente/0% HR y 40°C/93% HR) durante un periodo de hasta cerca de 85 días.

- En lo que respecta a la Forma cristalina B de la lenalidomida, la presente solicitud revela datos de estabilidad física a largo plazo en condiciones particulares de estrés de temperatura/humedad en la página 10, líneas 3-5. Estos datos indican que la Forma B no se convierte en una forma sólida diferente bajo dos condiciones diferentes de estrés de temperatura/humedad relativa (temperatura ambiente/0% HR y 40°C/93% HR) durante un periodo de hasta cerca de 85 días.
- En lo que respecta a la Forma cristalina E de la lenalidomida, la presente solicitud revela datos de estabilidad física a largo plazo en condiciones particulares de estrés de temperatura/humedad en la página 13, líneas 6-9. Estos datos indican que la Forma E típicamente no se convierte en una forma sólida diferente bajo dos condiciones diferentes de estrés de temperatura/humedad relativa (temperatura ambiente/0% HR y 40°C/93% HR) durante un periodo de hasta cerca de 85 días.

La estabilidad física beneficiosa a largo plazo es un factor importante para el almacenamiento y la vida útil de una forma sólida de un compuesto farmacéutico. Los datos beneficiosos de estabilidad física a largo plazo de las Formas A, B y E ilustran un aspecto clave del nivel inventivo asociado con las presentes reivindicaciones.

- v. La presente solicitud también proporciona datos en lo que respecta a propiedades de estabilidad mecánica asociadas con ciertas formas sólidas reivindicadas de la lenalidomida. Estas propiedades no son enseñadas o sugeridas por el estado de la técnica. Los datos de estabilidad mecánica indican la idoneidad de una forma sólida en particular para ciertas condiciones de procesamiento regularmente empleadas en el desarrollo farmacéutico, como molido y tableteado. La presente solicitud revela datos concernientes a la estabilidad mecánica de las Formas A y B con respecto a compresión y molienda. Ver la página 28, líneas 8-11 y la tabla 7. La estabilidad mecánica beneficiosa revelada para las Formas A y B indica la idoneidad para las condiciones típicas de procesamiento que se encuentran en operaciones de desarrollo farmacéutico. El hecho de que las Formas A y

9

B tengan estabilidad mecánica beneficiosa ilustra un aspecto del nivel inventivo asociado con las presentes reivindicaciones.

10. En resumen, las formas cristalinas reivindicadas poseen propiedades beneficiosas e inesperadas, las cuales no habrían sido predichas por una persona medianamente versada en la técnica. Por esta razón adicional, las formas cristalinas de la lenalidomida tienen nivel inventivo y representan un avance tecnológico significativo. Según esto, el objeto aquí reivindicado es inventivo por esta razón adicional.

11. Teniendo en cuenta la sentencia proferida dentro del Expediente No. 7684 el 22 de Octubre de 2004 y acogida por esa Superintendencia mediante memorando interno de fecha 22 de Diciembre de 2004 dirigido a los funcionarios de la Delegatura de Propiedad Industrial, no se hace necesario acompañar comprobante de pago por concepto de modificación de la solicitud por cuanto el nuevo pliego reivindicatorio se presenta a instancia de la Superintendencia en cumplimiento de lo ordenado por el artículo 45 de la Decisión 486.

Renuncio al término faltante de traslado, y les solicito ordenar la publicación del extracto.

De Ustedes Atentamente,


LUIS FELIPE CASTILLO GIBSONE
T.P. No. 68.995 del C.S.J.

Reivindicaciones

1. 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina que tiene un patrón de difracción de rayos X en el polvo que comprende picos aproximadamente a 8, 14,5 y 16 grados 2θ .
2. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de la reivindicación 1, en donde el patrón comprende picos en 7,9, 14,4 y 15,8 grados 2θ .
3. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de la reivindicación 1, en donde el patrón además comprende picos aproximadamente a 17,5, 20,5, 24 y 26 grados 2θ .
4. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de la reivindicación 3, en donde el patrón comprende picos en 17,6, 20,6, 24,1 y 26,0 grados 2θ .
5. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de cualquiera de las reivindicaciones 1-4, la cual tiene un termograma de calorimetría de barrido diferencial que comprende una endoterma con un máximo en cerca de 270°C .
6. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de cualquiera de las reivindicaciones 1-5, que no está solvatada.
7. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina que tiene un patrón de difracción de rayos X en el polvo que comprende picos aproximadamente a 16,22 y 27 grados 2θ .
8. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de la reivindicación 7, en donde el patrón comprende picos en 15,8, 22,2 y 26,7 grados 2θ .
9. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de la reivindicación 7, en donde el patrón además comprende un pico aproximadamente a 18 grados 2θ .
10. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de la reivindicación 9, en donde el patrón comprende un pico en 18,2 grados 2θ .
11. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de la reivindicación 7, que tiene un termograma de calorimetría de barrido diferencial que comprende endotermas con máximos en cerca de 146°C y cerca de 268°C .
12. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de la reivindicación 7, la cual pierde cerca de 3,1% de volátiles con base en el peso cuando se calienta a cerca de 175°C .
13. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de la reivindicación 7, que tiene aproximadamente 0,46 moles de agua por mol de 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona.
14. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de cualquiera de las reivindicaciones 7- 13, que está hemihidratada.

- 11
15. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina que tiene un patrón de difracción de rayos X en el polvo que comprende picos aproximadamente a 20,24.5 y 29 grados 2 θ .
 16. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de la reivindicación 15, en donde el patrón comprende picos en 20,0, 24,7 y 28.6 grados 2 θ .
 17. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de la reivindicación 15 o 16 que tiene un termograma de calorimetría de barrido diferencial que comprende endotermas con máximos en cerca de 99°C y cerca de 269°C.
 18. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de cualquiera de las reivindicaciones 15- 17, la cual está dihidratada.
 19. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de cualquiera de las reivindicaciones 1-1 8, la cual es más de 80% pura.
 20. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de cualquiera de las reivindicaciones 1-1 8, la cual es más de 90% pura.
 21. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de cualquiera de las reivindicaciones 1-1 8, la cual es más de 95% pura.
 22. La 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de cualquiera de las reivindicaciones 1 - 18, la cual es más de 97% pura.
 23. Una mezcla de la 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de cualquiera de las reivindicaciones 7-14 y la 3-(4-amino-1-oxo-1,3 dihidroisoindol-2-il)-piperidin-2,6-diona cristalina de cualquiera de las reivindicaciones 15- 18.
 24. Una composición farmacéutica que comprende 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona amorfa y la 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona de cualquiera de las reivindicaciones 1-18.
 25. La composición de la reivindicación 24, la cual comprende más de 50% en peso de 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina.
 26. Una composición farmacéutica que comprende la 3-(4-amino-1-oxo-1,3 dihidro-isoindol-2-il)-piperidin-2,6-diona cristalina de cualquiera de las reivindicaciones 1-22 y un excipiente farmacéuticamente aceptable.
 27. La composición farmacéutica de la reivindicación 26, en donde la composición es una forma de dosificación en dosis unitaria.

12

DECLARATION OF PROFESSOR WILLIAM JONES, Ph.D.

Sir:

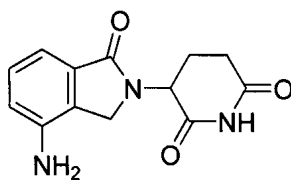
I, WILLIAM JONES, do hereby declare and state:

1. I am presently a Professor in the Department of Chemistry at the University of Cambridge, which is located in Cambridge, England.

2. I have over 35 years of experience conducting research in the field of chemistry. My academic and technical experience and honors, and a list of my publications, are set forth in my curriculum vitae, a copy of which is attached as Appendix A. As indicated by the publication list on my curriculum vitae, a significant portion of my current research involves the field of pharmaceutical solid-state chemistry.

3. I have read and am familiar with the disclosure of International Patent Application No. PCT/US2004/028736, which I will refer to as the "736 Application" in this Declaration. I understand that the earliest filing date of the '736 Application is September 4, 2003.

4. In my opinion, the technology described in the '736 Application relates to pharmaceutical solid-state chemistry and polymorphism, particularly with respect to the compound known as lenalidomide, which can be described by at least the following chemical names: 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione; 3-(4-amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione; 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline; and 3-(1-oxo-4-aminoisoindolin-2-yl)piperidine-2,6-dione. The '736 Application provides a chemical name for lenalidomide at page 1, lines 29–30, and depicts the chemical formula of lenalidomide at page 6, line 14. The chemical formula is also provided here as Formula (I).



(I)

13

5. I understand that Colombian Patent Application No. 06023144 corresponds to the '736 Application. I understand that an office action (which I will refer to as the "Colombian Office Action" in this Declaration) has been issued by the Colombian Patent Office in connection with this Colombian patent application. A copy of the Office Action and an English translation are attached as Appendix B. I understand that the Colombian Office Action contends that lenalidomide crystal forms described in this Colombian patent application are not innovative in view of the state of the art as of September 4, 2003. I understand and believe that the state of the art as of that date includes the information disclosed in U.S. Patent No. 6,281,230, which I will refer to as the '230 Patent in this Declaration. I understand that the Colombian Office Action also cites Chapter 76 of the 17th Edition of REMINGTON'S PHARMACEUTICAL SCIENCES (which I will refer to as the "Remington Reference" in this Declaration), which is published before September 4, 2003.

6. I have read and am familiar with the set of claims, attached hereto as Appendix C, which I understand will be submitted together with this Declaration and the response to the Colombian Office Action. I have been asked to provide my opinion about whether lenalidomide crystal forms described in the '736 Application are innovative in view of the state of the art as of September 4, 2003.

7. In the following paragraphs, I will provide my opinion that the lenalidomide crystal forms described in the '736 Application are innovative. I will support my opinion by discussing the state of the art as of September 4, 2003, including the '230 Patent and the Remington Reference. I will also discuss unexpected properties associated with particular lenalidomide crystal forms described in the '736 Application. Appendix D provides copies of the references I have cited in this Declaration.

I. THE LENALIDOMIDE CRYSTAL FORMS OF THE '736 APPLICATION ARE INNOVATIVE IN VIEW OF THE STATE OF THE ART

8. I understand that the Colombian Office Action contends that the lenalidomide crystal forms described in the '736 Application are not innovative in view of the state of the art as of September 4, 2003. As I explain in this Declaration, I disagree with this

124

contention. It is my opinion that the lenalidomide crystal forms described in the '736 Application could not have been predicted or expected in view of the state of the art as of September 4, 2003, including the '230 Patent and the Remington Reference.

A. The Existence of Particular Lenalidomide Crystal Forms Could Not Have Been Predicted From the Chemical Name and Formula of Lenalidomide

9. The '230 Patent discloses a chemical name for lenalidomide at, *e.g.*, column 9, lines 25–26, and a general chemical formula encompassing lenalidomide at column 4, lines 43–53. However, prior knowledge of the chemical name and formula of lenalidomide does not affect the novelty or innovation associated with the lenalidomide crystal forms described in the '736 Application. As of September 4, 2003, based only on a knowledge of the chemical name and formula of lenalidomide, an ordinary researcher would not have been able to predict the formation of particular lenalidomide crystal forms or their associated advantageous properties. It is my opinion that the crystal forms described in the '736 Application represent an important advance toward facilitating the use of lenalidomide as a pharmaceutical material, and are innovative.

10. It appears that the Colombian Office Action contends that the lenalidomide crystal forms of the '736 Application are not inventive because “the study of polymorphism is a routine task in the field of pharmaceuticals in the pre-formulation of pharmaceutical preparations, and that the variety of crystalline forms in a compound allows the properties of solubility, stability, and others to be varied.” Specifically, the Colombian Office Action contends that “it is a routine task for the expert (pre-formulator) to determine whether the compound exists in one or more crystalline forms, the number of polymorphs that may exist, and determine their character and physical and chemical characteristics . . . once they had been characterized, there are procedures by which the pre-formulator may prepare the various forms in larger quantities for additional evaluation . . . [t]herefore, the development of polymorphic forms of a compound to improve its physical and chemical properties is a problem which has already been solved in the state of the art, and is not considered to be inventive.” I disagree with these contentions.

11. As I explain in this declaration, in my opinion, the chemical formula of a particular organic compound does not disclose or suggest any specific solid form(s) in which the compound may precipitate. Based only on a knowledge of the chemical formula of a particular organic compound, it is generally not possible to predict whether the compound will form an oil, an amorphous solid, a crystalline solid, or a mixture thereof.

12. Predicting the nature of solid form(s) that might be generated for an organic compound can be further complicated by the possibility of solvate formation. Without detailed experimentation, an ordinary researcher cannot predict whether a particular organic compound will crystallize with or without the incorporation of solvent (e.g., water) in the crystal lattice. Determining whether a compound will crystallize as a solvate (e.g., a hydrate) requires substantial experimentation and cannot be predicted based only on the molecular formula of the compound.

13. As a result of the high degree of unpredictability in this field, researchers must perform detailed experimentation to ascertain and understand the formation of any crystal form(s) comprising a particular organic compound. The amount of experimentation required to ascertain and understand the formation of crystal forms comprising a particular organic compound can be substantial and extensive. The process is certainly not routine. As I explain in this Declaration, this is true in the case of lenalidomide crystal forms described in the '736 Application.

a. An Ordinary Researcher Could Not Have Predicted the Formation of the Lenalidomide Crystal Forms Described in the '736 Application

14. Each lenalidomide crystal form disclosed in the '736 Application is a new material with new properties, representing a significant advance toward understanding and controlling the solid-state chemistry of lenalidomide. In view of the state of the art as of September 4, 2003, an ordinary researcher would not have predicted the particular crystal forms described in the '736 Application, nor the particular conditions or experimental variables for forming the crystal forms, such as temperature or solvent, for example.

nb

15. As of September 4, 2003, an ordinary researcher could not have anticipated whether under a given set of experimental conditions lenalidomide would precipitate as, *e.g.*, an oil, an amorphous solid, an unsolvated crystalline material, a solvated crystalline material, or a mixture of one or more thereof. An ordinary researcher would also have been unable to predict the number of lenalidomide crystalline forms, if any, that could be obtained in the laboratory. I explained in 2006 that “it is not generally possible to computationally predict the number of observable polymorphs of even the simplest molecules.” Jones, W., *et al.*, Mater. Res. Bull. (2006), 31:875–79, at 875 (emphasis added). This statement applies to lenalidomide as of September 4, 2003.

16. In terms of chemical composition, certain of the lenalidomide crystal forms described in the '736 Application contain solvent (*e.g.*, water) in the crystal lattice, an unpredictable result that carries important implications for the development of a pharmaceutical compound. Lenalidomide crystal forms containing solvent in the crystal lattice have a different chemical composition from lenalidomide itself. For example, lenalidomide Form B is a hemihydrate ('736 Application at page 9, lines 15–19) and lenalidomide Form E is a dihydrate ('736 Application at page 7, lines 20–21). Thus, whereas the empirical formula of lenalidomide is $C_{13}H_{13}N_3O_3$, the Form B crystal form of lenalidomide has an empirical formula of $C_{13}H_{13}N_3O_3 \cdot \frac{1}{2}(H_2O)$, and the Form E crystal form of lenalidomide has an empirical formula of $C_{13}H_{13}N_3O_3 \cdot 2(H_2O)$. As of September 4, 2003, an ordinary researcher would not have predicted or expected these particular crystalline solvates (*e.g.*, hydrates), or others described in the '736 Application.

17. Molecular complexity, including molecular flexibility and the presence of competing hydrogen-bond donors and acceptors, is an obstacle to the prediction of possible crystal form(s) for a compound. See Day, G.M., *et al.*, Cryst. Growth Des. (2004), 4(6):1327–40. The lenalidomide chemical formula, as shown in FIG. 1 below, possesses both conformational flexibility and multiple hydrogen-bond donors and acceptors, each of which would have complicated any attempt at predicting the crystal forms of lenalidomide as of September 4, 2003.

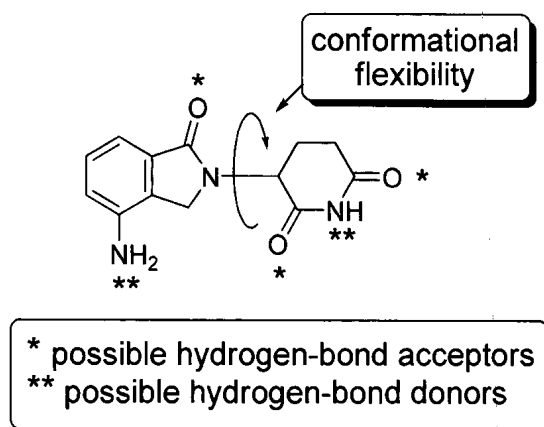


FIG. 1

18. In addition to molecular complexity, the number of molecule(s) in the crystallographic asymmetric unit was a significant limitation to structure prediction as of September 4, 2003. I explained in 2006 that “[t]he prediction of crystal structures with more than one molecule in the asymmetric unit . . . represents a major challenge in the field of crystal structure prediction.” Cruz Cabeza, A.J., *et al.*, J. Am. Chem. Soc. (2006), 128:14466–67, at 14466. In practice, this means that the formation and stoichiometric ratio of crystalline solvates, such as crystalline hydrates, were generally unpredictable as of September 4, 2003. Many of the lenalidomide crystal forms disclosed in the '736 Application, including Forms B and E, comprise solvent in the crystal lattice. It is my opinion that the demonstrated and unpredictable propensity of lenalidomide to incorporate solvent of crystallization would have represented a serious obstacle to the prediction of the crystal forms described in the '736 Application. Determining whether lenalidomide would crystallize as a solvate (such as, *e.g.*, a hydrate) would have required substantial laboratory experimentation and could not have been predicted or expected based on the chemical formula of lenalidomide.

b. An Ordinary Researcher Could Not Have Predicted the Conditions Required to Prepare the Lenalidomide Crystal Forms in the '736 Application

19. It is my opinion that as of September 4, 2003, an ordinary researcher could not have predicted the conditions required to prepare the crystal forms of lenalidomide disclosed in the '736 Application. Moreover, I believe that the lenalidomide crystallization

18

conditions described in the '736 Application represent an important advance toward facilitating the use of lenalidomide as a pharmaceutical material.

20. The '736 Application describes special difficulties that the inventors encountered in isolating pure lenalidomide crystal forms. The '736 Application explains that multiple batches of lenalidomide solid material were obtained as “apparent mixtures of polymorphs” of lenalidomide ('736 Application at page 17, line 20) and that various crystallizations resulted in a product containing mixtures of lenalidomide crystal forms ('736 Application at pages 19–21, Tables 1, 2, and 3). A reproducible, robust process for making pure batches of a single crystal form is critical to the successful development and manufacture of essentially all pharmaceutical compounds. Indeed, the '736 Application explained the importance of deriving “strict polymorphic controls in the validation batches and future manufacturing” of the lenalidomide active pharmaceutical ingredient ('736 Application at page 17, lines 23–24).

21. In view of the apparent propensity of lenalidomide to crystallize as a mixture of polymorphs, the inventors faced special challenges in developing reproducible, robust crystallization processes. For example, the '736 Application explained that lenalidomide Form B appeared to be metastable, *i.e.*, it was a kinetically controlled product of crystallization that subsequently, over time, may convert into a mixture of Forms B and E under certain conditions ('736 Application at page 18, lines 13–18). As the '736 Application describes, the inventors studied experimental variables including, for example, reaction scale, crystallization solvent, slurry temperature, solvent volume, cooling period, holding period, drying temperature, vacuum drying, filtration temperature, and filtration time ('736 Application at pages 18–19). Before the inventors performed these studies, the effects of each of these variables on the solid-state behavior of lenalidomide could not have been predicted.

22. It is my opinion that the conditions described in the '736 Application for obtaining pure batches of specific lenalidomide crystal forms, including the preferred process parameters for Forms B and E, represent important advances that facilitate the use of lenalidomide as a pharmaceutical compound.

9

B. The Existence of Particular Lenalidomide Crystal Forms Could Not Have Been Predicted from the '230 Patent and the Remington Reference

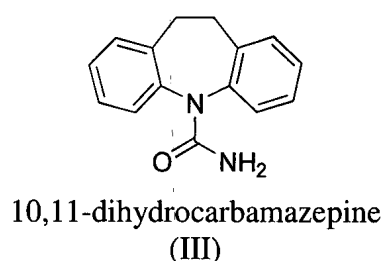
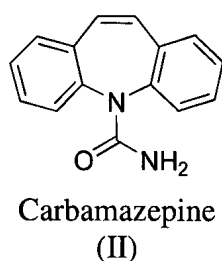
23. I understand that the Colombian Office Action contends that the lenalidomide crystal forms of the '736 Application are not innovative in view of the '230 Patent and the Remington Reference. I disagree with this contention.

24. The '230 Patent does not describe or suggest crystal forms of racemic lenalidomide, including the crystal forms described in the '736 Application. For example, the '230 Patent does not describe crystal forms of racemic lenalidomide, nor does it suggest whether or not racemic lenalidomide may be prepared as a crystalline material. In particular, the '230 Patent does not describe the particular crystal forms identified in the '736 Application. The '230 Patent also does not describe or suggest whether lenalidomide could form crystalline solvates, such as, the particular crystalline hydrates described in the '736 Application, including Forms B and E. In addition, the '230 Patent does not describe or suggest particular conditions for forming the lenalidomide crystal forms described in the '736 Application. Thus, in view of the '230 Patent, an ordinary researcher could not have predicted or expected the lenalidomide crystal forms described in the '736 Application.

25. The Remington Reference does not provide any specific information relating to the lenalidomide crystal forms described in the '736 Application. For example, the Remington Reference does not describe or suggest any particular compound related to lenalidomide, any particular crystal form of lenalidomide, or any particular condition for preparing the lenalidomide crystal forms described in the '736 Application. At most, the Remington Reference describes background information on drug formulation in general terms, which does not provide specific guidance on how to obtain lenalidomide crystal forms, and most certainly not those described in the '736 Application. Notably, the author of the Remington Reference stated that “[w]e are in an era in the pharmaceutical industry where we can no longer rely on past experience to formulate. We must have a thorough understanding of the physical-chemical properties as well as the pharmacokinetic and biopharmaceutical behavior of each drug substance being developed” (fourth paragraph of the Remington Reference). Thus, an ordinary researcher would not expect that a procedure that allegedly

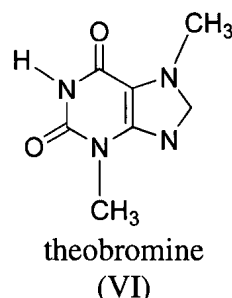
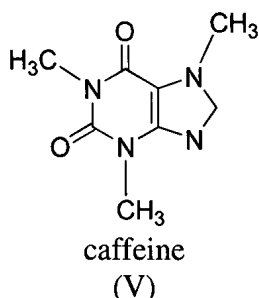
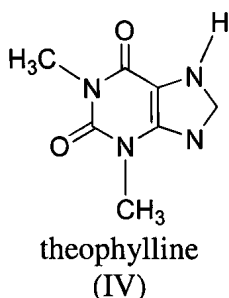
crystallizes one compound could be applied with comparable success to another compound, such as lenalidomide.

26. Studies published by my own research group illustrate that the crystallization behavior and solid-state characteristics of even structurally similar compounds can be remarkably different. I co-authored a publication describing the crystallization behavior of the pharmaceutical compounds carbamazepine and 10,11-dihydrocarbamazepine, depicted below as Formulae (II) and (III). See Cruz Cabeza, A.J., *et al.*, Cryst. Growth Des. (2007), 7(1):100–07.



27. The molecular formulae of these two compounds are very similar: carbamazepine differs from the dihydro compound by only two hydrogen atoms (*i.e.*, one double bond). Despite this close molecular similarity, however, these molecules possess significant differences in their solid-state characteristics and properties. For example, the dihydro compound readily crystallizes from ethanol to yield a crystal form consisting of hydrogen-bonded chains. However, even after extensive experimental effort that entailed subjecting carbamazepine to 66 different solvents (including ethanol) and 5 different crystallization protocols, a similar chain-like crystal form could not be obtained. Even our attempts at seeding a saturated solution of carbamazepine with the chain-like crystal form of the dihydro compound were unsuccessful.

28. The scientific literature provides many other instances of great differences in crystallization behavior arising between chemically similar molecules. For example, three xanthine compounds with pharmacological activity—theophylline, caffeine, and theobromine—differ by at most only one methyl group, as shown in Formulae (IV), (V), and (VI), below.



29. Despite the chemical similarity among these three compounds, they possess important solid-state differences regarding, *inter alia*, their ability to crystallize as hydrated crystal forms. Theophylline can form a relatively stable crystalline monohydrate. Caffeine, by contrast, does not form a monohydrate, but instead forms a non-stoichiometric 0.8-hydrate. Theobromine, unlike the other two compounds, does not form a hydrated crystal form at all. *See, e.g.,* Bruns, S., *et al.*, *Thermochimica Acta* (1984), 72:31–40, at 36.

30. As I have illustrated with the preceding examples, one would not expect that a crystallization procedure for one compound could be applied with comparable success to another compound, even for compounds with similar structures. Accordingly, an ordinary researcher would not expect that a procedure for crystallizing a compound other than lenalidomide could be applied with comparable success to lenalidomide. It is therefore my opinion that the lenalidomide crystal forms of the '736 Application are not described or suggested by the '230 Patent or the Remington Reference.

II. THE LENALIDOMIDE CRYSTAL FORMS OF THE '736 APPLICATION POSSESS SUPERIOR PROPERTIES UNEXPECTED FROM THE '230 PATENT AND THE REMINGTON REFERENCE

31. The lenalidomide crystal forms described in the '736 Application possess unexpected properties. For this additional reason, the lenalidomide crystal forms described in the '736 Application are innovative and represent a significant technological advance.

32. The '736 Application discloses advantageous properties associated with particular lenalidomide crystal forms. It is my opinion that as of September 4, 2003, the

172

properties associated with these lenalidomide crystal forms would not have been reasonably expected by an ordinary researcher based upon information in the '230 Patent and the Remington Reference.

33. I noted in 2006 that “the physical form of an [active pharmaceutical ingredient] represents a strategic opportunity for optimizing such physical properties as solubility, dissolution rate, hygroscopicity, physical stability and chemical stability.” Jones, W., *et al.*, *Mater. Res. Bull.* (2006), 31:875–79, at 875. The '736 Application represents an innovative step toward the optimization of lenalidomide as a solid-state pharmaceutical material. Examples of unexpected properties associated with particular lenalidomide crystal forms are summarized below.

34. The '736 Application provides data regarding thermal properties associated with the lenalidomide crystal forms; these properties could not have been predicted or expected based upon information in the '230 Patent and the Remington Reference. For example, for certain crystal forms, the '736 Application provides DSC, TGA, and/or TG-IR data (TG-IR is a thermal technique which couples TGA with IR spectroscopy for analyzing emitted vapors, such as volatile solvents). Thermal data such as these indicate the suitability of a particular crystal form for processing conditions regularly employed in pharmaceutical development, such as drying, milling, and storage, among others. For example, the DSC, TGA, and TG-IR plots for Form B, provided in the '736 Application at Figures 9 and 10, indicate that Form B does not undergo significant thermal transitions below about 146 °C, at which temperature it loses water of hydration. The '736 Application also provides DSC and TGA data for other crystal forms, such as Forms A and E at Figures 4 and 24. These data indicate that certain lenalidomide crystal forms, such as Forms A, B, and E, have beneficial thermal stability properties suitable for pharmaceutical processing and storage.

35. The '736 Application provides data regarding moisture sorption/desorption properties associated with certain lenalidomide crystal forms; these properties could not have been predicted or expected based upon information in the '230 Patent and the Remington Reference. Non-hygroscopicity is critical, *e.g.*, for formulation,

73

processing, and storage of pharmaceutical materials. For example, a moisture sorption/desorption isotherm for Form B is provided in the '736 Application at Figure 11. The '736 Application also provides moisture sorption/desorption data for Forms A and E at Figures 5 and 25. The plots indicate that Forms A, B, and E are substantially non-hygroscopic, suggesting that Forms A, B, and E possess properties desirable for the development of a pharmaceutical product.

36. The '736 Application provides data regarding solubility and dissolution rate associated with certain lenalidomide crystal forms; these properties could not have been predicted or expected based upon information in the '230 Patent and the Remington Reference. Solubility and dissolution rate properties can be critical for assessing the bioavailability and pharmacological efficacy of a solid form of a pharmaceutical compound. For example, the '736 Application discloses Form B solubility data at pages 27–28 and at Figure 52. These data indicate that Form B has appreciable solubility, about 5.8 mg/mL, in the selected dissolution medium. *See* Table 6 at page 27. This study also indicated that the undissolved Form B crystals did not convert to another solid form, therefore demonstrating that Form B has good stability in water. *See* page 27, lines 19–21, and Table 7 at page 28. The '736 Application reports dissolution data for Form B at pages 28–30 and at Figures 53–54. The '736 Application also provides solubility and dissolution data for Forms A and E at pages 27–28, pages 28–30, and at Figures 51 and 53–54. Beneficial solubility and dissolution rate data, such as those provided in the '736 Application, may be used to assess whether a particular solid form of a pharmaceutical compound will be bioavailable, an important characteristic affecting the biological and therapeutic properties associated with a solid form of a pharmaceutical compound.

37. The '736 Application provides data regarding long-term physical stability properties associated with certain lenalidomide crystal forms; these properties could not have been predicted or expected based upon information in the '230 Patent and the Remington Reference. Data such as these indicate the suitability of a particular crystal form with regard to important storage and shelf-life considerations. For example, the '736 Application provides the results of long-term physical stability studies for Form B under particular temperature/humidity stress conditions at page 10, lines 3–5. These data indicate

24

that Form B does not convert to a different solid form under two different conditions (room temperature/0%RH and 40°C/93%RH) for a period of up to about 85 days. The '736 Application also provides similar long-term physical stability data for Forms A and E, at page 8, lines 29–31, and page 13, lines 6–9. These results indicate that Forms A, B, and E have beneficial long-term physical stability suitable for long-term storage as a pharmaceutical product.

38. The '736 Application provides data regarding mechanical stability properties associated with certain lenalidomide crystal forms; these properties could not have been predicted or expected based upon information in the '230 Patent and the Remington Reference. Mechanical stability data indicate the suitability of a particular solid form for certain processing conditions regularly employed in pharmaceutical development, such as milling and tableting. For example, the '736 Application discloses data regarding the mechanical stability of Forms A and B with respect to compression and grinding at page 28, lines 8–11, and Table 7. The mechanical stability disclosed for Forms A and B indicates their suitability for typical processing conditions encountered in pharmaceutical development operations.

39. Thus, lenalidomide crystal forms described in the '736 Application, such as Forms A, B, and E, possess beneficial and unexpected properties. These specific beneficial properties would not have been predicted or expected by an ordinary research as of September 4, 2003. Accordingly, in my opinion, the claimed lenalidomide crystal forms are inventive for this additional reason.

III. CONCLUSION

40. In view of my analysis, I conclude that an ordinary researcher as of September 4, 2003 would not have been able to predict the formation or properties of the lenalidomide crystal forms described in the '736 Application, and that the lenalidomide crystal forms of the '736 Application possess unexpected properties, representing a significant advance in the solid-state chemistry of lenalidomide. I therefore disagree with the Colombian Office Action, and I conclude that the claimed lenalidomide crystal forms are novel and innovative.

25

41. I declare further that all statements made in this Declaration of my own knowledge are true, and that all statements made on information and belief are believed to be true.

Respectfully submitted,

May 3rd, 2011
Date

William Jones
William Jones

Attachments:

- Appendix A: Curriculum Vitae of Professor William Jones, Ph.D.
- Appendix B: Copy of Office Action in Colombian Patent App. No. 06023144 and an English translation
- Appendix C: Claims proposed for submission in Colombian Patent App. No. 06023144
- Appendix D: Copies of references cited herein

APOSTILLE

(Hague Convention of 5 October 1961 / Convention de La Haye du 5 octobre 1961)

UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND

1. Country: United Kingdom of Great Britain and Northern Ireland
Pays: Royaume-Uni de Grande-Bretagne et d'Irlande du Nord

This public document / Le présent acte public

2. Has been signed by Neil Roger Burton
a été signé par

3. Acting in the capacity of Notary Public
agissant en qualité de

4. Bears the seal/stamp of The Said Notary Public
est revêtu du sceau/timbre de

5. at London/à Londres
Certified/Attesté
6. the/le 11 May 2011

7. by Her Majesty's Principal Secretary of State for Foreign and Commonwealth Affairs /
par le Secrétaire d'Etat Principal de Sa Majesté aux Affaires Etrangères et du Commonwealth.

8. Number/sous No 1868482

9. Stamp:
timbre:
10. Signature: G.Barrie-Shilson



For the Secretary of State / Pour le Secrétaire d'Etat

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NEIL R. BURTON
Notary Public

Francis House
112 Hills Road
Cambridge CB2 1PH

NOTARIAL CERTIFICATE

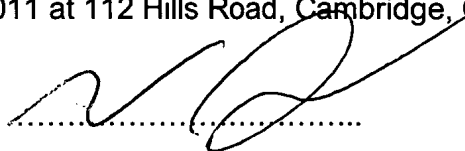
To: ALL TO WHOM THESE PRESENTS SHALL COME

I **NEIL ROGER BURTON** of 112 Hills Road, Cambridge, Notary Public duly authorised, admitted and sworn and practising within the United Kingdom of Great Britain and Northern Ireland do hereby certify that William Jones (identified by passport number 060252426) appeared before me and did declare and sign the annexed document in my presence.

I accept no responsibility for the contents of the annexed document.

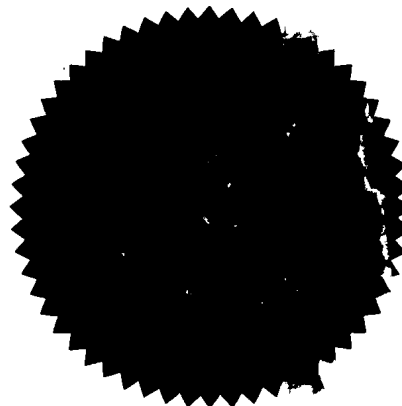
In witness whereof I have hereunto subscribed my name and affixed my seal of office this 3 day of May 2011 at 112 Hills Road, Cambridge, CB2 1PH, England

Signed



Neil R. Burton
Notary Public
My commission expires with life

Doc No. CNB/255/2011

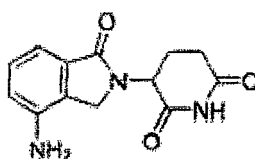


DECLARACIÓN DEL PROFESOR WILLIAM JONES, PH.D.

Apreciados señores:

Yo, WILLIAM JONES, por medio de la presente declaro y manifiesto:

1. En el presente soy Profesor del Departamento de Química de la Universidad de Cambridge, situada en Cambridge, Inglaterra.
2. Tengo más de 35 años de experiencia en investigación en el campo de la química. Mi experiencia académica y técnica, los honores que he recibido y una lista de mis publicaciones, aparecen en mi hoja de vida, una copia de la cual aparece anexa identificada como Apéndice A. Como lo indica la lista de publicaciones de mi hoja de vida, una parte significativa de mi investigación actual involucra el campo de la química farmacéutica del estado sólido.
3. He leído y estoy familiarizado con la revelación de la Solicitud internacional de patente No. PCT/US2004/028736, a la cual me referiré como "Solicitud 736" en la presente Declaración. Entiendo que la fecha primera de radicación de la Solicitud 736 es el 4 de septiembre de 2003.
4. En mi opinión, la tecnología descrita en la Solicitud 736 se relaciona con la química farmacéutica del estado sólido y el polimorfismo, en especial en lo que respecta al compuesto conocido como lenalidomida, el cual puede ser descrito con por lo menos los siguientes nombres químicos: 3-(4-amino-1-oxo-1,3-dihidro-isoindol-2-il)-piperidin-2,6-diona; 3-(4-amino-1-oxoisoindolin-2-il)piperidin-2,6-diona; 1-oxo-2-(2,6-dioxopiperidin-3-il)-4-aminoisoindolina; y 3-(1-oxo-4-aminoisoindolin-2-il)piperidin-2,6-diona. La Solicitud 736 proporciona un nombre químico para la lenalidomida en la página 1, líneas 29-30 e ilustra la fórmula química de la lenalidomida en la página 6, línea 14. La fórmula química también se da aquí como Fórmula (I).



(I)

5. Entiendo que la solicitud colombiana de patente No. 06023144 corresponde a la Solicitud 736. Entiendo que un Oficio (al cual haré referencia como el "Oficio colombiano" en la presente Declaración) ha sido emitido por la Superintendencia de Industria y Comercio en relación con esta solicitud colombiana de patente. Se anexan una copia del Oficio de la SIC y una traducción al inglés que se identifican como Apéndice B. Entiendo que el Oficio colombiano alega que las formas cristalinas de la lenalidomida descritas en esta solicitud colombiana de patente carecen de nivel

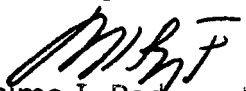

Jaime I. Pedraza F.
Traductor e Intérprete Oficial
Res. Minjusticia 1025 de 1995

inventivo a la luz del estado de la técnica a 4 de septiembre de 2003. Entiendo y creo que el estado de la técnica en esa fecha incluía la información revelada en la patente estadounidense No. 6.281.230, a la cual me referiré como la Patente 230 en la presente Declaración. Entiendo que el Oficio colombiano también cita el Capítulo 76 de la 17ª Edición de REMINGTON'S PHARMACEUTICAL SCIENCES (al cual haré alusión como "Referencia a Remington" en la presente Declaración), la cual fue publicada antes del 4 de septiembre de 2003.

6. He leído y estoy familiarizado con el juego de reivindicaciones, que se anexa a la presente en forma de Apéndice C, el cual entiendo que será radicado junto con la presente Declaración y la respuesta al Oficio colombiano. Se me ha pedido que dé mi opinión acerca de si las formas cristalinas de la lenalidomida descritas en la Solicitud 736 tienen nivel inventivo a la luz del estado de la técnica existente el 4 de septiembre de 2003.
7. En los siguientes párrafos consignaré mi opinión de que las formas cristalinas de la lenalidomida descritas en la Solicitud 736 tienen nivel inventivo. Respaldaré tal opinión con una discusión del estado de la técnica prevalente el 4 de septiembre de 2003, incluyendo la Patente 230 y la Referencia a Remington. También examinaré las propiedades inesperadas asociadas con formas cristalinas particulares de la lenalidomida descritas en la Solicitud 736. En el Apéndice D se dan copias de las referencias que he citado en la presente Declaración.

I. LAS FORMAS CRISTALINAS DE LA LENALIDOMIDA DE LA SOLICITUD 736 TIENEN NIVEL INVENTIVO A LA LUZ DEL ESTADO DE LA TÉCNICA

8. Entiendo que el Oficio colombiano alega que las formas cristalinas de la lenalidomida descritas en la Solicitud 736 carecen de nivel inventivo a la luz del estado de la técnica existente el 4 de septiembre de 2003. Como explico en la presente Declaración, estoy en desacuerdo con esa afirmación. En mi opinión las formas cristalinas de la lenalidomida descritas en la Solicitud 736 no podrían haber sido predichas ni eran de esperar a la luz del estado de la técnica existente el 4 de septiembre de 2003, incluida la Patente 230 y la Referencia a Remington.
- A. La existencia de Formas cristalinas particulares de la lenalidomida No podría haber sido predicha a partir del nombre químico y la fórmula de la lenalidomida
9. La Patente 230 revela un nombre químico para lenalidomida, p.ej., en la columna 9, líneas 25-26 y una fórmula química general que abarca a la lenalidomida en la columna 4, líneas 43-53. No obstante, el conocimiento previo del nombre químico y la fórmula de la lenalidomida no afectan la novedad o el nivel inventivo que se asocian con las formas cristalinas de la lenalidomida descritas en la Solicitud 736. Al 4 de septiembre de 2003, con base únicamente en un conocimiento del nombre químico y la fórmula de la lenalidomida, la persona medianamente versada en la técnica no habría sido capaz de predecir la formación de formas cristalinas particulares de la lenalidomida o sus


 Jaime I. Pedraza F.
 Traductor e Intérprete Oficial
 Res. Minjusticia 1025 de 1995

propiedades provechosas asociadas. En mi opinión, las formas cristalinas descritas en la Solicitud 736 representan un importante avance hacia la facilitación del uso de la lenalidomida como material farmacéutico y tienen nivel inventivo.

10. Parece ser que el Oficio colombiano alega que las formas cristalinas de la lenalidomida de la Solicitud 736 carecen de nivel inventivo porque "el estudio de polimorfismo es una tarea habitual en el campo de la farmacéutica en la preformulación de preparaciones farmacéuticas y que la variedad de formas cristalinas en un compuesto permite variar las propiedades de solubilidad, estabilidad y otras". Específicamente, el Oficio colombiano alega que "es una tarea habitual para el experto (preformulador) determinar si el compuesto existe en una o más formas cristalinas, el número de polimorfos que puedan existir y determinar su carácter y características físicas y químicas... una vez que han sido caracterizadas, hay procedimientos por medio de los cuales el preformulador puede preparar las diversas formas en cantidades más grandes para evaluación adicional... [p]or consiguiente, el desarrollo de formas polimórficas de un compuesto para mejorar sus propiedades físicas y químicas es un problema que ya ha sido resuelto en el estado de la técnica y no se considera inventivo". Estoy en desacuerdo con estas afirmaciones.
11. Como explico en la presente Declaración, en mi opinión, la fórmula química de un compuesto orgánico en particular no revela o sugiere ninguna forma sólida específica en la cual el compuesto se pueda precipitar. Con base solamente en un conocimiento de la fórmula química de un compuesto orgánico en particular, en general no es posible predecir si el compuesto va a formar un aceite, un sólido amorfo, un sólido cristalino, o una mezcla de los mismos.
12. Predecir la naturaleza de forma(s) sólida(s) que pudieran haber sido generadas para un compuesto orgánico puede resultar todavía más complicado por la posibilidad de la formación de solvatos. Sin experimentación detallada, la persona medianamente versada en la técnica no puede predecir si un compuesto orgánico en particular se va a cristalizar con o sin la incorporación de solvente (p.ej., agua) en el entramado cristalino. Determinar si un compuesto se va a cristalizar como un solvato (p.ej., un hidrato) requiere sustancial experimentación y no es posible predecirlo con base solamente en la fórmula molecular del compuesto.
13. Como resultado del alto grado de imprevisibilidad en este campo, los investigadores tienen que llevar a cabo experimentación detallada para verificar y entender la formación de cualquier forma cristalina que comprende un compuesto orgánico en particular. La cantidad de experimentación requerida para verificar y entender la formación de formas cristalinas que comprende un compuesto orgánico en particular puede ser sustancial y extensa. El proceso por supuesto dista de ser habitual. Como explico en la presente Declaración, esto es cierto en el caso de las formas cristalinas de la lenalidomida descritas en la Solicitud 736.


Jaime I. Pedraza F.
Traductor e Intérprete Oficial
Res. Minjusticia 1025 de 1995

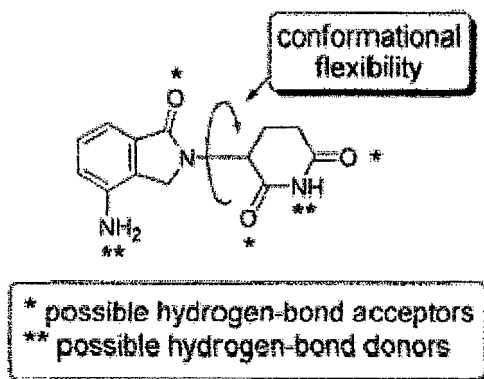
a. *La persona medianamente versada en la técnica no podría haber predicho la formación de las formas cristalinas de la lenalidomida descritas en la Solicitud 736*

14. Cada forma cristalina de la lenalidomida revelada en la Solicitud 736 es un nuevo material con nuevas propiedades, que representa un significativo avance hacia el entendimiento y el control de la química del estado sólido de la lenalidomida. A la luz del estado de la técnica existente el 4 de septiembre de 2003, la persona medianamente versada en la técnica no habría predicho las formas cristalinas particulares descritas en la Solicitud 736, ni las condiciones o variables experimentales particulares para la obtención de las formas cristalinas, como la temperatura o el solvente, por ejemplo.
15. A 4 de septiembre de 2003, la persona medianamente versada en la técnica no podría haber previsto si dado un conjunto de condiciones experimentales la lenalidomida se precipitaría como, p.ej., un aceite, un sólido amorfo, un material cristalino no solvatado, un material cristalino solvatado, o una mezcla de uno o más de los anteriores. La persona medianamente versada en la técnica tampoco habría podido predecir el número de formas cristalinas de la lenalidomida, si existen, que podrían haber sido obtenidas en el laboratorio. En 2006 expliqué que “en general no es posible predecir de forma computacional el número de polimorfos observables incluso de las moléculas más simples”. Jones, W., et al., Mater. Res. Bull. (2006), 31:875-79, at 875 (el énfasis es mío). La presente Declaración se aplica a la lenalidomida prevalente el 4 de septiembre de 2003.
16. En lo tocante a la composición química, algunas de las formas cristalinas de la lenalidomida descritas en la Solicitud 736 contienen solvente (p.ej., agua) en el entramado cristalino, un resultado impredecible que comporta importantes implicaciones para el desarrollo de un compuesto farmacéutico. Las formas cristalinas de la lenalidomida que contienen solvente en el entramado cristalino tienen una composición química diferente de la lenalidomida misma. Por ejemplo, la Forma B de la lenalidomida es un hemihidrato (Solicitud 736 en la página 9, líneas 15-19) y la Forma E de la lenalidomida es un dihidrato (Solicitud 736 en la página 7, líneas 20-21). Así, aunque la fórmula empírica de la lenalidomida es $C_{13}H_{13}N_3O_3$, la Forma cristalina B de la lenalidomida tiene una fórmula empírica de $C_{13}H_{13}N_3O_3 \cdot \frac{1}{2}(H_2O)$ y la Forma cristalina E de la lenalidomida tiene una fórmula empírica de $C_{13}H_{13}N_3O_3 \cdot 2(H_2O)$. Al 4 de septiembre de 2003, la persona medianamente versada en la técnica no habría predicho o esperado estos solvatos cristalinos en particular (p.ej., hidratos), u otros descritos en la Solicitud 736.
17. La complejidad molecular, incluida la flexibilidad molecular y la presencia de donantes y aceptores de enlaces hidrógeno competidores, supone un obstáculo para la predicción de posibles formas cristalinas para un compuesto. Ver Day, G.M., et al., Cryst. Growth Des. (2004), 4(6): 1327-40. La fórmula química de la lenalidomida, como se muestra en la FIG. 1 más adelante, posee tanto flexibilidad conformacional como múltiples donantes y aceptores de enlaces hidrógeno, cada uno de los cuales habría complicado


Jaime I. Pedraza F.
Traductor e Intérprete Oficial
Res. Minjusticia 1025 de 1995

31

cualquier intento de predecir las formas cristalinas de la lenalidomida existentes el 4 de septiembre de 2003.



Flexibilidad conformacional

* Posibles aceptores de enlaces hidrógeno

** Posibles donantes de enlaces hidrógeno I

FIG. I

18. Además de la complejidad molecular, el número de molécula(s) en la unidad cristalográfica asimétrica fue una limitación significativa a la predicción de la estructura existente el 4 de septiembre de 2003. En 2006 expliqué que “[l]a predicción de estructuras cristalinas con más de una molécula en la unidad asimétrica... supone un gran desafío en el campo de la predicción de las estructuras cristalinas”. Cruz Cabeza, A.J., et al., J. Am. Chem. Soc. (2006), 128: 14466-67, en 14466. En la práctica, esto significa que la formación y el cociente estequiométrico de los solvatos cristalinos, como hidratos cristalinos, en general eran impredecibles a 4 de septiembre de 2003. Muchas de las formas cristalinas de la lenalidomida reveladas en la Solicitud 736, incluidas las Formas B y E, comprenden solventes en el entramado cristalino. En mi opinión la predisposición demostrada e impredecible de la lenalidomida a incorporar solvente de cristalización habría representado un serio obstáculo para la predicción de las formas cristalinas descritas en la Solicitud 736. Determinar si la lenalidomida cristalizaría como un solvato (como, p.ej., un hidrato) habría exigido sustanciales experimentación de laboratorio y no podría haber sido predicho ni era de esperar con base en la fórmula química de la lenalidomida.

a. *La persona medianamente versada en la técnica no podría haber predicho las condiciones requeridas para preparar las formas cristalinas de la lenalidomida presentes en la Solicitud 736*

19. En mi opinión, antes del 4 de septiembre de 2003, la persona medianamente versada en la técnica no podría haber predicho las condiciones requeridas para preparar las formas cristalinas de la lenalidomida reveladas en la Solicitud 736. Además, creo que las condiciones de cristalización de la lenalidomida descritas en la Solicitud 736


Jaime I. Pedraza F.
Traductor e Intérprete Oficial
Res. Minjusticia 1025 de 1995

representan un importante avance hacia la facilitación del uso de la lenalidomida como un material farmacéutico.

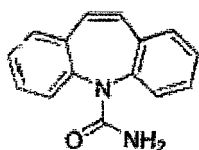
20. La Solicitud 736 describe dificultades especiales que los inventores encontraron para el aislamiento de formas cristalinas puras de la lenalidomida. La Solicitud 736 explica que se obtuvieron múltiples lotes del material sólido de la lenalidomida como “mezclas aparentes de polimorfos” de la lenalidomida (Solicitud 736 en la página 17, línea 20) y que diversas cristalizaciones dieron lugar a un producto que contenía mezclas de formas cristalinas de la lenalidomida (Solicitud 736 en las páginas 19-21, Tablas 1, 2 y 3). Un proceso reproducible y robusto para obtener lotes puros de una única forma cristalina es crítico para el éxito en el desarrollo y la manufactura prácticamente de todos los compuestos farmacéuticos. En efecto, la Solicitud 736 explicó la importancia de derivar “controles polimórficos estrictos en los lotes de validación y futura manufactura” del ingrediente farmacéutico activo de la lenalidomida (Solicitud 736 en la página 17, líneas 23-24).
21. A la luz de la aparente predisposición de la lenalidomida a cristalizar como una mezcla de polimorfos, los inventores enfrentaron retos especiales para el desarrollo de procesos reproducibles y robustos de cristalización. Por ejemplo, la Solicitud 736 explicó que la Forma B de la lenalidomida parecía ser meta-estable, es decir, era un producto de cristalización cinéticamente controlado que más adelante, con el paso del tiempo, se puede convertir en una mezcla de Formas B y E en ciertas condiciones (Solicitud 736 en la página 18, líneas 13-18). Como lo describe la Solicitud 736, los inventores estudiaron variables experimentales incluidas, por ejemplo, la escala de reacción, solvente de cristalización, temperatura de la lechada, volumen del solvente, periodo de enfriamiento, periodo de retención, temperatura de secado, secado al vacío, temperatura de filtración y tiempo de filtración (Solicitud 736 en las páginas 18-19). Antes de que los inventores realizaran estos estudios, no se habrían podido predecir los efectos de cada una de estas variables sobre el comportamiento en el estado sólido de la lenalidomida.
22. En mi opinión las condiciones descritas en la Solicitud 736 para obtener lotes puros de formas cristalinas específicas de la lenalidomida, incluidos los parámetros preferidos de proceso para las Formas B y E, representan importantes avances que facilitan el uso de la lenalidomida como un compuesto farmacéutico.
- B. La existencia de formas cristalinas particulares de la lenalidomida no podría haber sido prevista a partir de la Patente 230 y la Referencia a Remington
23. Entiendo que el Oficio colombiano alega que las formas cristalinas de la lenalidomida de la Solicitud 736 carecen de nivel inventivo a la luz de la Patente 230 y la Referencia a Remington. Estoy en desacuerdo con esa afirmación.
24. La Patente 230 no describe o sugiere formas cristalinas de lenalidomida racémica, incluidas las formas cristalinas descritas en la Solicitud 736. Por ejemplo, la Patente


Jaime I. Pedraza F.
Traductor e Intérprete Oficial
Res. Minjusticia 1025 de 1995

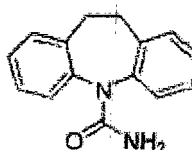
230 no describe formas cristalinas de lenalidomida racémica, ni sugiere si la lenalidomida racémica puede o no ser preparada como un material cristalino. En particular, la Patente 230 no describe las formas cristalinas particulares identificadas en la Solicitud 736. La Patente 230 tampoco describe o sugiere si la lenalidomida podría formar solvatos cristalinos, como, los hidratos cristalinos particulares descritos en la Solicitud 736, incluidas las Formas B y E. Además, la Patente 230 no describe o sugiere condiciones particulares para la formación de las formas cristalinas de la lenalidomida descritas en la Solicitud 736. Así las cosas, a la luz de la Patente 230, la persona medianamente versada en la técnica no podría haber predicho o esperado las formas cristalinas de la lenalidomida descritas en la Solicitud 736.

25. La Referencia a Remington no da ninguna información específica relacionada con las formas cristalinas de la lenalidomida descritas en la Solicitud 736. Por ejemplo, la Referencia a Remington no describe o sugiere ningún compuesto en particular relacionado con la lenalidomida, ninguna forma cristalina en particular de la lenalidomida, o ninguna condición particular para la preparación de las formas cristalinas de la lenalidomida descritas en la Solicitud 736. En el mejor de los casos, la Referencia a Remington describe información de fondo sobre la formulación de fármacos en términos generales, sin dar orientación específica sobre cómo obtener las formas cristalinas de la lenalidomida y casi con absoluta seguridad no las que se describen en la Solicitud 736. Cabe anotar que el autor de la Referencia a Remington afirma que "[e]stamos en una era de la industria farmacéutica en la cual ya no podemos basarnos en las experiencias del pasado para formular. Hemos de tener un encendimiento detallado de las propiedades fisicoquímicas así como del comportamiento farmacocinético y biofarmacéutico de cada sustancia medicamentosa que se está desarrollando (cuarto párrafo de la Referencia a Remington). Así pues, la persona medianamente versada en la técnica no esperaría que un procedimiento que presuntamente cristaliza un compuesto pudiera ser aplicado con éxito comparable a otro compuesto, como la lenalidomida.

26. Los estudios publicados por mi propio grupo de investigación ilustran que el comportamiento de cristalización y las características del estado sólido de compuestos incluso similares en su estructura puede ser notoriamente diferente. Soy coautor de una publicación que describe el comportamiento de cristalización de los compuestos farmacéuticos carbamazepina y 10,11-dihidrocarbamazepina, que se ilustran a continuación como Fórmulas (II) y (III). Ver Cruz Cabeza, A.J., et al., Cryst. Growth Des. (2007), 7(1): 100-07.



Carbamazepina
(II)

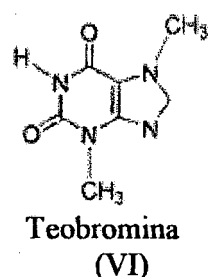
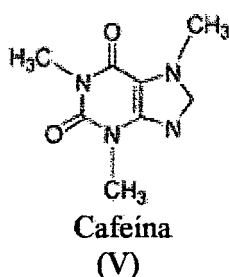
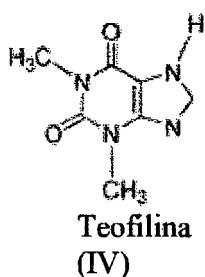


10,11-dihidrocarbamazepina
(III)


Jaime I. Pedraza F.
Traductor e Intérprete Oficial
Res. Minjusticia 1025 de 1995

32

27. Las fórmulas moleculares de estos dos compuestos son muy similares: La carbamazepina difiere del compuesto dihidro solamente por dos átomos de hidrógeno (es decir, un doble enlace). Sin embargo, a pesar de esta estrecha similitud molecular, estas moléculas tienen significativas diferencias en sus características y propiedades de estado sólido. Por ejemplo, el compuesto dihidro se cristaliza rápidamente en etanol a dando una forma cristalina compuesta por cadenas unidas por enlaces de hidrógeno. Con todo, aun después de extensos esfuerzos experimentales que involucraron someter a la carbamazepina a 66 solventes diferentes (incluido el etanol) y 5 diferentes protocolos de cristalización, no fue posible obtener una forma cristalina similar parecida a una cadena. Incluso nuestros intentos de sembrar una solución saturada de carbamazepina con la forma cristalina parecida a una cadena del compuesto dihidro no rindieron frutos.
28. La bibliografía científica proporciona muchos otros ejemplos de grandes diferencias en el comportamiento de cristalización que surgen entre moléculas con similitud química. Por ejemplo, tres compuestos de xantina con actividad farmacológica (teofilina, cafeína y teobromina) difieren máximo solo por un grupo metilo, como se muestra en las Fórmulas (IV), (V) y (VI), que se presentan a continuación.



29. A pesar de la similitud química entre estos tres compuestos, ellos tienen importantes diferencias en el estado sólido en lo que respecta a, entre otras cosas, su capacidad de cristalizar como formas cristalinas hidratadas. La teofilina puede formar un monohidrato cristalino relativamente estable. La cafeína, por contraste, no forma un monohidrato, sino más bien un 0.8-hidrato no estequiométrico. La teobromina, a diferencia de los otros dos compuestos, no produce en absoluto una forma cristalina hidratada. Ver, p.ej., Bruns, S., et al., *Thermochimica Acta* (1984), 72:3 1-40, en 36.
30. Como he ilustrado con los ejemplos precedentes, uno no esperaría que un procedimiento de cristalización para un compuesto pudiera ser aplicado con éxito comparable a otro compuesto, incluso en si tuvieran estructuras similares. Según esto, la persona medianamente versada en la técnica no esperaría que un procedimiento para cristalizar un compuesto diferente a la lenalidomida pudiera ser aplicado con éxito comparable a la lenalidomida. Por tanto, en mi opinión las formas cristalinas de la lenalidomida de la Solicitud 736 no están descritas o sugeridas en la Patente 230 ni en la Referencia a Remington.


Jaime I. Pedraza F.
Traductor e Intérprete Oficial
Res. Minjusticia 1025 de 1995

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II. LAS FORMAS CRISTALINAS DE LA LENALIDOMIDA DE LA SOLICITUD 736 POSEEN PROPIEDADES SUPERIORES E INESPERADAS CON RESPECTO A LA PATENTE 230 Y LA REFERENCIA A REMINGTON

31. Las formas cristalinas de la lenalidomida descritas en la Solicitud 736 poseen propiedades inesperadas. Por esta adicional razón, las formas cristalinas de la lenalidomida descritas en la Solicitud 736 tienen nivel inventivo y representan un avance tecnológico significativo.
32. La Solicitud 736 revela propiedades provechosas asociadas con formas cristalinas particulares de la lenalidomida. En mi opinión, antes del 4 de septiembre de 2003, las propiedades asociadas con estas formas cristalinas de la lenalidomida no habrían sido razonablemente esperadas por la persona medianamente versada en la técnica con base en información de la Patente 230 y la Referencia a Remington.
33. Anoté en 2006 que "la forma física un [ingrediente farmacéutico activo] representa una oportunidad estratégica para optimizar propiedades físicas tales como solubilidad, tasa de disolución, higroscopicidad, estabilidad física y estabilidad química". Jones, W., et al., Mater. Res. Bull. (2006), 31:875-79, at 875. La Solicitud 736 representa un paso innovador hacia la optimización de la lenalidomida como un material farmacéutico en estado sólido. Ejemplos de propiedades inesperadas asociado con formas cristalinas particulares de la lenalidomida se resumen a continuación.
34. La Solicitud 736 proporciona datos concernientes a las propiedades térmicas asociadas con las formas cristalinas de la lenalidomida; estas propiedades no podrían haber sido predichas ni eran de esperar con base en información de la Patente 230 y la Referencia a Remington. Por ejemplo, para ciertas formas cristalinas, la Solicitud 736 proporciona datos de DSC, TGA y/o TG-IR (TG-IR es una técnica térmica que acopla TGA con espectroscopia IR para analizar los vapores emitidos, como solventes volátiles). Los datos térmicos como estos indican la idoneidad de una forma cristalina en particular para las condiciones de procesamiento regularmente empleadas en el desarrollo farmacéutico, como secado, molido y almacenamiento, entre otras. Por ejemplo, las gráficas de DSC, TGA y TG-IR de la Forma B, que se dan en la Solicitud 736 en las Figuras 9 y 10, indican que la Forma B no sufre transiciones térmicas significativas por debajo de cerca de 146°C, temperatura a la cual pierde agua de hidratación. La Solicitud 736 también proporciona datos de DSC y TGA para otras formas cristalinas, como las Formas A y E en las Figuras 4 y 24. Estos datos indican que ciertas formas cristalinas de la lenalidomida, como las Formas A, B y E, tienen propiedades beneficiosas de estabilidad térmica adecuadas para procesamiento farmacéutico y almacenamiento.
35. La Solicitud 736 proporciona datos concernientes a propiedades de sorción/desorción de humedad asociados con ciertas formas cristalinas de la lenalidomida; estas propiedades no podrían haber sido predichas ni eran de esperar con base en información de la Patente 230 y la Referencia a Remington. Ninguna higroscopicidad es crítica,


Jaime I. Pedraza F.
Traductor e Intérprete Oficial
Res. Minjusticia 1025 de 1995

p.ej., para la formulación, procesamiento y almacenamiento de materiales farmacéuticos. Por ejemplo, una isoterma de sorción/desorción de humedad para la Forma B se da en la Solicitud 736 at Figura II. La Solicitud 736 también proporciona datos de sorción/desorción de humedad para las Formas A y E en las Figuras 5 y 25. Las gráficas indican que las Formas A, B y E son sustancialmente no higroscópicas, lo cual sugiere que las Formas A, B y E poseen propiedades deseables para el desarrollo de un producto farmacéutico.

36. La Solicitud 736 proporciona datos concernientes a la tasa de solubilidad y disolución asociada con ciertas formas cristalinas de la lenalidomida; estas propiedades no podrían haber sido predichas ni eran de esperar con base en información de la Patente 230 y la Referencia a Remington. Las propiedades de la tasa de solubilidad y disolución pueden ser críticas para valorar la biodisponibilidad y eficacia farmacológica de una forma sólida de un compuesto farmacéutico. Por ejemplo, la Solicitud 736 revela datos de solubilidad de la Forma B en las páginas 27-28 y en la Figura 52. Estos datos indican que la Forma B tiene solubilidad apreciable, cercana a 5,8 mg/mL, en el medio de disolución seleccionado. Ver la Tabla 6 en la página 27. Este estudio también indicó que los cristales no disueltos de la Forma B no se convierten en otra forma sólida, lo cual demuestra que la Forma B tiene buena estabilidad en agua. Ver la página 27, líneas 19-21 y tabla 7 en la página 28. La Solicitud 736 contiene datos de disolución para la Forma B en las páginas 28-30 y las Figuras 53-54. La Solicitud 736 también proporciona datos de solubilidad y disolución para las Formas A y E en las páginas 27-28, páginas 28-30 y en las Figuras 51 y 53-54. Los datos de una tasa beneficiosa de solubilidad y disolución, como los que aparecen en la Solicitud 736, se pueden usar para valorar si una forma sólida en particular de un compuesto farmacéutico será biodisponible, una importante característica que afecta las propiedades biológicas y terapéuticas asociadas con una forma sólida de un compuesto farmacéutico.
37. La Solicitud 736 proporciona datos concernientes a las propiedades de estabilidad física a largo plazo asociadas con ciertas formas cristalinas de la lenalidomida; estas propiedades no podrían haber sido predichas ni eran de esperar con base en la información de la Patente 230 y la Referencia a Remington. Datos como estos indican la idoneidad de una forma cristalina en particular con respecto a importantes consideraciones de almacenamiento y vida útil. Por ejemplo, la Solicitud 736 proporciona los resultados de estudios de estabilidad física a largo plazo para la Forma B en condiciones particulares de estrés de temperatura/humedad en la página 10, líneas 3-5. Estos datos indican que la Forma B no se convierte en una forma sólida diferente en dos condiciones diferentes (temperatura ambiente/0% HR y 40°C/93% HR) durante un periodo de hasta cerca de 85 días. La Solicitud 736 también proporciona datos similares de estabilidad física a largo plazo para las Formas A y E, en la página 8, líneas 29-31 y página 13, líneas 6-9. Estos resultados indican que las Formas A, B y E tienen estabilidad física beneficiosa a largo plazo adecuada para almacenamiento a largo plazo como producto farmacéutico.



Jaime I. Pedraza F.
Traductor e Intérprete Oficial
Res. Minjusticia 1025 de 1995

38. La Solicitud 736 proporciona datos concernientes a las propiedades de estabilidad mecánica asociadas con ciertas formas cristalinas de la lenalidomida; estas propiedades no podrían haber sido predichas ni eran de esperar con base en la información de la Patente 230 y la Referencia a Remington. Los datos de estabilidad mecánica indican la idoneidad de una forma sólida en particular para ciertas condiciones de procesamiento regularmente empleadas en el desarrollo farmacéutico, como molido y tableado. Por ejemplo, la Solicitud 736 revela datos concernientes a la estabilidad mecánica de las Formas A y B con respecto a compresión y molienda en la página 28, líneas 8-11 y tabla 7. La estabilidad mecánica revelada para las Formas A y B indica su idoneidad para condiciones típicas de procesamiento encontradas en operaciones de desarrollo farmacéutico.

39. Así las cosas, las formas cristalinas de la lenalidomida descritas en la Solicitud 736, como Formas A, B y E, poseen propiedades beneficiosas e inesperadas. Estas propiedades beneficiosas específicas no habrían sido previstas o esperadas por una investigación ordinaria realizada antes del 4 de septiembre de 2003. Según esto, en mi opinión, las formas cristalinas reivindicadas de la lenalidomida son inventivas por esta razón adicional.

III. CONCLUSIÓN

40. Teniendo en cuenta mi análisis anterior, concluyo que la persona medianamente versada en la técnica existente antes del 4 de septiembre de 2003 no habría sido capaz de predecir la obtención o las propiedades de las formas cristalinas de la lenalidomida descritas en la Solicitud 736 y que las formas cristalinas de la lenalidomida de la Solicitud 736 poseen propiedades inesperadas, que representan un avance significativo en la química del estado sólido de la lenalidomida. Por tanto, estoy en desacuerdo con el Oficio de la autoridad colombiana y concluyo que las formas cristalinas reivindicadas de la lenalidomida cumplen los requisitos de novedad y nivel inventivo.

41. Además, hago constar que todas las declaraciones consignadas en la presente Declaración hechas con base en mi propio conocimiento son ciertas y que creo que todas las declaraciones hechas con base en información y opinión son ciertas.

Respetuosamente,
Fecha

William Jones

Anexos:

Apéndice A: Hoja de vida del Profesor William Jones, PhD

Apéndice B: Copia del oficio sobre la solicitud colombiana de patente. No. 06023 144 y su traducción al inglés

Apéndice C: Reivindicaciones propuestas para ser presentadas en Solicitud colombiana de patente. No. 06023 144

Apéndice D: Copias de las referencias aquí citadas


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38

NEIL R. BURTON
Notario Público

Francis House
112 Hills Road
Cambridge CB2 1PH

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Yo, NEIL ROGER BURTON, con domicilio en 112 Hills Road, Cambridge, Notario Público debidamente autorizado, registrado y juramentado y con práctica en el Reino Unido de la Gran Bretaña e Irlanda del Norte por la presente certifico que William Jones (identificado con Pasaporte No. 060252426) compareció ante mí y declaró y firmó el documento que se anexa en mi presencia.

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39

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210

Appendix A

Curriculum Vitae of Professor William Jones, Ph.D.

2/1

Curriculum Vitae
Professor William Jones

Nationality: British

Date of Birth: October 25th 1949

Present Address: Chemistry Department, University of Cambridge
Lensfield Road, Cambridge CB2 1EW,
Tel. 01223 336468
Fax 01223 336362
Email: wj10@cam.ac.uk

Current Appointments: Head, Chemistry Department, Professor of Materials Chemistry,
University of Cambridge

Professorial Fellow, Sidney Sussex College

Education: BSc 1971 University of Wales (Aberystwyth)

PhD 1974 University of Wales (Aberystwyth)

MA 1980 University of Cambridge

Professional History: Staff Demonstrator, University of Wales, Aberystwyth (1974/1975 and
1976/1978)
SERC Post-doctoral Associate, Cambridge (1978/1980)
SAIR, University of Cambridge (1980-1985)
ADR University of Cambridge (1985-1999)
Reader, University of Cambridge (1999-2006)
Fellow (1980), Admissions Tutor, Tutor and Senior Tutor, Sidney
Sussex College (1982-1992)

Professional Qualifications: Fellow of the Royal Society of Chemistry (1987 onwards)
Chartered Chemist (1987 onwards)
Fellow of the Learned Society of Wales (2011)

Other Appointments:

Editorships:

Book "Organic Molecular Solids Properties and Applications", CRC Press, 1997.

Book: "Supramolecular Organization and Materials Design" with C. N. R. Rao, Cambridge University Press, 2001.

Journal: "Proceedings of the International Symposium on the Chemistry of the Organic Solid State", Molecular Crystals Liquid Crystals, 2000.

Journal: European Editor of *Journal of Crystal Engineering*, Elsevier Press. (2001-2004)

Journal: Editor of *Materials Chemistry*, *Chemistry Central*.

Peer reviewing:

Grant application reviewing for: EPSRC, Royal Society, Leverhulme Trust, European Union, American Chemical Society. *Publication reviewing* for numerous journals including those of the Royal Society of Chemistry, the American Chemical Society, Nature and Science.

Promotion applications: numerous UK, EU and US Universities, National University of Singapore, the UK Scientific Civil Service.

Affiliations:

Royal Society of Chemistry, British Crystallographic Association, Royal Pharmaceutical Society, University and College Union.

Awards:

Jacob London Fellow, Weizmann Institute, Israel, 1975/76

Corday Morgan Medal, Royal Society of Chemistry, 1983

Visiting Professor, State University of New York, 1988

Publications:

380 articles; 2 Edited Books, various US and EC patents

Scientific Committees:

Current and previous membership of various standing committees for International Symposia including: *International Conference on Intercalation Compounds* (every 2 years), *International Conference on the Chemistry of the Organic Solid State* (every 2 years), *International Conference on Ion-Exchange*, *IUPAC Conference on Physical Organic Chemistry*. Member of Scientific Committee for *ad hoc* meetings of the Royal Society of Chemistry, The International Union of Crystallography, The International Clay Minerals Society.

112

213

Professor William Jones

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Appendix D

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THERMOCHEMICAL INVESTIGATION OF THEOPHYLLINE,
THEOPHYLLINE HYDRATE AND THEIR AQUEOUS SOLUTIONS

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ABSTRACT

Theophylline, although structurally very similar to caffeine, does not exhibit a solid state phase transition. In contrast to caffeine, it forms a monohydrate, of which the quadruple point and enthalpy of hydration have been determined. Furthermore, solubility and enthalpy of solution measurements are discussed.

INTRODUCTION

Purines are widespread in nature and play an important role in animal and human metabolism. Of the methylated dioxo-purines caffeine, theophylline and theobromine are the most important, Fig. 1. Theophylline, the subject of this paper, occurs inter alia in green tea, maté and other plants and may be a by-product in the decaffeination of coffee. It is an important drug in the treatment of asthma and cardiac diseases and is used as a diuretic. It shows as well similarities with as distinct differences from its 7-methyl derivative caffeine.

MATERIALS

At 60°C, a saturated solution of theophylline (ICN Pharmaceuticals, Inc.) in dist. water was prepared, which on slow cooling yielded fine, long needles of theophylline hydrate. After centrifuging the rest of adhering solution was removed by storing the hydrate for several weeks at 92 % rel. humidity over a saturated solution of $\text{Na}_2\text{CO}_3 \cdot 10 \text{H}_2\text{O}$ ("conditioning"). Desiccation at 90°C gave anhydrous theophylline. The purity was determined by DSC as 99,98 mol-%.

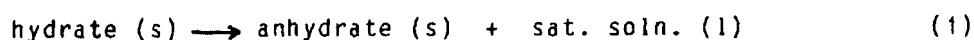
THEOPHYLLINE HYDRATE

Composition

In the literature and pharmacopoe, theophylline and caffeine are both reported to form monohydrates. Since we had previously observed that caffeine in fact forms a 4/5-hydrate (ref. 1), we decided to check also the composition of theophylline hydrate. Both isothermal and dynamic TG measurements, however, showed that theophylline hydrate (if conditioned as described above) is indeed a monohydrate. The compositions theophylline·H₂O and caffeine·0.8 H₂O found are in accord with the X-ray structural analyses, see Fig. 2 (ref. 2, 3, 4).

Stability point

The stability point, often also referred to as the quadruple point, is the temperature T_D up to which a hydrate is thermodynamically stable in the presence of its own saturated solution and vapour pressure. Literature data of T_D show considerable discrepancies. From measurements of solubility resp. rate of solution of theophylline hydrate and theophylline SHEFTER and HIGUCHI deduce $T_D = 73^\circ\text{C}$ (ref. 5) and WADKE and REIER $T_D = 73.6^\circ\text{C}$ (ref. 6). ABOU-SHAABAN and SIMONELLI from TG and DTG curves obtain a range 52...80°C (ref. 7). FOKKENS et al. have undertaken powder X-ray diffraction, DSC and vapour pressure measurements and report $T_D = 63.8 \pm 1.3^\circ\text{C}$ (ref. 8). We have determined the stability point in two ways. Theophylline hydrate was encapsulated in hermetic crucibles and the reaction



followed by DSC at various heating rates. Fig. 3 shows that the extrapolated temperature of peak onset decreases with decreasing heating rate. For vanishing heating rate, a value of $T_D = 67...68^\circ\text{C}$ can be deduced. This value was checked by isothermal measurements: Samples of theophylline hydrate, enclosed in hermetically sealed crucibles, were held at constant temperatures for periods of 6...13 days and subsequently analysed by DSC whether the dehydration peak still showed up or not. Having approached T_D first in greater steps, it was observed that after tempering 168 h at 66°C resp. 166 h at 66.9°C the hydrate was stable, where as a sample held 189 h at 67°C to about 99 % had converted.

24

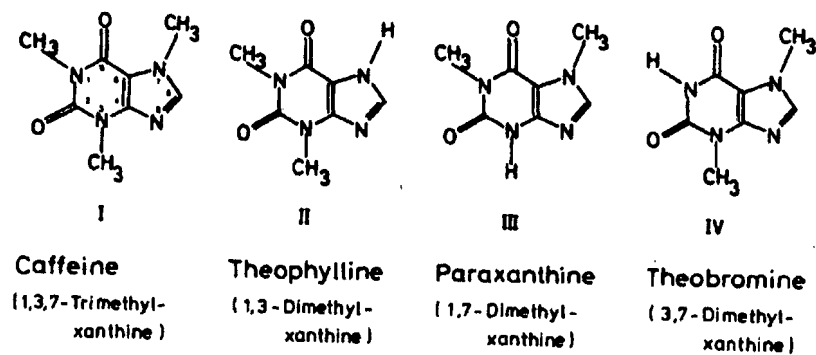


Fig. 1. Structural formulae of some methyl-substituted xanthines.

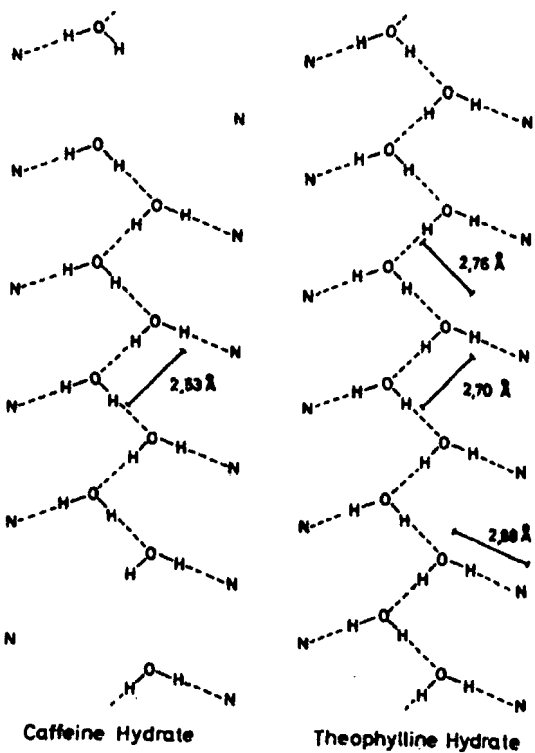


Fig. 2. The hydrogen bonding in the crystal structures of caffeine hydrate and theophylline hydrate. Projection on the (001) plane (ref. 2, 3, 4).

We thus conclude that $T_D = 67.0 \pm 0.2^\circ\text{C}$.

Fig. 3 shows the dependence of T_D on the heating rate for the hydrates of theophylline and caffeine. It is seen that the T_D -curve for caffeine hydrate for vanishing heating rate by far does not approach the stability point as determined by isothermal long-time measurements, which yield $T_D = 51.5^\circ\text{C}$. BOTHE and CAMMENG (ref. 1, 9, 10, 11) have shown by DSC, X-ray diffraction and solution calorimetry that during the dehydration of caffeine hydrate besides the stable β -caffeine a considerable fraction of the metastable α -polymorph is formed, which subsequently reconverts to the β -form. This obviously results in a kinetic hindrance of the dehydration reaction. This example shows again that one should be careful in deducing solid state transition temperatures from DSC measurements even when having varied the heating rate. Thus CESÀRO and STAREC report $T_D = 86^\circ\text{C}$ for the stability point of caffeine hydrate, which is 35°C too high (ref. 12)!

Enthalpy of dehydration

The enthalpy of dehydration was determined by DSC with samples of ≈ 10 mg at a heating rate of $2 \text{ K}\cdot\text{min}^{-1}$ in hermetically sealed capsules. The data observed were corrected for sat. solution and water vapour formation within the capsule as described previously (ref. 1). For the enthalpy of dehydration we obtain $\Delta H_D = 10.5 \pm 0.3 \text{ kJ}\cdot\text{mol}^{-1}$. As is seen from Table 1, this is 50 % higher than for caffeine hydrate. A higher value is expected from stronger hydrogen bonding of the water molecules in the crystal lattice of theophylline hydrate compared to that in caffeine hydrate, see Fig. 2. Theophylline hydrate shows continuous chains of bound water molecules whereas in caffeine hydrate the chains are interrupted.

Theobromine does not form a hydrate, whereas paraxanthine still has to be investigated in detail.

ANHYDROUS THEOPHYLLINE

Polymorphism

Caffeine shows a first order polymorphic phase transition at $140\ldots 141^\circ\text{C}$ (ref. 9). GRABOWSKA and KALISZAN from structural considerations have predicted polymorphism in a number of purines, inter alia in caffeine and theophylline (ref. 13). DOSER believed to have found some evidence for a polymorphic transition near 260°C , when theophylline was obtained by desiccation of its hydrate

86

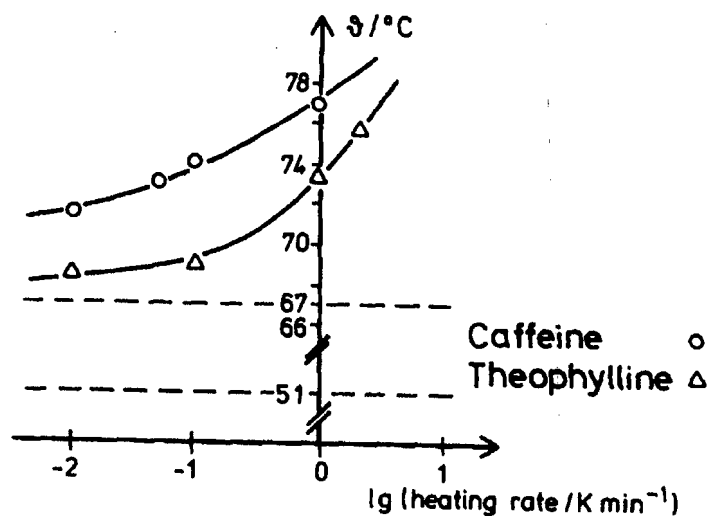


Fig. 3. Extrapolated temperatures of the onset of the dehydration peak (DSC curve) as a function of the heating rate, under which the measurements had been made.

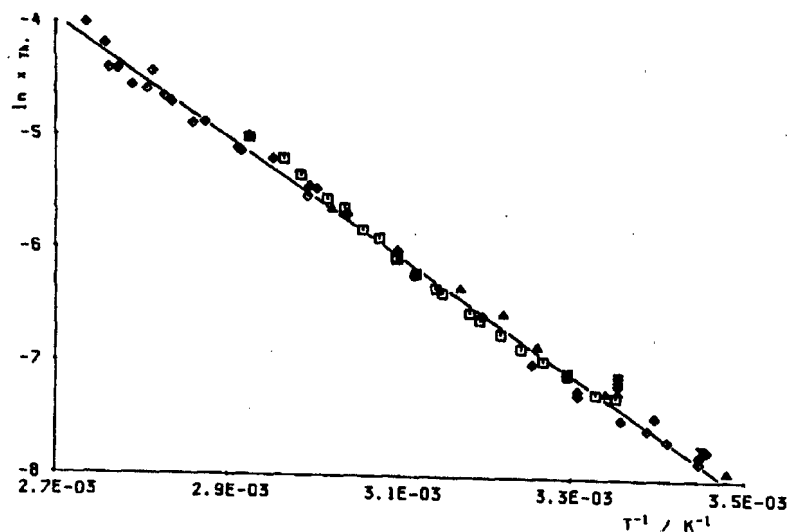


Fig. 4. Solubility of theophylline hydrate and theophylline in water. $\circ$ data of FOKKENS (ref. 8, 15); Δ data of SHEFTER and HIGUCHI (ref. 5); $\times$ scattered literature data; $\square$ data of this work.

TABLE 1

Comparison of some thermochemical data of important purines.
The enthalpy of solution data are given in $\text{kJ}\cdot\text{mol}^{-1}$

Property	Theophylline	Caffeine	Theobromine
Phase transition	—	141°C (DSC) 140°C (TMA)	—
$\Delta H_t^\circ/\text{kJ}\cdot\text{mol}^{-1}$		4.1	
Melting point	271.2°C	236.1°C	348.5°C
$\Delta H_m^\circ/\text{kJ}\cdot\text{mol}^{-1}$	30.9	21.6	(13.1)
Thermal stability (DSC)	medium	very good	poor
Hydrate (TG)	1 H ₂ O	0.8 H ₂ O	—
Quadruple point	67°C	51.5°C	
$\Delta H_D^\circ/\text{kJ}\cdot\text{mol}^{-1}$	10.5	7.06	
Solubility in mol per 1000 g of water at 25°C	$3.72\cdot 10^{-2}$	$1.092\cdot 10^{-1}$	$(2.6\cdot 10^{-3})$
Anhydrate			
$\Delta H_{\text{sol}}^\circ, c \rightarrow o, 298$	19.83	16.32	in prep.
$\Delta H_{\text{sol}}^\circ, c_s, 298$	in prep.	in prep.	in prep.
Hydrate			
ΔH_{298}°	30.6	36.7	in prep.
$\Delta H_{\text{sol}}^\circ, c \rightarrow o, 298 + \Delta H_D^\circ$	30.3	23.38	in prep.

(ref. 14). FOKKENS et al. report another uncertain observation (ref. 8, 15). We, however, found no evidence for a polymorphic phase transition in theophylline: neither quick dehydration of the hydrate nor fast crystallisation from non-aqueous solvents or quenching of the melt resulted in a sample showing thermal events other than melting.

Fusion

Melting of theophylline occurs at $271.5 \pm 0.5^\circ\text{C}$. The enthalpy of fusion is $30.9 \pm 1.0 \text{ kJ}\cdot\text{mol}^{-1}$; this is about 40 % higher than for caffeine.

Thermal stability

The thermal stability, which is of great importance inter alia in food technology, has also been determined. Pure samples were -in the absence of oxygen- encapsulated in hermetic aluminium crucibles or sealed into glass ampoules. These were held isothermally for 1 h at various fixed temperatures, then cooled quickly and analysed by computer-assisted DSC purity analysis. The thermal stability is astonishing for organic compounds and markedly decreases in the order caffeine > theophylline > theobromine.

THEOPHYLLINE SOLUTIONS

FOKKENS has measured the solubility of theophylline hydrate and theophylline in aqueous buffer solutions of pH=5 (ref. 8, 15). Since it occurred to him that a plot of his data gave no evidence of a quadruple point, he fitted an equation

$$\ln x_c = - \frac{\Delta H^{\circ'}}{R \cdot T} + \text{const.} \quad (2)$$

to all his data, from which he deduced an "enthalpy of solution" of $43.1 \text{ kJ}\cdot\text{mol}^{-1}$. We had found, however, that aqueous caffeine solutions are far from being ideal (ref. 11). This is due to solvent/solute and solute/solute interactions, the latter resulting in molecular sandwiching between two or more purine molecules ("base-stacking"). Since one knows from osmotic coefficient measurements that the activity coefficient is a strong function of concentration, the $\Delta H^{\circ'}$ from equation (2) is far from the last enthalpy of solution of caffeine hydrate $\Delta H_{\text{sol},c_s,298}^{\circ}$ = $18.6 \text{ kJ}\cdot\text{mol}^{-1}$, see Table 1. Furthermore, equation (2) is only a

very rough fitting equation for solubility data of non-ideal solutions, e.g. when the measurement accuracy is not good, Fig. 4. As we have shown e.g. in the case of caffeine, a much better fit for $\ln x$ can often be obtained with a polynomial of second power in $1/T$ (ref. 11). The same proved true for theophylline (Fig. 5) and from this equation $\Delta H_{298}^{\circ} = 30.6 \text{ kJ}\cdot\text{mol}^{-1}$ is calculated. If $\ln x$ for the solubility measurements of FOKKENS (ref. 8, 15) is fitted by polynomials of second power in $1/T$ for theophylline hydrate and anhydrate separately, the curves intersect at 66°C , close to the stability point of $\lambda_D = 67^{\circ}\text{C}$ as found by us, Fig. 6.

The enthalpy of solution at infinite dilution for the hydrates is

$$\Delta H_{\text{sol}, c \rightarrow o, 298}^{\circ} + \Delta H_D^{\circ}.$$

One obtains $30.3 \text{ kJ}\cdot\text{mol}^{-1}$ for theophylline hydrate and $23.38 \text{ kJ}\cdot\text{mol}^{-1}$ for caffeine hydrate. This is close to ΔH_{298}° in the case of theophylline ($30.6 \text{ kJ}\cdot\text{mol}^{-1}$) but far from the value for caffeine ($36.7 \text{ kJ}\cdot\text{mol}^{-1}$), which means that the solute/solute interactions in theophylline solutions must be much smaller. The much higher solubility (and its high temperature increment) of caffeine must thus be attributed to better "base-stacking" of caffeine molecules in solution. This is in accord with conclusions drawn by GUTTMAN and HIGUCHI from their partition coefficient measurements (ref. 16), which they defend against controversial but doubtful results obtained by NMR (ref. 17).

Such structure/interaction relationships in purine and other solutions are the subject of further studies under way in our laboratory.

ACKNOWLEDGEMENTS

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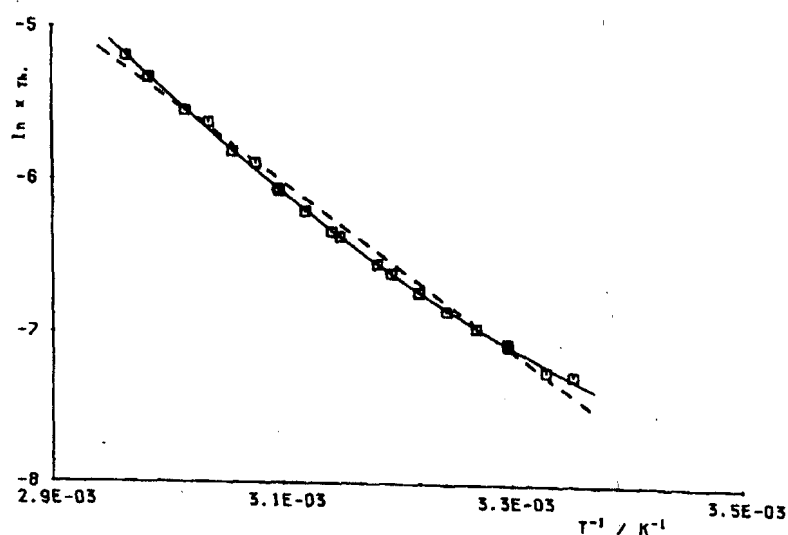


Fig. 5. Solubility of theophylline hydrate of this work alone. The data have been fitted as well by equation (2) (dotted line) as by a polynomial of second power in $1/T$ (full line).

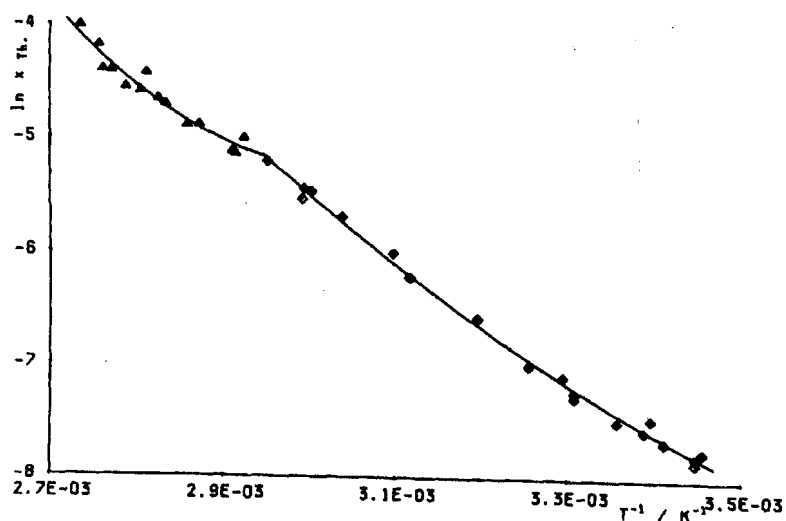


Fig. 6. In this figure the solubility data of FOKKENS (ref. 8, 15) have been fitted for theophylline hydrate and theophylline separately by polynomials of second power in $1/T$. The intersection is at 66°C , see text.

91

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Prediction and Observation of Isostructurality Induced by Solvent Incorporation in Multicomponent Crystals

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The prediction of crystal structures with more than one molecule in the asymmetric unit ($Z' > 1$) represents a major challenge in the field of crystal structure prediction (CSP).¹ The increasingly complex searchable phase space for crystal structures with multiple crystallographically independent molecules results in a much higher computational cost than for $Z' = 1$. This is the case for even the simplest multicomponent systems. As a result, although the properties of such systems (which include cocrystals, solvates² and salts)^{3,4} are of key importance to the pharmaceutical industry, as well as other areas of materials chemistry, their study has been principally experimental—with the exception of only three studies on the prediction of various sugar hydrates⁵ and diastereoisomeric salts.^{6,7}

Carbamazepine (CBZ), a first generation antiepileptic drug, is a well-studied crystal system known to crystallize in various polymorphic forms.⁸ CBZ is also well-known for its promiscuity in forming cocrystals and solvates;⁹ CBZ:acetic acid (AcOH) is one of many. On the other hand, its 10,11-dihydro derivative (DHCBZ) has very rarely been investigated, with no studies addressing its cocrystallization/solvation tendencies. In a recent study,¹⁰ we rationalized how the small difference in molecular shape between CBZ and DHCBZ induces the systems to hydrogen bond in completely different manners: dimers are consistently observed in all CBZ polymorphs,⁸ whereas chains are preferred in the DHCBZ ones.^{11,12} In this context, two main points are addressed in this study: first, on the predictability of the crystal structures of these two pharmaceutical molecules with AcOH, and second, on the influence of the incorporation of AcOH in the crystal lattices on the observed hydrogen bonding and crystal structures of these, to date, very different crystal systems.

We initially generated crystal structures of 1:1 CBZ:AcOH in six of the most common space groups ($C2/c$, $P2_1$, $P2_12_12_1$, $P2_1/c$, $P1$, and $R\bar{3}$) using a Monte Carlo simulated annealing sampling of packing space, as implemented in the Polymorph Predictor package in Cerius2.¹³ Because of the stochastic nature of the Monte Carlo algorithm, independent searches in each space group were repeated until no new structures were generated, requiring up to 15 independent runs in some space groups. Such calculations required approximately 2 months of computer time (on a single 600 MHz SGI machine). Meanwhile, an assessment of the space group occurrences using the Cambridge Structural Database¹⁴ (CSD) was also undertaken. This assessment showed that more than 85% of the observed multicomponent systems of nonchiral molecules with AcOH crystallize within either $P2_1/c$ or $P\bar{1}$ space groups.¹⁵ This information was then used for the prediction of our second system, DHCBZ:AcOH. Limiting the calculations to these two space groups reduced computational time significantly. Ranking of the crystal structures was on the basis of lattice energy, using the W99 potential and an atomic multipole model for the evaluation of intermolecular

Table 1. Crystal Structure Information for the Experimental and Predicted CBZ:AcOH and DHCBZ:AcOH Crystal Structures. Both Systems Belong to the $P2_1/c$ Space Group

	CBZ:AcOH		DHCBZ:AcOH	
	predicted	exp, $T = 100$ K	predicted	exp, $T = 120$ K
E_{int} (kJ/mol)	-175.75		-171.71	
ρ (g/cm ³)	1.295	1.331	1.241	1.300
a (Å)	19.135	18.499	19.163	18.720
b (Å)	15.808	15.714	16.043	15.425
c (Å)	5.034	5.121	5.215	5.299
β (°)	93.49	96.55	94.78	95.17

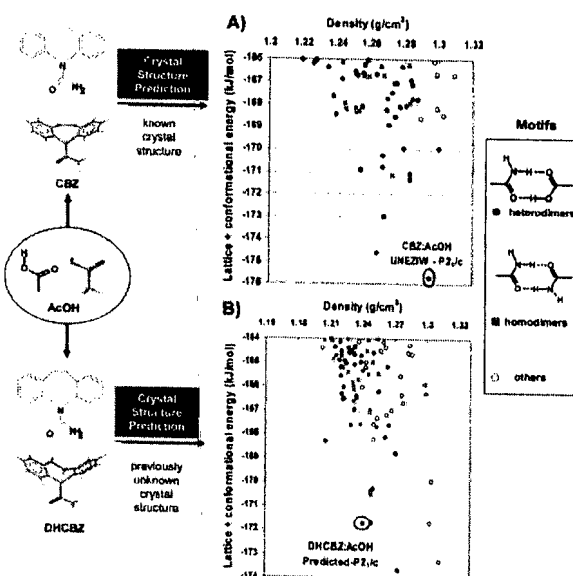


Figure 1. Crystal structure prediction results for the two multicomponent systems CBZ:AcOH (A) and DHCBZ:AcOH (B). Structures are classified by hydrogen bond motif: black dots show heterodimers, crossed squares homodimers, and open circles other motifs (e.g., homo- or heterochains).

repulsion–dispersion and electrostatic interactions, respectively. Molecular flexibility due to amide pyramidalization was taken into account during energy minimization in the same manner as reported for our study of the pure CBZ polymorphs.¹⁶

An experimental crystal structure of CBZ:AcOH (CSD refcode UNEZIW)⁹ was known beforehand. For the DHCBZ:AcOH system, no experimental work was undertaken until all calculations were completed and a set of possible structures was established. Subsequently, possible formation of crystals of DHCBZ with AcOH was assessed by simple grinding¹⁷ of the two components. The resulting structure was identified by comparison of the experimental PXRD pattern with the simulated ones for the most stable predicted

[†] Cambridge Crystallographic Data Center.

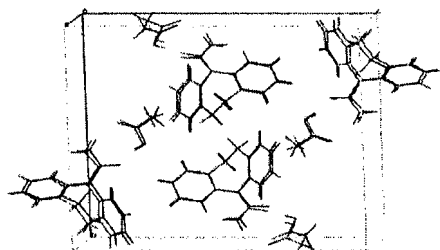


Figure 2. Overlay of the predicted (red) and experimentally determined (blue) DHCbz:AcOH crystal structures.

crystal structures of the system. As final confirmation, single crystals were grown from a solution of DHCbz in AcOH, and the structure was confirmed by single-crystal XRD at 120 K (Table 1).

Excellent agreement between prediction and experiment was obtained for the CBZ:AcOH system (Figure 1A). Not only was the experimental structure found by the search method, but it also emerged as the most stable among the thousands of possibilities generated during the sampling of packing space. Upon analysis of the DHCbz:AcOH prediction results (Figure 1B), it was noticed that the third most stable generated structure for DHCbz:AcOH was isomorphic with the experimental CBZ:AcOH. Furthermore, the energy gain predicted upon formation of the DHCbz:AcOH multicomponent crystal ($E_{\text{DHCbz:AcOH}} - E_{\text{DHCbz}} = -52.7$ kJ/mol) was of comparable magnitude to that of the known CBZ:AcOH system ($E_{\text{CBZ:AcOH}} - E_{\text{CBZ}} = -60.8$ kJ/mol),¹⁸ thereby suggesting a high likelihood of formation. The subsequent grinding experiment revealed the formation of a DHCbz:AcOH crystal structure which was readily identified as the third most stable predicted structure, the structure isomorphic with CBZ:AcOH (Figure 2). Although the structure observed would not have been first choice in terms of calculated stability, falling within the lowest three was encouraging, and it still remains to be seen whether additional polymorphs, corresponding to the two lower energy structures, may be produced.

From a crystal engineering point of view,²⁰ analysis of the hydrogen bond motifs in all of the hypothetical structures (Figure 1) may be considered a way of assessing the synthon preferences in this type of multicomponent system. The amide–carboxylic acid heterosynthon is notably most favored within the low-energy structures of both systems (Figure 1); the calculations agree with the synthon rationalization proposed by Fleischman et al.⁹ for the design of a series of CBZ cocrystals and solvates.

Remarkably, we noticed that even though small changes in molecular shape appear to dictate the preference between chain and dimer motifs for the CBZ and DHCbz single-component crystals, incorporation of AcOH resulted not only in the formation of identical heterosynthons but also in isomorphic crystal structures (Table 1 and Figure 3) for the two systems. In this case, the presence of the second component overrides the influence of the change in molecular shape on crystal packing and hydrogen bonding.

In conclusion, we have reported the first successful prediction of the crystal structures of multicomponent crystals of two pharmaceutically active molecules, demonstrating that CSP methods are useful in understanding multicomponent crystals; the methods are, of course, subject to the same limitations as single-component studies—limited accuracy of model potentials and our lack of understanding of kinetic influences on crystal structure. Further-

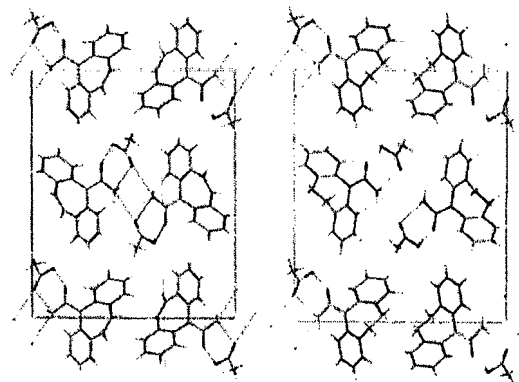


Figure 3. Unit cell representations of the isomorphic CBZ:AcOH (left) and DHCbz:AcOH (right) crystal structures.

more, we stress the fact that, although CBZ and DHCbz show very different hydrogen bonding and packing arrangements in their respective single-component polymorphs, formation of a multicomponent crystal with AcOH results in isomorphic structures.

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Supporting Information Available: Crystallographic data of the DHCbz:AcOH crystal structure. CIFs and further information of the 10 most stable predicted crystal structures of CBZ:AcOH and DHCbz:AcOH. Complete list of authors for ref 1. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Importance of Molecular Shape for the Overall Stability of Hydrogen Bond Motifs in the Crystal Structures of Various Carbamazepine-Type Drug Molecules

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ABSTRACT: Carbamazepine, a first generation anticonvulsant, is known to crystallize in various polymorphic forms, all of which exhibit an *anti*-carboxamide hydrogen bond dimer motif. Furthermore, unless cocrystallized with carboxylic acids, these dimers are also present in most crystal structures of the known carbamazepine solvates. On the other hand, two derivatives of the drug (oxcarbazepine and 10,11-dihydrocarbamazepine) have been reported to adopt hydrogen bond chain motifs in their crystal structures, whereas the epoxy derivative (10,11-epoxycarbamazepine) shows a third mode of hydrogen bonding, *syn*-dimers. In order to rationalize the differences in hydrogen bonding caused by the small changes in molecular structure, computational searches for the low-energy crystal structures were analyzed. In addition, interaction energies between pairs of molecules were calculated using the SCDS-PIXEL approach, which partitions the intermolecular interaction energy into its different contributions (Coulombic, polarization, dispersion, and repulsion). The importance of overall molecular shape and the influence that this has on the hydrogen bond arrangements in these structures is emphasized.

1. Introduction

Carbamazepine (CBZ) is a first generation anticonvulsant used in the treatment of epilepsy and trigeminal neuralgia. Metabolized in the liver, the first step in its degradation consists of the oxidation of the C₁₀–C₁₁ double bond (Figure 1) to the carbamazepine 10,11-epoxide (ECBZ),¹ a metabolite responsible for some of the side effects associated with consumption of the drug.² The derivative 10,11-dihydro 10-oxo carbamazepine, known as oxcarbazepine (OXCZ), has been shown to have several advantages as a drug, including improved efficacy and tolerability and fewer side effects.^{3,4}

CBZ is an interesting system not only from a pharmacological view but also as a model compound for the study of polymorphs,⁵ solvates,⁶ and cocrystals.⁷ To date, however, no reports have appeared dealing with the possible polymorphs or cocrystals of CBZ derivatives such as ECBZ and OXCZ. However, during preparation of the manuscript, a second polymorph of one CBZ derivative, 10,11-dihydrocarbamazepine (DHCZ), was reported. (In this paper, where we refer to the crystal structure of DHCZ, we mean the first reported form, unless otherwise indicated.) Four polymorphs are reported for carbamazepine: form I⁸ (*P*1, Cambridge Structure Database⁹ Refcode = CBZMZN11), form II⁹ (*R*3, CSD Refcode = CBZMZN03), form III¹⁰ (*P*2₁/*c*, CSD Refcode = CBZMZN01), and form IV¹¹ (*C*2/*c*, CSD Refcode = CBZMZN12). All the polymorphs exhibit similar hydrogen bonding: that is *anti*-carboxamide dimers (Figure 2). Furthermore, unless cocrystallized with a carboxylic acid, most of the reported CBZ solvates show a similar hydrogen bond *anti*-dimer motif.⁷ Recently, however, crystal structure prediction studies for CBZ have suggested that *anti*-dimers and chains are of comparable stability.^{6,12} Despite an extensive experimental screen using high-

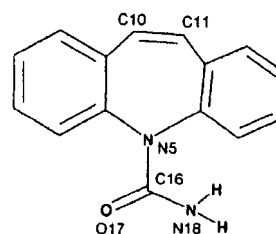


Figure 1. Sketch of carbamazepine and numbering of some of the atoms.

throughput crystallization methods,⁶ no additional polymorphs were obtained and, in particular, none exhibiting the chain motif. Interestingly, we noticed that two derivatives of the drug, OXCZ (*P*2₁/*c*, whose crystal structure was only recently reported¹³) and 10,11-dihydrocarbamazepine¹⁴ (DHCZ) (*P*2₁/*c*, CSD Refcode = VACTAU01) exhibit chain motifs in their crystal structures (Figure 2). The epoxy derivative ECBZ¹⁵ (*P*ben, CSD Refcode = ZIPTEX) shows a third mode of hydrogen bonding, twisted *syn*-dimers (Figure 2).

The questions we address here are as follows: how can we explain the dramatic influences that small changes in molecular structure (remote from the hydrogen-bonding functional group) have on the observed hydrogen bonding in the crystal structures and how “predictable” are the structures adopted by the various derivatives?

Crystal structure prediction (CSP) is a growing area of research whose main aim is to predict the observable crystal structures of a compound starting from only a knowledge of molecular connectivity.^{16–18} Such a powerful technique is of great applicability especially in pharmaceutical sciences and other areas (e.g., fine chemicals and pigments). If the crystal form used for drug delivery is not the most stable, phase transitions may occur to another polymorph with unpredictable and potentially serious changes in physical properties.^{19–21} In

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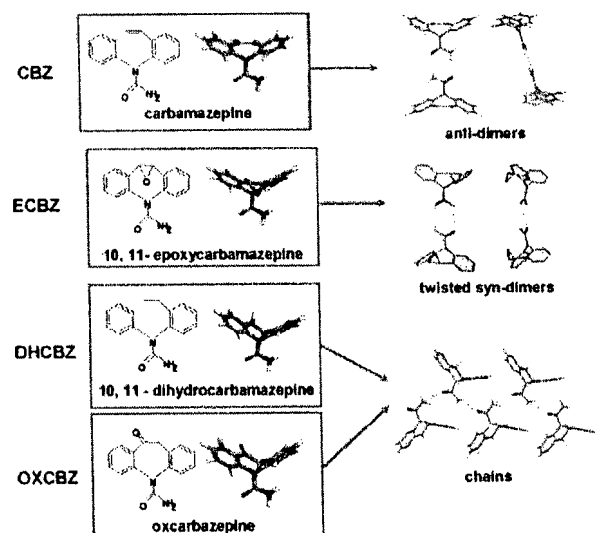


Figure 2. Carbamazepine (CBZ), 10,11-epoxycarbamazepine (ECBZ), 10,11-dihydrocarbamazepine (DHCBZ), and oxcarbazepine (OXCBZ) molecular structures and hydrogen bond motifs observed in the crystals.

In this study, we present crystal structure prediction calculations for ECBZ, DHCBZ, and OXCBZ and compare the results with those recently reported for CBZ itself.¹² Of special interest are the CSP calculations for OXCBZ, because it is a second generation antiepileptic drug currently in use and no studies have been reported on the stability of the observed crystal structure or tendency for polymorphism. Additionally, the distributions of hydrogen bond patterns, along with other molecular interactions, are analyzed. SCDS-PIXEL^{22,23} calculations of the strengths of these competing interactions are used to rationalize their effect on the observed hydrogen bonding on the various crystal structures. Finally, we discuss the importance of molecular shape and its influence on the hydrogen bonding interactions for these molecules in the crystal structures.

2. Computational Methods

Molecular Models. All molecular models were treated as rigid during the crystal structure search and lattice energy minimizations. The initial molecular structures were taken from density functional theory (DFT) isolated molecule geometry optimizations using the code *Dmol3* as implemented in the Accelrys package *Materials Studio*,²⁴ using the PW91 functional²⁵ and the double numerical polarized (DNP) basis set.²⁶

Model Potential. The repulsion–dispersion contributions to the intermolecular potential were evaluated using the W99 empirically derived atom–atom potential parameters^{27–29} and interactions were summed to a 15 Å cutoff. The center of interaction for all hydrogen atoms was shifted 0.1 Å along the X–H bond (X = C, N, or O) toward the heavy atom; X–H distances were shortened by 0.1 Å after DFT optimization. These adjusted hydrogen atom positions were also used as centers for the electrostatic model (see below).

Electrostatic Models. Electrostatic contributions to the intermolecular potential were calculated from two models of the molecular charge distribution: atomic charges and atomic multipoles. Atom centered point charges were derived to reproduce the molecular electrostatic potential (ESP charges)^{30,31} using the fitting procedure implemented in *Dmol3*. Atomic multipoles up to $l = 4$ (charge, dipole, quadrupole, octupole, and hexadecapole) on each atom were obtained by performing a distributed multipole analysis³² of a B3P91/6-31G** (d,p) electron density using the *CADPAC* code.³³ These atomic multipoles were derived for the adjusted hydrogen atom sites of the W99 model potential, from the wavefunction calculated before shortening X–H distances. All charge–charge, charge–dipole, and

dipole–dipole contributions to the lattice energy were evaluated using Ewald summation, while higher order terms (up to R^{-5}) were summed to a 15 Å cutoff on whole molecules.

Crystal Structure Prediction. Crystal structures were generated in the nine most common space groups ($P2_1/c$, $P1$, $P2_12_12_1$, $P2_1$, $C2/c$, $Phca$, $Pnma$, $Pna2_1$, and $Pben$), as well as in $R3$, the space group of one of the observed CBZ polymorphs, using the simulated annealing algorithm of Karfunkel and Gdanitz,^{34–36} as implemented in the Accelrys *Polymorph Predictor* (PP) module of the Cerius² software suite.³⁷ Searches were carried out with one molecule in the asymmetric unit ($Z' = 1$), followed by lattice energy minimization (W99 + ESP charges) and clustering to remove identical structures. Up to four independent searches were performed within each space group until the generation of structures converged. This initial clustering of structures was performed using the algorithm implemented in the Cerius² software.³⁸ The simulated annealing adjustable parameters were taken from previous studies.³⁸

Refinement of the Calculations. As described in a recent publication,¹² amide pyramidalization is an important flexibility problem to consider in crystal structure prediction. For consistency in the methodology and improvement of the calculations, amide pyramidalization was considered in the same way as reported recently in our study for CBZ.¹² For further details and a diagram of the methodology, see Supporting Information. Final results presented here correspond to crystal structures that were lattice energy minimized with the molecular model showing the optimal amide geometry (in each particular crystal structure) using the W99 potential (for the description of dispersion and repulsion) and the atomic multipole model (for the description of the electrostatics) using the program *DMAREL*.³⁹

Structure Comparison and Final Clustering. The *COMPACT*⁴⁰ algorithm was used for the final clustering and comparison of the computer generated and the experimentally observed crystal structures (taken from the Cambridge Structural Database⁴¹) with a tolerance of 0.15 Å on atomic separations in a coordination sphere of 15 molecules, a central molecule and its 14 nearest neighbors.

Synthon Calculations—SCDS-PIXEL Method. The valence electron densities of the molecular models were calculated with the package Gaussian at the MP2/6-31G** level of theory using appropriate box sizes and steps of 0.08 Å in the calculations. Pixels were then condensed into superpixels. Molecular clusters, taken from the various *DMAREL* minimized crystal structures, were generated by using the appropriate crystal symmetry operations. Single-point energy calculations of those molecular clusters were performed using the parameters described by Gavezzotti.⁴¹

3. Results

3.1. Similarities and Dissimilarities of the Molecular Structures in the Experimentally Observed Crystals. The main chemical differences between the molecular structures of CBZ and its derivatives are located in the azepine ring at carbons 10 and 11. While those atoms are double bonded in CBZ, they are epoxidated in ECBZ (the oxygen of the epoxide lying below the azepine ring), hydrogenated in DHCBZ, and carbonylated at C10 and hydrogenated at C11 in OXCBZ. The four derivatives are therefore chemically very different around the azepine ring, but none exhibit any extra hydrogen bond donors, although the carbonyl in OXCBZ could act as a hydrogen bond acceptor.

From the point of view of the molecular shape, ECBZ retains the symmetry and the shape of the azepine ring and is superimposable on the CBZ molecular structure (Figure 3), although the extra oxygen of the epoxy function constitutes an important change in terms of shape in the region below the azepine ring. By comparison, however, there is a clear break in the symmetry of the azepine ring in the other two derivatives and therefore a distortion in the shape of the “butterfly” ring system. Besides the hydrogen and oxygen atoms at C10, DHCBZ and OXCBZ themselves are almost perfectly superimposable.

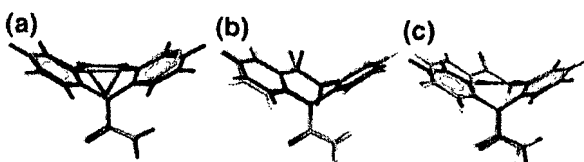


Figure 3. Superposition of the molecular structures of (a) CBZ (blue) and ECBZ, (b) DHCBZ (red) and OXCBBZ, and (c) CBZ (blue) and OXCBBZ. The molecular structures were taken directly from the experimentally solved crystal structures, CSD refcodes CBMZPN02, ZIPTEX, and VACTAU and the crystal structure of oxcarbazepine.

3.2. Molecular Models. When the symmetry of the azepine ring is broken in the molecular structures of DHCBZ and OXCBBZ, the conformations obtained by a 180° rotation about the N5–C16 bond (Figure 1) are no longer related by inversion. As a result, two molecular minima with different energies can exist (we denote them as SYN and ANTI, Figure 4). The SYN conformations are those where the NH_2 groups are located on the same side as C10 (see Figure 1) whereas the ANTI conformation represents the opposite arrangement. In both cases, the SYN conformations are less stable than the ANTI conformations, by 1.67 and 4.17 kJ/mol for DHCBZ and OXCBBZ, respectively (based on DFT calculations). Crystal structure prediction calculations using the ANTI or the SYN conformations would give rise to two completely different sets of crystal structures. After two independent crystal structure searches with each conformer, the most stable generated crystal structures for the SYN sets were located 7 and 5 kJ/mol higher in total energy than the most stable crystal generated with the ANTI conformations for DHCBZ and OXCBBZ, respectively (see Supporting Information). The crystals generated with the SYN conformation were not, therefore, energetically competitive with those generated with the ANTI and were not considered further.

3.3. Crystal Structure Predictions. Crystal structures prediction results on the four systems are shown in Figure 5, where relative total energy (lattice and conformational) versus packing coefficient of the structures are classified by hydrogen bond motifs. Computer-generated crystal structures within 5 kJ/mol of the global minimum for each molecule are available in CIF format as Supporting Information.

All experimentally observed crystal structures (with the exception of form I CBZ, which has four molecules in the asymmetric unit) were successfully generated during the CSP calculations (circled structures, Figure 5). Nevertheless, their predicted stabilities, relative to the alternative computer-generated crystal structures, vary among the four derivatives. For CBZ, the lattice energy calculations successfully predict two of the polymorphs (form III and form IV); their lattice energies are lowest among the predicted possibilities, along with an energetically competitive third structure, which has not yet been observed experimentally. On the other hand, form II and the energy-minimized form I polymorphs were predicted not to be as stable (8.3 and 6.2 kJ/mol above the global minimum, for further discussions see ref 12). Predictions were not as successful for ECBZ, where the experimentally observed crystal structure was found to be almost 5 kJ/mol above the global minimum in energy. To date, no other polymorphs have been reported for this system, although predictions indicate that other more stable crystal structures are possible. Interestingly for DHCBZ, there are two very similar (but distinct) and almost isoenergetic generated structures (ranked number 2 and 3 in the final set of crystals) that match the experimentally observed crystal structure within the tolerances used in the structure comparison algorithm. These two apparent minima at 0 K would

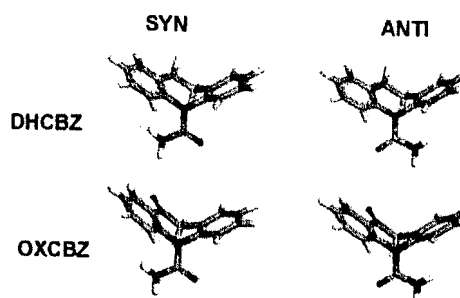


Figure 4. Molecular models of the ANTI and SYN conformations of DHCBZ and OXCBBZ.

probably converge to one free energy minimum at higher temperatures and this “structure delocalization” may result in a higher entropic contribution lowering its free energy relative to other structures. Nevertheless, according to these results, there is still a hypothetical crystal structure predicted to be more stable (at 0 K) than the structure found in the CSD. In fact, while this paper was in preparation, the report of a new polymorph of DHCBZ was published.⁴² To our satisfaction, this new polymorph corresponded to the global minimum structure from our search. This polymorph shows the same type of hydrogen bonding as the previously known crystal structure. By way of contrast, for the oxo derivative OXCBBZ, the experimental crystal was predicted to be the most stable, with no other competitive structures in a range of 2.5 kJ/mol. This observation is important since it suggests that no polymorphs of competitive energy appear to be possible, suggesting that if this drug is commercialized with this form, no phase transformations to another polymorph would be expected.

Concerning the type of hydrogen-bond motifs (Figure 5), while both *anti*-dimers and chains are found among the most stable predictions for CBZ, chains are notably favored for the DHCBZ and OXCBBZ crystal structures (all the stable crystals up to 4 kJ/mol above the global minimum contain chains, Figure 5). It is therefore not surprising that the experimentally observed crystal structures of both DHCBZ and OXCBBZ show hydrogen bond chains. On the other hand, chains, *anti*-dimers, and other types of motifs, where the epoxide oxygen is also involved as acceptor (see Supporting Information), were found among the most stable of the ECBZ predicted structures. Unexpectedly, the experimental crystal structure shows *syn*-dimers (Figure 2), which are rarely found in any of the four sets of predictions (crossed squares in Figure 5).

As a complementary analysis, the packing of the molecules in the different structures is also of great interest; there are two fundamental ways in which the azepine ring can pack, sandwich herringbone and π – π stacking (Figure 6) [for further discussion on packing similarities in the observed CBZ crystal structures and some of its analogues see ref 43].

The three most stable structures predicted for carbamazepine (including form III and form IV) showed sandwich herringbone interactions between azepine rings whereas both form I and form II exhibited π – π stacking interactions (Figure 7). This π – π stacking interaction is not favorable in the case of epoxycarbamazepine, because the epoxide oxygen lying below the azepine ring sterically prevents this contact; thus a sandwich herringbone arrangement is the most commonly found among the most stable predicted crystal structures, in agreement with the experimentally observed crystal structure. Both the $P2_1/c$ polymorph of DHCBZ and the crystal structure of OXCBBZ show π – π stacking. We observed that the best π – π stacked structures

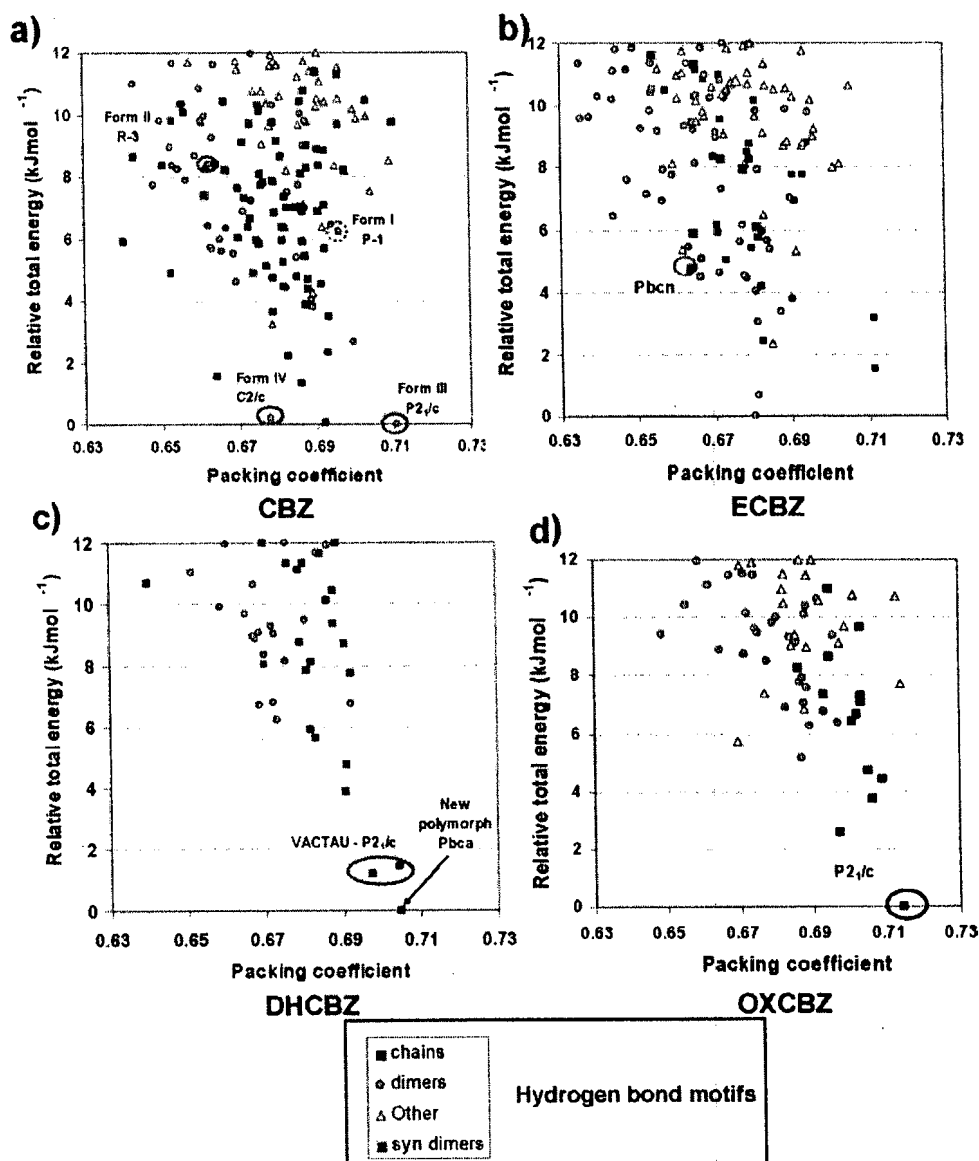


Figure 5. Final predictions classified by hydrogen-bonding motifs: (a) carbamazepine,¹² (b) epoxycarbazepine, (c) 10,11-dihydrocarbamazepine, and (d) oxcarbazepine.

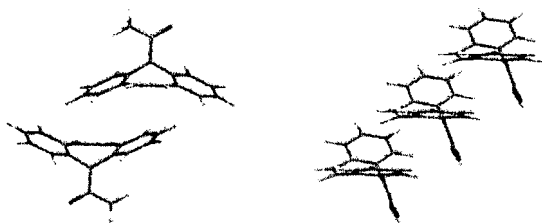


Figure 6. Types of recognizable packing of the azepine rings: sandwich herringbone (left) and π - π stacking (right).

are both 4.7 kJ/mol more stable than the best sandwich herringbone structures in this case. It is to be noticed that the recently discovered lower energy structure for DHCbz has neither of these packing patterns.

Finally, differences in the packing efficiencies of the four sets of crystal structures are also noticed. We find that OXCbz packs more efficiently than the other derivatives with the observed crystal structure having a packing coefficient of 0.714. On the other hand, a large range of packing coefficients (from 0.677 to 0.710) is observed within the most stable crystal structures predicted for CBZ, of which the P2₁/c polymorph is the most efficiently packed. For ECBZ, only two crystal structures pack in an exceptionally efficient way (black squares in Figure 5 at packing coefficients of 0.711). Neither of these structures has been observed, but it would not be surprising if the most stable of them was obtained after crystallization under high-pressure conditions; high pressure has been used to access high-density predicted crystal structures of other small molecules.⁴⁴ In comparing the packing efficiency of the different hydrogen bond motifs, we find that structures exhibiting

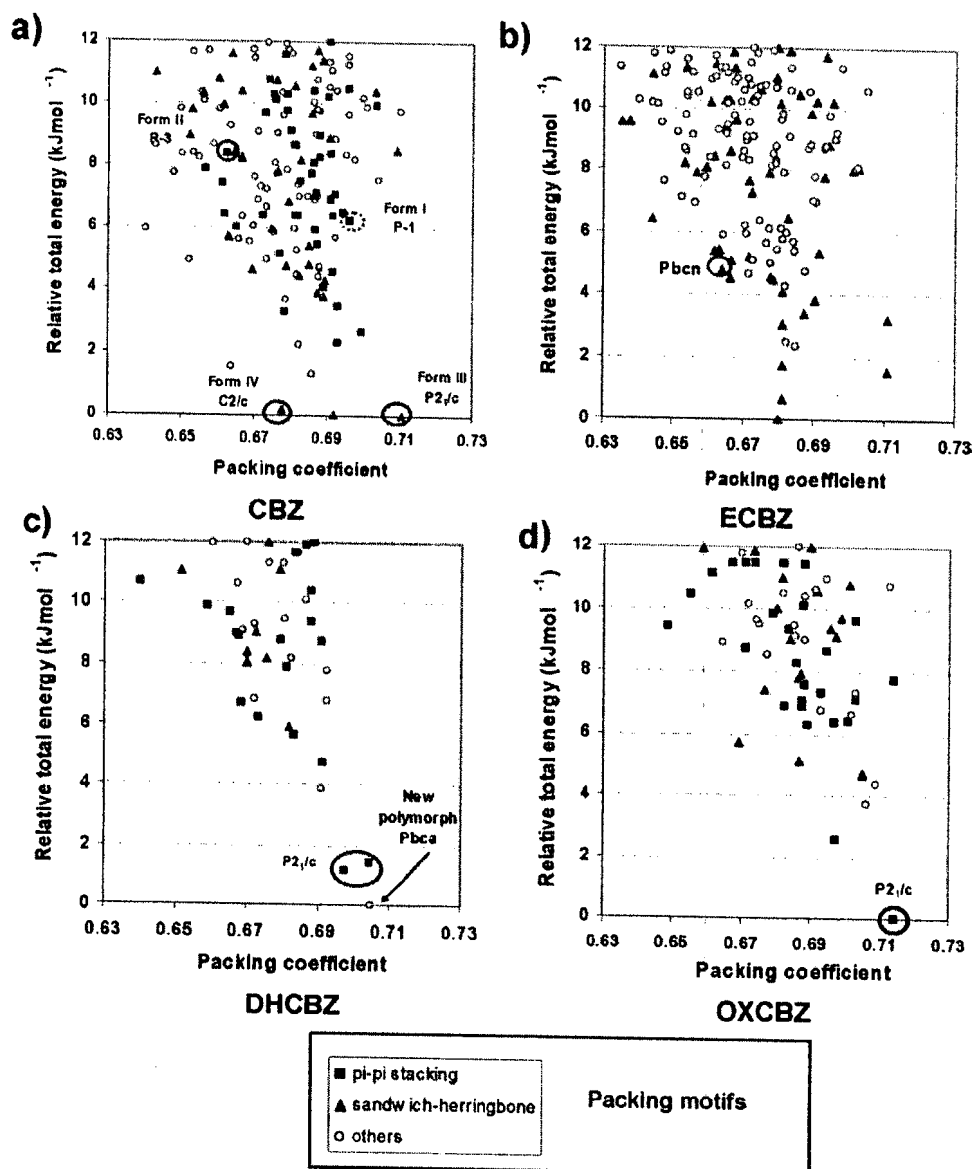


Figure 7. Final predictions classified by packing motifs: (a) carbamazepine, (b) epoxycarbazepine, (c) 10,11-dihydrocarbamazepine, and (d) oxcarbazepine.

hydrogen bond chains generally pack more efficiently than those containing dimers, with the exception of CBZ where the distinction in packing coefficient between chains and dimers is less clear.

3.4. Synthon Calculations—SCDS-PIXEL method. Although the geometries of molecular pairs taken from the crystal structures may not be energy minima when isolated from crystal forces, single-point energy calculations of interaction energies in such pairs may help us determine which interactions are dominant in certain crystals and, therefore, which interactions are more important during crystal formation. For example, comparing the different interactions among polymorphic forms may be an important factor in understanding why a particular polymorph, over other possible ones, is observed under certain conditions. Also, a comparison of the interaction energies of the same motifs found in the crystal structures of the different

CBZ derivatives may clarify why chains are favored in the crystal structures of some derivatives but not others.

We took molecular pair geometries from CBZ form III (the most stable polymorph) and the known crystal structures of DHCbz, OXCbz, and ECBz. For comparison, we took molecular pairs from the most stable predicted hypothetical crystals with alternate hydrogen bonding to the observed structures: CBZ structure ranked 2 (the most stable chain), ECBz global minimum (*anti*-dimers), and the most stable structures showing *anti*-dimers for DHCbz and OXCbz (ranked 8 and 6, respectively). Interaction energies calculated with the PIXEL method are summarized for these pairs in Table 1. The interaction energies are given per molecule, which requires halving the dimer interaction energy; the calculated interaction between a pair of molecules taken from a chain is not halved because each molecule would be involved in two such molecule—

Table 1. Interaction Energies Per Molecule for the Most Stable (Experimental or Hypothetical) Dimers and Chains Calculated Using the PIXEL Method

interaction ^a	molecule	crystal structure	packing ^b	E_{coul} (kJ/mol)	E_{pol} (kJ/mol)	E_{disp} (kJ/mol)	E_{rep} (kJ/mol)	E_{total} (kJ/mol)
<i>anti</i> -dimers	CBZ	form III	SH	-32.7	-11.3	-6.0	25.2	-24.8
	ECBZ	hyp. rank 1	SH	-29.2	-9.6	-6.1	21.8	-23.0
	DHCBZ	hyp. rank 8	π - π	-35.1	-11.6	-6.4	28.8	-24.2
	OXCBCZ	hyp. rank 6	SH	-27.2	-8.0	-5.8	17.8	-23.1
<i>syn</i> -dimers	ECBZ	<i>Phen</i>	SH	-17.8	-5.2	-5.5	9.4	-19.0
	CBZ	hyp. rank 2	SH	-21.5	-9.8	-15.5	22.2	-24.7
	DHCBZ	$P2_1/c$	π - π	-25.8	-11.7	-16.4	25.4	-28.6
	OXCBCZ	$P2_1/c$	π - π	-25.5	-10.9	-16.4	23.8	-28.9

^a For interaction energies per molecule, the calculated dimer energies are divided by 2. ^b SH = sandwich herringbone, π - π = π - π stacking of the azepine rings.

molecule interactions in the complete chain. The chain energies do not account for next nearest neighbor interaction energies (hydrocarbon-hydrocarbon), but we expect these to be somewhat balanced by similar interdimer interactions in the crystal.

The calculated interaction energies of the *anti*-dimer motifs are very similar for all four derivatives (approximately -23 to -25 kJ/mol). However this is not the case for hydrogen bond chains, where the interaction energy is found to be favored for DHCBZ and OXCBCZ over CBZ by almost 4 kJ/mol. Chains and dimers in the CBZ crystals show comparable interaction energies (-24.8 and -24.7 kJ/mol respectively), whereas chains are clearly favored over dimers for both DHCBZ (by ~4.5 kJ/mol) and OXCBCZ (by ~6 kJ/mol). These energies are in line with the observations we made based on total lattice energies of the observed and predicted crystal structures and show that much of the energetic advantage for chains in DHCBZ and OXCBCZ results from the strength of the interaction between hydrogen-bonded molecules. For dimers, this interaction energy is dominated by the Coulombic contribution, which is weaker in the chains, but compensated for by a greater dispersion energy (Table 1). On the other hand, *anti*-dimer interaction energies are also greatly favored (by ~4.5 kJ/mol) over the *syn*-dimers in the ECBZ crystal structures.

4. Discussion

In light of the results described in sections 3.3 and 3.4, various noteworthy points can be addressed in an overall comparison of the four molecular systems.

4.1 On the Possible Latent Polymorphism of CBZ Derivatives. Limited by the type of calculations (static and at 0 K) and unable to consider kinetic effects, we cannot yet predict the formation of metastable polymorphs and cannot computationally study growth condition effects on the occurrence of polymorphs. Nevertheless, if the observed crystal structure happens not to be the global minimum in a CSP calculation or many structures are found to be of very similar lattice energy, we may expect polymorphism for the system. For example, we are confident that no thermodynamically more stable polymorphs are to be expected for OXCBCZ (because there are no competitive structures within approximately 3 kJ/mol), whereas for DHCBZ, we predicted a lower energy structure than the known crystal structure, and this has now been experimentally observed.⁴² Similarly, polymorphism in ECBZ is expected according to the results of the CSP calculations, where many structures were found to be more stable in terms of lattice energy, and the PIXEL calculations show that the *anti*-dimer interaction is favored over the *syn*-dimer interaction (Table 1).

On the other hand, we also notice differences in the density of generated structures (i.e., the number of energy minima in a certain energy range above the global minimum) for the various

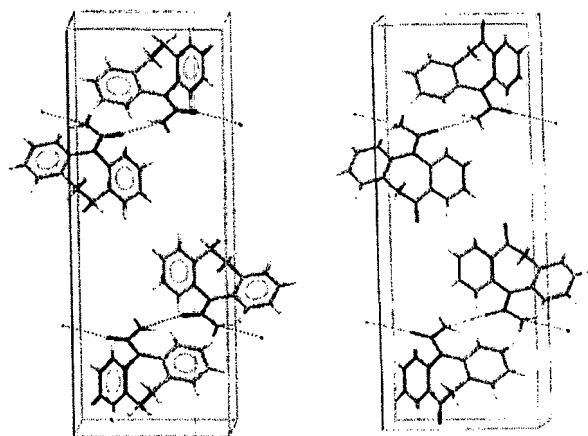


Figure 8. Unit cells of the crystal structures of dihydrocarbamazepine¹⁴ ($P2_1/c$, $a = 5.51$ Å, $b = 9.16$ Å, $c = 24.27$ Å) and oxcarbazepine¹³ ($P2_1/c$, $a = 5.28$ Å, $b = 9.31$ Å, $c = 24.84$ Å).

derivatives; while many energetically competitive crystal structures are generated for CBZ and ECBZ (the two molecules where symmetry around the azepine ring is maintained), the number of generated structures of low energy for DHCBZ or OXCBCZ (the two molecules where symmetry around the azepine ring is broken) is much lower. To what extent is molecular shape or molecular symmetry determining the number of low-energy packing possibilities and in a way its potential polymorphism? Could we then state that OXCBCZ is less likely to be polymorphic than ECBZ? Unfortunately, definitive answers are not possible at this stage of the calculations, and future work is also needed in order to give an appropriate answer, because much experimental work has focused on CBZ but very little for any of its derivatives.

4.2. On Molecular Shape as the Most Important Factor Dictating Molecular Packing. Interestingly, we noticed that not only the hydrogen bond arrangements and the packing motifs but also the observed crystal structures of DHCBZ (the $P2_1/c$ polymorph) and OXCBCZ were very similar, if not isostructural (Figure 8). Indeed, if the DHCBZ molecule is replaced by that of OXCBCZ in VACTAU01 (the crystal structure of DHCBZ) and lattice energy minimized, the crystal structure of OXCBCZ is obtained and vice versa. This is an important observation because, even though the molecular structures of both are different chemically, nearly isomorphous crystals are observed. This highlights the importance of overall molecular shape in determining crystal packing; despite the opportunity for extra hydrogen bonding in OXCBCZ (using the carbonyl group), the two geometrically superimposable molecules adopt the same crystal structure. Indeed, the most stable crystal structure in

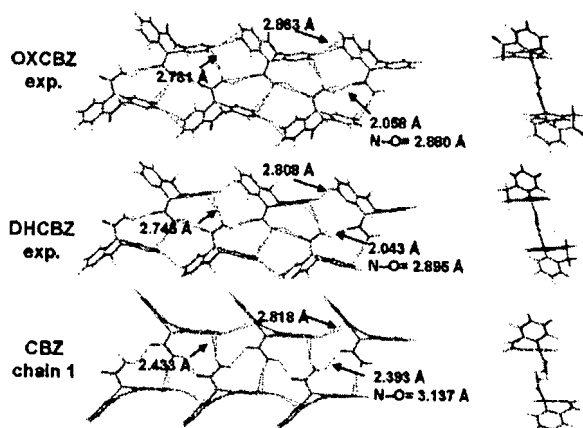


Figure 9. Comparison of the chain structures of the observed OXCBZ and DHCBBZ crystals and the hypothetical most stable chain structure of CBZ.

which the carbonyl oxygen does participate in hydrogen bonding is 4.7 kJ/mol less favored than the experimentally observed crystal. Also, by examining the set of predicted crystal structures of CBZ, we found a crystal structure close to isomorphous with the crystal structure of DHCBBZ and OXCBZ (relative energy of about 2 kJ/mol above the monoclinic polymorphs). The growth of this crystal structure was attempted without success by seeding a saturated CBZ solution in ethanol with crystals of DHCBBZ.

4.3. On the Rationalization of the Observed Hydrogen Bond Motifs. As Dunitz and Gavazzotti recently suggested,⁴⁵ a detailed analysis of intermolecular motifs in virtual and experimental crystal structures is a unique way of assessing which motifs are common, which motifs are obligatory, and which motifs are unlikely for a certain system. As stated previously, both the CSP and PIXEL calculations indicated that the chain motifs are favored over *anti*-dimers in the most stable generated crystal structures of DHCBBZ and OXCBZ (the only molecules where the azepine ring is distorted). This is not the case for CBZ, where chains and *anti*-dimers are present in the most stable predicted structures, nor for ECBZ where *anti*-dimers are more popular in the lowest energy region. However, as shown by the PIXEL calculations, the calculated interaction energies of the *anti*-dimer motifs are very similar in all four cases because the structural differences between the molecules are remote from the amide group and they do not affect the dimer interaction. Rather than dimers being energetically disfavored for DHCBBZ and OXCBZ, it would appear, therefore, that the small changes in molecular shape by the azepine ring distortion favor the chain interaction. But, why do such small changes in molecular shape favor chains over dimers and why has a chain structure not been observed for CBZ, even after a quite extensive experimental screening?⁴⁶

Almost 40 years ago, Kitaigorodskii suggested that the structure of a crystal is mainly dictated by repulsive forces.⁴⁶ If the chain structures of the different crystals are analyzed carefully (Figure 9), important geometric differences can be observed. For similar $C_{ar}H-C_{ar}$ close contacts in all chain structures (Figure 9), the observed OXCBZ and DHCBBZ crystals allow the best hydrogen bond contacts, leading to a 5 kJ/mol advantage in Coulombic and polarization energy over chains in CBZ. Furthermore, the predicted N—O distance for the two observed chain structures (OXCBZ and DHCBBZ) lie in the highest probability of the N—O distance distribution of amide

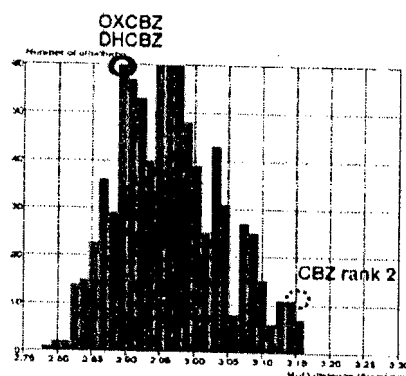


Figure 10. Histograms of N—O distances in the crystal structures of amides (384 entries, excluding dimer motifs) found in the CSD.⁸

structures showing hydrogen bonds distinct to dimers in the CSD. In contrast, the N—O distance in the predicted CBZ chain structure lies in the tail of the histogram (Figure 10), suggesting that it is energetically strained.

As pointed out in section 3.1, the changes in molecular shape of DHCBBZ and OXCBZ with respect to CBZ are most important around the azepine ring (Figure 3), since for DHCBBZ and OXCBZ, one of the benzene rings is twisted slightly inward. This small deformation of the azepine ring allows these two molecules to come closer in the chain structure and thus optimize the hydrogen bonding. Similar optimal hydrogen bonds cannot be formed in CBZ chains (and neither in ECBZ) without generating very short, repulsive, CH—C close contacts.

This analysis offers an explanation (at least in part) as to why the OXCBZ and DHCBBZ chains are more stable than the CBZ chain. Even though the total lattice energy of a chain structure of CBZ is competitive with the observed forms III and IV, the geometrically strained hydrogen bond chains might be at a disadvantage during crystallization. If it was found to be the only stable structure by far, then it would be expected to be found experimentally under certain conditions. However, its accessibility during nucleation is disfavored, because other isoenergetic crystals showing stronger hydrogen bonding are possible. This may explain why, even after extensive experimental effort,⁶ crystal structures containing chains have not been observed for CBZ.

5. Conclusions

Successful crystal structure prediction (CSP) calculations have been presented for two of the CBZ polymorphs and the crystal structures of OXCBZ and DHCBBZ. Additionally, metastability of the known ECBZ crystal structure is proposed, because both CSP and PIXEL calculations suggest that neither the crystal structure nor the *syn*-dimer interaction were among the most favored possibilities. Analysis of the low-energy calculated crystal structures helps rationalize why the small changes in molecular structure result in completely different hydrogen bonding arrangements in their crystal structures. Furthermore, PIXEL calculations on the different hydrogen bond motifs showed how the small distortion in the azepine rings in DHCBBZ and OXCBZ resulted in chain motif preference, because the molecules were able to approach closer and therefore maximize the hydrogen bond interaction in the hydrogen-bonded chains. With a combined CSP and PIXEL calculation study, we have shown how, although hydrogen bonds are crucial in the stability of these crystal structures, it is indeed the repulsions and the

molecular shape (even if the changes are very small) that dictate the final packing of the structures and, in the end, the hydrogen bonding.

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Supporting Information Available: Descriptions of the molecular models and the CSP methodology, results of calculations with ANTI and SYN conformations, unusual motifs found in the predicted crystal structures, density of structures in CSP plots, information on lowest energy predicted crystal structures for each of the four molecules, and CIF files for these structures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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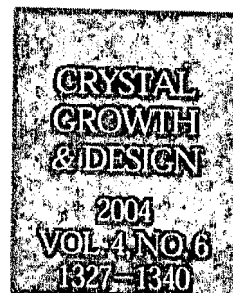
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An Assessment of Lattice Energy Minimization for the Prediction of Molecular Organic Crystal Structures

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ABSTRACT: Lattice energy searches for theoretical low-energy crystal forms are presented for 50 small organic molecules, and we compare the experimentally observed crystal forms to these lists of hypothetical polymorphs. For each known crystal, the relative stability is calculated with respect to the global minimum energy structure, and we determine the number of unobserved structures lower in energy than the experimental form. The distributions of these relative energies and their rankings in the predicted lists are used to determine the efficacy of lattice energy minimization in crystal structure prediction. Although a simple form for the interaction energies has been used, the calculations produce almost a third of the known crystals as the global minimum in energy, and approximately a half of the known structures are within 1 kJ/mol of the global minimum. Molecules with no hydrogen-bonding capacity are most likely to be found close to the global minimum in lattice energy, while increasing the number of possible hydrogen-bond donor–acceptor combinations leads to less reliable predictions.

1. Introduction

Computational methods of predicting the most likely crystal structures of a molecule from its atomic connectivity alone could be of great value in several areas of materials chemistry. The predicted properties of such hypothetical polymorphs could be used as a screen for molecular crystals with novel properties, such as a high nonlinear optical response. Furthermore, the forecasting of energetically competitive polymorphs may help avoid the potentially disastrous problems associated with unanticipated crystal forms appearing late in the processing cycle of a pharmaceutical molecule.

The field of crystal structure prediction (CSP) has benefited from many detailed studies of well-chosen molecules, such as paracetamol¹ and *p*-dichlorobenzene,² as well as collaborations comparing alternate methodologies for small sets of molecules.^{3–5} Surveys of larger groups of molecules^{6–11} have also been valuable, but have usually been limited to specific classes of molecules, often in the development of model potentials. Such detailed studies of single molecules or small families have the advantage of being able to fine-tune the prediction methodology as well as the possibility of performing extensive experimental investigations in parallel with the theoretical study.

A more general survey does not allow for fine-tuning of the method for each molecular system and so may not give as reliable results for the individual molecules. However, it can provide a broader picture that can answer different questions:

(i) How often have experimentally observed crystals not found the global free energy minimum (i.e., are

kinetic products of crystallization) and, so, what success rate can we expect from CSP by global minimization methods?

(ii) Crystals of which types of molecules are more likely to be predictable by lattice energy minimization (see the recent survey asking this question¹²), and are there molecular characteristics that make them amenable to this most common of prediction methods?

(iii) Where the energy is not the sole factor determining the final crystal structure, what other calculated properties or nonenergetic descriptors can help determine the most likely observable structures generated by CSP searches?

Differences in methodology among the growing number of prediction studies make these questions difficult to answer through literature surveys. Predictions are especially dependent on the model used to calculate energies, and, indeed, different schemes for calculating intermolecular energies can give contrasting results for a given molecule. Pyridine is a good example—an atom–atom model potential with electrostatics described by atomic multipoles found that there are many lower energy structures than the known polymorphs,¹³ but by calculating interaction energies for the same hypothetical crystals by the semiclassical density sums (SCDS) pixel approach,^{14,15} the global minimum does correspond to one of the known forms.¹⁵ The approaches are different and each describes certain contributions to the interaction energy very well. The discrepancies are therefore noteworthy and highlight the sensitivity of crystal structure predictions to the approach used to describe the intermolecular energy. Such variations in results make it difficult to draw general conclusions from individual studies of particular molecules. However, we hope that, over a large set of molecules, using a consistent computational method, such variations will not obscure the overall picture and we can make

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conclusions about the general approach to crystal structure prediction. We have, therefore, performed a study of a large group of organic molecules, with a variety of shapes, sizes, and constituent functional groups. This diverse group of molecules is limited only by the criteria of being (a) small and reasonably rigid (less than 25 atoms) and (b) composed of common atom types, for which high-quality parameters are available for the model potential.

There are numerous software packages that generate lists of hypothetical crystal packings for a given molecule, many of which are described in Verwer and Leusen's review of crystal structure prediction methods.¹⁶ The problem of searching phase space is being solved by the use of clever algorithms and the rapid increase in available computing power. At least for small, rigid molecules, the generation of all of the plausible structures should now rarely be a stumbling point, at least when there is only one symmetrically independent molecule ($Z' = 1$). It is the post-search ranking of structures that is the main obstacle to reliable crystal structure prediction for small, rigid molecules. In the present study, we only consider the lattice energy of the hypothetical crystals. However, the collection of these predicted crystal structures can act as a future test set for other methods of ranking the probabilities of observing computed structures.

We chose to start with a form of model potential that requires no particularly specialized modeling software—the pairwise additive, isotropic repulsion–dispersion model with the charge distribution described by atomic point charges. Higher quality models, such as those explicitly including polarization,^{17,18} using atomic multipoles for more accurate electrostatics,^{19–21} anisotropic atom–atom repulsion,^{2,22} or more detailed partitioning of the molecular charge density,^{14,15} are certain to yield better results, but we leave the effects of such improvements in the model for future study. Furthermore, lattice energy calculations ignore the potentially important effects of lattice vibrational energy differences, especially the entropy – enantiotropic polymorphs are proof that the temperature dependence of relative stabilities is important. A computational study of known polymorphs²³ suggests that entropy differences of up to 15 J/mol·K between polymorphs are possible, although almost two-thirds of polymorphic pairs differ by less than 4 J/mol·K—just over 1 kJ/mol at room temperature. Inclusion of vibrational contributions to the energy will be important in fine-tuning the energy ranking of predicted structures, but we have not calculated these contributions in this work.

2. Choice of Molecules

As we have mentioned, we chose a set of small, rigid organic molecules for our test set. Many practical applications of CSP will need to deal simultaneously with conformational flexibility and the search for close packed structures of the molecule. This increases the difficulty of the problem because all relevant conformations must be searched in the generation of the trial crystal structures, and the models for the inter- and intramolecular energies must be accurate and well balanced. Some of the difficulties in combining inter-

and intramolecular energy calculations have been highlighted by case studies of conformational polymorphism.^{24,25} Flexible molecule force fields are continually improving, but, as we see from the results of recent blind tests,^{3–5} the added difficulties dramatically lower the success rates of CSP for flexible molecules. With this in mind, we initially chose to study rigid molecules and we searched the Cambridge Structural Database for suitable small molecules, using the following criteria.

(i) Molecules containing only the elements C, H, N, and O and a range of common functional groups, molecular sizes, masses, and shapes.

(ii) We defined “small” as less than 25 atoms and only took molecules that we judged as reasonably rigid. Some of the molecules we chose do have a potentially flexible torsion angle or rotation, but it was necessary to include these to cover a range of functional groups (for example, amide substituted aromatic rings). We are not addressing the problems related to molecular flexibility in this work, so when choosing molecules with potentially flexible functional groups, we only included molecules whose calculated gas phase conformation was close to the conformation observed in the crystal, e.g., torsion angles within about 5°. For example, the calculated out-of-plane torsion angle of the amide group in benzamide is about 21°, which is reasonably close the 25–28° observed in the various determinations of its crystal structure.

(iii) The molecule must have at least one known crystal structure with all atomic positions accurately located and no disorder.

As a final criterion, we chose to limit ourselves to the nine most common space groups: $P2_1/c$, $P1$, $P2_12_12_1$, $P2_1$, $C2/c$, $Pbca$, $Pnma$, $Pna2_1$, and $Pbcn$; about 95% of homomolecular organic crystals are observed in these space groups.²⁶ Furthermore, we have only considered crystals with $Z' \leq 1$. While these restrictions would not be necessary when studying a particular molecule, they restrict the search space and allowed us to study a greater number of molecules. As searching the less common space groups is only a technical detail, the exclusion of molecules that crystallize with less common symmetries is a small sacrifice for allowing a larger sample of molecular types and, so, more general results and conclusions.

The set that we chose consists of 50 molecules (Table 1), ranging in molecular weights from 28 to 181 amu. We deliberately chose some molecules that are known to be polymorphic so that the total number of crystals is 62 (ignoring those in the less common space groups that we are not considering). The sampling of space groups within this set (58% $P2_1/c$, 11% $Pbca$, 10% $P1$, 6% $Pna2_1$, 6% $P2_12_12_1$, 5% $P2_1$, and 3% $C2/c$) is reasonably representative of what is observed in the CSD.²⁶

3. Computational Methods

One of our criteria for the choice of molecules was that they were reasonably rigid, so that we could treat the molecular structure as fixed during crystal structure prediction. Crystal structure searches were performed using idealized molecular structures, generated from density functional theory calculations. We optimized the gas-phase structure using the program Dmol3,²⁷ with

103

Table 1. Test Set of Small Organic Molecules and Results of the Crystal Structure Prediction

molecule	molecular formula	CSD Refcode	space group	diagram	N_{inner}^1	ΔE (kJ/mol) ¹
ethylene	C ₂ H ₄	ETHLEN01 (ETHLEN02)	$P2_1/c$ ($Im3m$) ¹	H ₂ C=CH ₂	2	+0.06
acetonitrile	C ₂ H ₃ N	QQQCIV01 (QQQCIV02)	$P2_1/c$ ($Cmc2_1$) ²	N≡C—	8 0	+0.55 -0.20
methylamine	CH ₅ N	METAMI	$Pcab$	H ₃ C—NH ₂	3	+0.25
nitromethane	CH ₃ NO ₂	NTROMA	$P2_12_12_1$	H ₃ C—NO ₂	4	+0.95
formamide	CH ₃ NO	FORMAM	$P2_1/c$	H ₂ N—C(=O)H	30	+1.55
acetic acid	C ₂ H ₄ O ₂	ACETAC	$Pna2_1$	CH ₃ —C(=O)OH	3	+1.25
tetrollic acid	C ₄ H ₆ O ₄	TETROL TETROL01	$P\bar{1}$ $P2_1$	HOOC—C≡C—COOH	12 4	+1.40 +0.86
glycine	C ₂ H ₅ NO ₂	GLYCIN02 GLYCIN (GLYCIN01)	$P2_1/c$ $P2_1$ ($P3_1$) ²	H ₂ N—CH ₂ —COOH	2 98	+1.27 +5.19
(Z)-2-nitroethenamine	C ₂ H ₄ N ₂ O ₂	EDAWIP	$P2_1/c$	H ₂ N—CH=CH—NO ₂	8	+1.83
2-azetidinone	C ₃ H ₅ NO	FEPNAP	$P\bar{1}$		0	-0.61
1,2,4-triazole	C ₂ H ₃ N ₃	TRAZOL	$Pbca$		3	+0.51
isoxazol-3-ol	C ₂ H ₃ NO ₂	NEZMUA	$P2_1/c$		7	+2.23
succinic anhydride	C ₄ H ₄ O ₃	SUCANH	$P2_12_12_1$		0	-3.18
succinimide	C ₄ H ₇ NO ₂	SUCCIN	$Pbca$		12	+2.54
oxazolidine-2,5-dione	C ₃ H ₅ NO ₃	OXAZDO	$C2/c$		11	+4.23
vinylene carbonate	C ₃ H ₂ O ₃	VINYLC	$P2_1/c$		0	-0.74
2-oxazolidinone	C ₃ H ₅ NO ₂	OXAZIL	$P2_1/c$		0	-1.64
5-methylene-oxazolidin-2-one	C ₄ H ₇ NO ₂	YOBQAH	$P2_1/c$		31	+1.84
3,5-pyrazolidinedione	C ₃ H ₄ N ₂ O ₂	DUNVEN	$P2_1/c$		66	+5.06
1,2,4-triazolidine-3,5-dione	C ₂ H ₃ N ₃ O ₂	KOXRIY	$P2_1/c$		45	+4.23
parabanic acid	C ₃ H ₃ N ₃ O ₃	PARBAC	$P2_1/c$		8	+2.44

Table 1 (Continued)

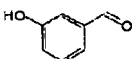
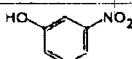
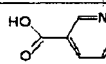
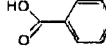
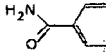
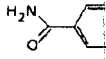
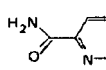
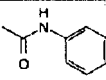
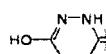
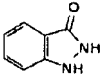
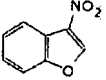
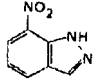
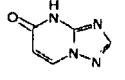
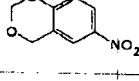
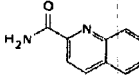
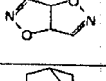
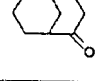
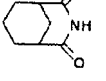
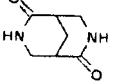
molecule	molecular formula	CSD Refcode	space group	diagram	N_{iso} ¹	ΔE (kJ/mol) ¹
bicyclo(3.2.0)hept-1(5)ene	C ₇ H ₁₀	JIZREP	$P2_1/c$		10	+0.48
3,4-cyclobutylfuran	C ₆ H ₆ O	XULDUD	$Pbca$		0	-0.36
		XULDUDX01	$P2_1/c$		24*	+2.48*
benzene	C ₆ H ₆	BENZEN	$Pbca$		2	+0.16
		BENZEN03	$P2_1/c$		0	-0.16 (-1.12 with PV ²)
pyrimidine	C ₄ H ₄ N ₂	PRMDIN	$Pna2_1$		5	+0.61
<i>m</i> -hydroxybenzaldehyde	C ₇ H ₆ O ₂	XAYCIJ	$Pna2_1$		Only including observed conformation	
					5	+0.61
<i>m</i> -nitrophenol	C ₆ H ₅ NO ₂	MNPHOL01 MNPHOL02	$P2_12_12_1$ $P2_1/c$		Including all 4 possible conformations	
					25	+2.24
<i>o</i> -nitrobenzaldehyde	C ₇ H ₅ NO ₂	NIBZAL	$P2_1$		3	+1.61
					12	+2.70
2-amino-4-nitropyridine	C ₅ H ₄ N ₂ O ₂	SEGRUR	$P2_1/c$		43	+7.30
2-amino-3-nitropyridine	C ₅ H ₄ N ₂ O ₂	AMNTPY	$P2_1/c$		0	-1.18
		AMNTPY01	$P2_1/c$		0	-0.67
		AMNTPY02	$P2_1/c$		-	-
nicotinic acid	C ₆ H ₅ NO ₂	NICOAC	$P2_1/c$		0	-0.14
isonicotinic acid	C ₆ H ₅ NO ₂	ISNICA	$P\bar{1}$		6	+1.35
benzamide	C ₇ H ₇ NO	BZAMID	$P2_1/c$		3 *	+3.15 *
nicotinamide	C ₆ H ₆ N ₂ O	NICOAM	$P2_1/c$		0	-0.96
pyrazinamide	C ₅ H ₄ N ₂ O	PYRZIN	$P2_1/c$		24	+2.72
		PYRZIN01	$P2_1/c$		104 *	+5.88 *
		PYRZIN02	$P\bar{1}$		59	+4.19
		(PYRZIN05)	$(Pa)^2$			
paracetamol	C ₈ H ₉ NO ₂	HXACAN	$Pbca$		11	+3.33
		HXACAN01	$P2_1/c$		0	-1.32
1,2-dihydropyridazine-3,6-dione	C ₄ H ₄ N ₂ O ₂	MALEHY01	$P2_1/c$		2	+0.84
		MALEHY10	$P\bar{1}$		2	+0.55
		MALEHY12	$P2_1/c$		19	+3.59

Table 1 (Continued)

molecule	molecular formula	CSD Refcode	space group	diagram	N_{lower}^1	ΔE (kJ/mol)
phthalide	$C_8H_6O_2$	HEZQUY	$P2_1/c$		2	+0.82
indazolinone	$C_7H_6N_2O$	FADMIG	$P2_1/c$		9	+2.22
3-nitrobenzofuran	$C_8H_5NO_3$	NIVBUP	$P\bar{1}$		0	-0.13
7-nitroindazole	$C_7H_5N_3O_2$	FULKUS	$P2_1/c$		0	-0.03
4,5-dihydro-5-oxo-(1,2,4)-triazolo(1,5-a)pyrimidine	$C_7H_6N_4O$	QAJYIJ	$Pna2_1$		0	-0.01
6-nitro-1,3-benzodioxin	$C_8H_7NO_4$	GEYWIQ	$Pbca$		1	+0.73
1,8-naphthyridine	$C_8H_6N_2$	NAPTYR	$P2_1/c$		0	-0.93
quinoline-2-carboxamide	$C_{10}H_8N_2O$	QUINCB10	$P2_1/c$		6	+2.15
3a,6a-dihydro-isoxazolo(5,4)isoxazole	$C_4H_4N_4O_2$	DIHXL10	$P2_1/c$		2	+0.68
norbornene	C_7H_{10}	HOBBOP	$P2_1/c$		4	+0.58
bicyclo(3.3.1)nonane-2,6-dione	$C_8H_{12}O_2$	HEBBEV	$C2/c$		0	-4.39
3-aza-bicyclo(3.3.1)nonane-2,4-dione	$C_8H_{11}NO_2$	BOQQUT	$P2_1/c$		6	+3.37
3-diazabicyclo(3.3.1)nonane-2,6-dione	$C_8H_{10}N_2O_2$	DOGTIC	$P2_12_12_1$		0	-0.30

¹ ΔE is the calculated energy difference between the experimentally observed crystal structure and the lowest energy unobserved crystal structure. N_{lower} is the number of predicted, but unobserved, crystal structures that are lower in energy than the experimentally observed crystal structure. These measures are described in section 4.3. ² Polymorphs in space groups that were not considered in this work are listed in parentheses. ³ For the high-pressure polymorph of benzene, we added the PV energy term to the calculated energies of all the predicted structures to determine its position in the ranked list of hypothetical structures. ⁴ AMNTPY02 relaxed to the same potential energy minimum as AMNTPY01 (see text). ⁵ Indicates a crystal that was not located in the first four repeats of the simulated annealing search and the search was repeated until the structure was found.

the PW91 functional²⁸ and the double numerical polarized (DNP) basis set.²⁹ Several of the molecules in our list have multiple possible "rigid" conformations. For example, in *m*-nitrophenol, the proton of the alcohol

group could point away from (as is observed in the known polymorphs) or toward the nitro group; both planar geometries are stable in the gas phase and separated by a ~ 5 kJ/mol energy barrier. For the 1.1

molecules where there are such alternate conformations, we optimized geometries for each conformer (see section 4.4).

3.1 Model Potential. Besides the molecular structures, the other main ingredient in crystal structure prediction is a model for the interaction energies between molecules. Much of the variability of results in previous prediction studies results from differences in the choice of model potential.³⁻⁵ As this choice is crucial to the success of a study, we tested several models on a subset of 15 crystals from our test set.

There are many choices for the model potential, not only in the functional form used, but in the approach used to parametrize the function. As already mentioned, we restricted ourselves to simple functional forms of intermolecular potential in the present study, leaving improvements requiring more specialized software and increased computational expense to later investigations. We chose a set of model potentials that we consider suitable for the type of molecules that we are studying here.

Two model potentials derived specifically for rigid molecules were tested, both using the *exp-6* (Buckingham) functional form for nonbonded interactions.

(a) The FIT model uses the C, N, O, and nonpolar H parameters from Cox and Williams' work.^{30,31} The parameters were fitted to reproduce the structures and lattice energies of a large set of non-hydrogen-bonded hydrocarbons, oxohydrocarbons and azahydrocarbons. To complete the FIT model, parameters for polar hydrogen atoms, H(-N) and H(-O), were later fitted by Price and co-workers to sets of hydrogen-bonded organic molecular crystals, in conjunction with an accurate electrostatic model.^{9,20}

(b) The W99 model³²⁻³⁴ is a more recent set of parameters, derived in much the same way as the FIT model, but allowing the parameters for each atom type to depend on the bonding environment of the atom. For example, the parameters describing aromatic and aliphatic carbon were varied separately in the fitting procedure. Another detail of the W99 model potential is that the center of the interaction for all hydrogen atoms is shifted 0.1 Å along the X-H bond (X = C, N, O), toward the heavy atom. Thus, for all of our calculations with the W99 model, we foreshortened all DFT optimized X-H bond lengths by 0.1 Å.

We also tested the use of the nonbonded parameters from three flexible molecule force fields: Dreiding, CVFF95 and COMPASS.

(c) Dreiding³⁵ is the oldest of these three and one of the most commonly used force fields for molecular simulation. We tested the Dreiding-X variation of the potential, which uses the same *exp-6* functional form as FIT and W99. A specific 12-10 model for hydrogen bonding is included within the Dreiding model, although the use of an explicit expression for hydrogen bonding is probably redundant when the repulsion, dispersion, and electrostatic models are sufficiently accurate. Thus, we tested the Dreiding model with and without this hydrogen bond term.

(d) CVFF95³⁶⁻³⁹ force field was developed specifically for materials science applications and the nonbonded parameters were parametrized to the crystal structures

of small organic molecules, employing a 12-6 (Lennard-Jones) form.

(e) The final model that we tested is COMPASS,⁴⁰ a force field for condensed phase simulations. The non-bonded parameters of the 9-6 model were parametrized to structures and properties of the condensed phase. In contrast to the normal approach of parametrization by comparing lattice energy minimized models to experiment, molecular dynamics simulations were used to explicitly include temperature when fitting the model to experiment.

Both CVFF95 and COMPASS can supply their own atomic charges, based on bond increments. We tested the models with their own charges as well as atomic charges specifically fitted for each molecule. The charges were derived by fitting to the molecular electrostatic potential (ESP) of the DFT calculated charge density, calculated within the Dmol3 program.²⁷ The grid of points used in the fitting was taken from the internal and external atomic radii suggested in Dmol3 and smoothly varying weighted fitting points across border layers at the internal and external edges of the area around each atom.²⁷ These ESP atomic charges were used as the electrostatic model for the FIT, W99, and Dreiding calculations.

To test the models, we took the known crystal structures from the Cambridge Structural Database and replaced the molecular structure with the DFT optimized geometry. The crystal was then energy minimized using the Cerius2 software, keeping the DFT geometry fixed. We measured the root-mean-squared change in the lattice parameters *a*, *b*, and *c* and used the overall root-mean-squared change in the lattice parameters to judge the performance of the potential (Table 2).

The ESP atomic charges are a clear improvement over those calculated from transferable bond increments and the cost of calculating the molecular electrostatic potential for these small molecules is minimal. The best performance was with the W99 model, which was developed specifically for these types of molecules, while COMPASS+ESP followed closely behind. We proceeded with the W99+ESP model potential for all of the crystal structure searches presented here. We only used structural comparisons to guide our choice of model potential in this work, so are not making definite conclusions regarding the accuracy of these models. Sublimation enthalpies have been determined for a few of the crystals in our list and these are given, along with the W99+ESP calculated lattice energies, in the Supporting Information. There is good agreement for some of the crystals, while the lattice energy underestimates the sublimation enthalpy for several of the hydrogen-bonded crystals. Previous studies^{11,42} suggest that an improved electrostatic model would improve the agreement between lattice energies and sublimation enthalpies.

3.2 Crystal Structure Search. There are numerous methods of generating the possible crystal packings of a molecule,¹⁶ as compared in blind tests of crystal structure prediction.³⁻⁵ Some of these methods build up crystals from stable molecular clusters,⁴³⁻⁴⁶ while others search phase space systematically,^{6,7,47,48} by Monte Carlo,⁴⁹⁻⁵³ Molecular Dynamics,⁵⁴ or genetic algorithms⁵⁵⁻⁵⁷ or even completely randomly.^{58,59} Some of these methods may be more efficient or more effective

Table 2. Root Mean Squared % Changes in Lattice Parameters on Energy Minimization for the Model Potentials Tested

crystal Refcode	FIT	W99	Dreiding		CVFF95		COMPASS	
			with H-bond	without H-bond	bond increment charges	ESP charges	bond increment charges	ESP charges
ETHLEN	6.55	2.64	3.07	3.07	12.72	2.48	11.03	3.21
NTROMA	4.81	2.09	2.75	2.75	5.73	4.60	2.03	0.89
TRAZOL	6.88	4.68	6.87	6.22	17.09	6.78	21.17	5.67
NEZMUA	5.64	3.80	3.03	2.50	3.37	2.19	4.19	2.22
OXAZIL	2.23	3.12	3.90	1.15	1.98	1.94	2.90	3.25
SEGRUR	2.35	2.55	2.00	1.93	5.59	2.11	6.11	3.53
α PYRZIN	2.34	1.09	2.60	2.42	4.95	3.83	2.70	3.53
β PYRZIN	6.86	2.69	4.38	4.79	2.41	2.99	0.59	2.84
γ PYRZIN	5.23	2.80	2.61	2.89	0.40	0.92	1.72	1.73
δ PYRZIN	4.92	2.79	3.36	4.13	6.26	1.85	6.67	1.98
HEZQUY	2.75	1.42	1.39	1.39	1.85	1.28	1.79	1.77
QAJYLJ	6.57	3.50	2.83	2.83	17.72	4.23	12.52	4.31
GEYWIQ	2.25	1.38	1.41	1.41	2.43	1.80	7.78	0.92
QUINCB	2.45	0.97	1.56	2.27	2.31	2.72	1.96	2.22
HEBBEV	1.58	0.91	0.60	0.60	2.65	1.35	1.41	2.30
overall	4.65	2.66	3.18	3.04	7.91	3.12	7.83	2.96

for different types of molecules, but our aim is not to compare search methods. Instead, we are interested in the ranking of structures, given a list generated by one of these types of searches. We chose the simulated annealing algorithm of Karfunkel and Gdanitz,^{16,51–53} as implemented in the Accelrys Polymorph Predictor module of the Cerius2 software suite.⁶⁰ This search method is well tested and seems to be effective for a range of types of molecules.

The search consists of several steps and is repeated separately for each space group.

(i) The first stage is the simulated annealing, during which crystal structures are built and randomly modified. During a heating phase, high energy barriers can be crossed and so the whole of the potential energy surface is sampled. This is followed by a cooling phase, during which the probability of crossing energy barriers decreases.

(ii) After the simulated annealing, the resulting structures are clustered to eliminate duplicates.

(iii) The hypothetical structures are energy minimized.

(iv) The structures are clustered again to remove duplicates that converged to the same minimum during energy minimization.

There are several parameters that can be adjusted during this routine. The simulated annealing is governed by the heating rate (T_h), cooling rate (T_c), and two parameters to signal the end of the heating phase: a maximum temperature (T_{max}) and a limit on the number of consecutive accepted structures, N_{accept} . The heating is ended when either the temperature reaches T_{max} or N_{accept} consecutive random moves are accepted. The clustering is controlled by a tolerance on the measure of similarity. The lower this number, the closer structures must be to be considered identical, so high tolerances risk throwing away distinct crystals. During the first clustering, a maximum number of structures, N_{max} , are chosen for energy minimization. Although it is possible that a high energy trial structure can relax to be one of the most stable crystals, the number of energy minimizations can be limited, by discarding any structures above N_{max} in the energy ranked list following the simulated annealing. The settings for the parameters are a balance of computing time and con-

Table 3. Simulated Annealing and Clustering Parameters

simulation parameter	value used
heating rate, T_h	0.025
cooling rate, T_c	0.001
maximum temperature, T_{max}	100000 K
maximum number of consecutive accepted structures, N_{accept}	12
clustering tolerance	0.10
structures kept after clustering	10000

fidence that the search generates a complete set of possible low-energy hypothetical crystals.

We took most of our settings from the default values in the highest performance (and most computationally intensive) settings provided by the Cerius2 software. The heating and cooling rates as well as maximum temperature and number of consecutive accepted moves in the heating were left unchanged (Table 3). We have developed our own algorithm for the clustering of crystal structures, so used the Cerius2 clustering with very tight settings. The tolerance for comparing structures was decreased to 0.10 from the default value of 0.13, which resulted in very few structures being discarded. We tested the performance of the search on a subset of our molecules and found that, with this clustering tolerance, the default number of structures to energy minimize (1000 per space group), was too low, missing about 5% of structures within 5 kJ/mol of the global minimum in the final list. While doubling N_{max} to 2000 lowered the number of missed structures to about 1% of those in the lowest 5 kJ/mol, we decided to include all distinct simulated annealing structures in the energy minimization by increasing N_{max} to 10000.

The simulation was repeated several times for a subset of our molecules, combining final lists after each repeat of the simulated annealing. We kept track of the number of new structures in the low energy list after each repeat and found that the search was converged after three repeats for this subset of molecules. To be more confident of adequately searching phase space, we added an extra repeat and performed four searches for each molecule in our set. On average, one repeat of the search (simulated annealing, clustering, and energy minimization) for the nine space groups took about 6–8 hours on our workstation (one 600 MHz processor of an SGI Octane), so we used about 1 day of computing time per molecule with the final settings.

For those molecules with multiple rigid conformations, the search was repeated for any conformations with molecular energy within 10 kJ/mol of the most stable. The low energy lists of crystals structures for each conformation were then combined, using the sum of molecular (gas-phase DFT energy) and lattice (from the model potential) energies as a measure of their relative stabilities.

3.3 Symmetry Constraints and Unstable Structures. Energy minimizers generally do not guarantee that a minimum in the energy is found, but stop at any stationary point (i.e., the forces are all zero to within some tolerance). Such points on the potential energy surface could be minima or saddle points, which are a minimum in energy in at least one direction, but a maximum along at least one other path. Such structures are especially likely to be found when using space group symmetry in the energy minimizations, as is done in the energy minimizations within the Polymorph Predictor package. Therefore, there may be many such cases in the final lists we generate with Polymorph Predictor. These saddle point structures are not observable crystals, so must be removed from the lists of hypothetical structures or pushed downhill toward the nearest minimum on the energy surface. This task is automated in another crystal structure modeling program, DMAREL,^{19,61} which can calculate elastic constants and phonon frequencies for rigid molecules. Either an unstable elastic constant matrix or a negative phonon frequency indicates that the structure is at a saddle point. In either case, DMAREL performs a line search in the direction of decreasing energy and resumes the energy minimization at the lowest point along this path.

The final structures within 15 kJ/mol of the global minimum from the Polymorph Predictor calculations were reminimized using DMAREL, initially using space group symmetry. Their stabilities (phonon frequencies and elastic constants) were then checked and structures were pushed to a minimum where required. In some cases, the original space group symmetry is conserved, but some structures required at least one symmetry constraint to be removed to reach a stable minimum. In these cases, all symmetry was removed from the structure and we identified the remaining symmetry after energy minimization. In some cases, this leads to space groups that were not included in the initial searches and even to structures with more than one molecule in the asymmetric unit. As yet, only $\mathbf{k} = 0$ phonon calculations are implemented in DMAREL, so it is still possible that some structures at the end of this procedure are unstable to unit cell doubling.

For comparison to the lists of predicted crystals, the known crystal structures for each molecule were taken from the CSD and lattice energy minimized, with the molecular structure exchanged for the idealized gas-phase geometry. A table of unit cell parameters for the lattice energy minimized structures is available as supplementary information.

3.4 Clustering. The list of optimized predicted structures often contains several duplicate structures, and it is helpful if these duplicates are removed to produce a list of unique structures. To cluster our list, we have developed an algorithm, COMPACT,⁶² to identify whether two crystal structures are the same

to within specified tolerances. The algorithm avoids the use of space group symmetry but rather defines a crystal structure in terms of its molecular packing environment. A molecule together with its coordination shell, say the closest 14 closest molecules, is chosen to represent the reference crystal structure. Relationships between these molecules are described by distance and angle constraints which can then be searched for in a target structure. Tolerances are placed on distances and angles to control the level to which relative positions and orientations of the molecules must match. This method was also used to locate the position of the observed structure within the predicted list.

4. Results and Discussion

4.1 Are All of the Experimental Structures Generated? Of the 62 crystals in the space groups that we searched, all but four were located in the final lists: benzamide (BZAMID), the monoclinic polymorph of 3,4-cyclobutylfuran (3-oxabicyclo(3.2.0)hepta-1,4-diene) (XULDUD01), the β polymorph of pyrazinamide (PYRZIN01), and form III of 2-amino-3-nitropyridine (AMNTPY02). The result is pleasing; if we were studying a particular molecule on its own, more than four repeats of the simulated annealing would be run to be more confident in having a complete search of phase space. Furthermore, an experienced user of the software could perhaps adjust other settings (e.g., the heating and cooling rates) to further optimize the efficiency of the search. All of the missed structures are in the space group $P2_1/c$. As this is the most common space group for molecular organic crystals, it is probably a good idea for future prediction studies to search this symmetry more intensely than others. For three of these four missed crystals, we added additional repeats of the simulated annealing in $P2_1/c$ until the known structure was found. For XULDUD01, the known structure was located in the fifth search, BZAMID in the 12th, and PYRZIN01 in the 13th. The simulated annealing algorithm clearly has difficulties with the latter two crystals; they may inhabit narrow potential energy wells in otherwise inaccessible areas of phase space. We have not compared search methods in this work, but if the cause of the difficulty in finding these structures is the narrowness of the potential energy well, then other search methods would probably have similar problems in finding these crystal structures.

The four missed structures remind us that we have not produced complete sets of crystal structures for our molecules; there may be other low-energy structures missing. We know this in advance, because of our limiting of the space groups considered. Furthermore, few of the molecules have been subjected to polymorph screens, so it is likely that undiscovered structures exist. More extensive calculations could show up more unrealized low-energy structures, while more extensive experiments could discover more real polymorphs. We expect, however, that the results of either would have a small influence on the overall results of our study.

The lack of success with the remaining crystal (2-amino-3-nitropyridine, AMNTPY02) is due to shortcomings of lattice energy minimization with a simple model potential. When relaxing the experimental crystal on the W99+point charge potential energy surface, the

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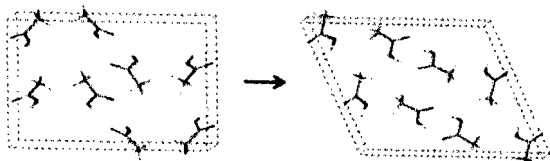


Figure 1. Relaxing one of the acetic acid saddle point (unstable) crystals.

stepped sheet structure relaxes to the energy minimum of one of the other polymorphs (AMNTPY01), with planar hydrogen-bonded molecular sheets. The model fails to reproduce the energy barrier between the two forms, so we can only expect to be able to predict the polymorph with the stable minimum. A similar observation was made with a similar trimorphic molecule, 2-amino-5-nitropyrimidine,⁶³ where a polymorph with buckled hydrogen-bonded sheets collapses to another known polymorph on energy minimization, even with an elaborate model of the electrostatic interactions. Such crystals, with closely related polymorphs, are a challenge for computer modeling. AMNTPY02 is excluded from the results reported in the rest of this study.

Although we only searched nine space groups, the structures do have the opportunity to adopt higher symmetry space groups during energy minimization, or lower symmetry space groups during the stability checking and reminimizations (section 3.3). As a result, we did find one known crystal structure that is not in our list of space groups, the *Cmc*2₁ low-temperature phase of acetonitrile was located in searches of both *P*2₁ and *Pna*2₁ and is the global minimum of the lattice energy search for this molecule (Table 1). Therefore, in total (excluding AMNTPY02 and including the *Cmc*2₁ polymorph of acetonitrile), we consider the prediction of 62 crystal structures in the remainder of this study.

4.2 Reranking of Structures after Reminimization. The use of DMAREL and the calculation of elastic constants and phonon frequencies to test the stability of the predicted structures proved to be very important. Of the 50 molecules, the stability testing and reminimizations introduced new low-energy crystal structures in most cases. On average, 2 of the 10 lowest energy structures for each molecule resulted from this process and would not have been located had we not performed these extra calculations beyond the initial energy minimizations. The "top 10" list of structures of only 6 of the 50 molecules was unaffected by these calculations.

As an example, four of the 10 lowest energy crystal structures of acetic acid were found during this process and would have been missed if the search had been stopped after the first energy minimizations immediately following the simulated annealing search. One of these structures is shown in Figure 1. On the left is structure 80 (counting upward from the global minimum) of the list from Polymorph Predictor, an orthorhombic (*Pbcn*) crystal 4.48 kJ/mol above the global minimum. The stability calculations showed that this structure is unstable to breaking the symmetry constraints of the mirror plane that keeps the methyl groups in close contact. The symmetry was relieved and the lattice parameters shifted during energy minimization to allow the methyl groups to reorient to the symmetry relaxed structure on the right of Figure 1, a

triclinic (*P* $\bar{1}$, *Z'* = 4) crystal 2.56 kJ/mol lower in energy than its orthorhombic "parent" and seventh in the final list.

4.3 Location of the Observed Structures in the Ranked Lists. The locations of the observed crystal structures in the lists of predicted crystals (Table 1) are described by their energy relative to the most stable unobserved crystal in the list (ΔE) and the absolute position of the observed crystals in the list, counting upward from the global minimum. Instead of presenting the position of the observed crystal by its absolute rank, we report the number of lower energy hypothetical, but unobserved, crystal structures, N_{lower} . In this way, a "perfect" prediction yields a negative ΔE and $N_{\text{lower}} = 0$. Furthermore, all the known crystal forms for polymorphic systems can reach such a ranking simultaneously. The three structures that were not found in the original search, but were located after an increased number of simulated annealing repeats, are included in Table 1 and are indicated with an asterisk.

A positive relative energy tells how much the experimentally observed crystal is apparently less stable than the theoretical global minimum structure, while a negative value indicates that there are no lower energy unobserved structures in the list. We illustrate this with two contrasting results, using plots of the lattice energy against density for the lowest energy structures to summarize the results of the CSP for these two molecules (Figure 2). The upper plot shows the results for bicyclo(3.3.1)nonane-2,6-dione. For this rather bulky molecule, the lowest energy structure corresponds to the known crystal (CSD refcode HEBBEV) and there are no competing structures within 4.4 kJ/mol. For this crystal, ΔE is -4.4 kJ/mol and $N_{\text{lower}} = 0$; this crystal is easily predicted and polymorphs seem unlikely.

The lower plot in Figure 2 summarizes results for the much smaller 3,5-pyrazolidinedione. Here, there is no large gap in energy separating any of the lowest energy structures and, with the model potential used here, the known crystal (refcode DUNVEN) is 67th in the list ($N_{\text{lower}} = 66$), 5.1 kJ/mol above the global minimum. With such small energy differences between structures, this crystal is a much greater challenge for prediction by lattice energy minimization. Furthermore, if the energy calculations are correct, and entropy differences between structures are small, the known crystal is metastable to many more densely packed potential polymorphs.

Of the 62 known crystals, 18 are predicted with lower energy than any of the unobserved structures; 17 are global minima and the two polymorphs of 2-amino-3-nitropyridine rank 1 and 2 in energy. This represents a success rate of 29% for such a "perfect" prediction. Of these, most (12) are within 1 kJ/mol of the nearest unobserved crystal and a further three are within 2 kJ/mol; results as in Figure 2a, where the known crystal is far more stable than any others, are quite rare. The worst ranked crystal is the β form of pyrazinamide (refcode PYRZIN01); there are 105 lower energy hypothetical crystals in the list. On relative energy, all of the structures are within 7.3 kJ/mol of the global minimum. The known crystal of 2-amino-4-nitropyridine (CSD refcode SEGRUR) has this highest ΔE . Considering all of the predicted structures as potential poly-

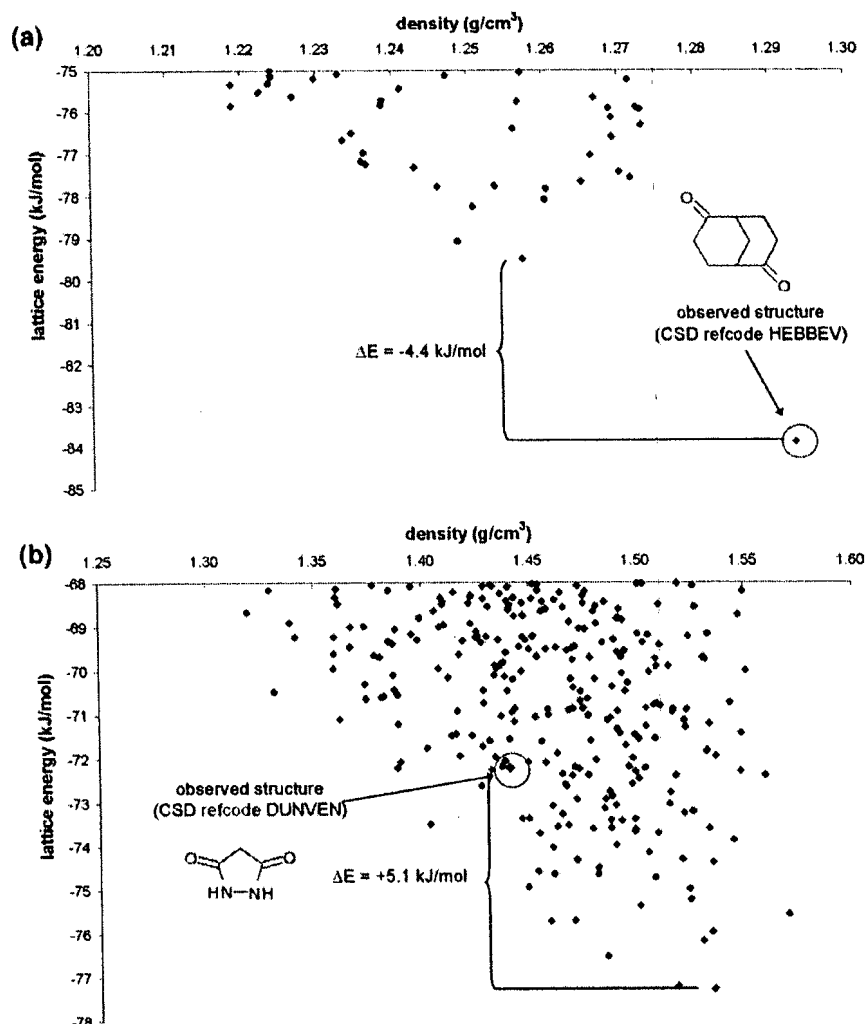


Figure 2. Predicted structures of (a) bicyclo(3.3.1)nonane-2,6-dione and (b) 3,5-pyrazolidinedione.

morphs, these results are consistent with estimates that lattice energy differences between polymorphs are usually smaller than 10 kJ/mol.^{64,65}

Our overall results are summarized in Figure 3a,b, where the predictions are binned into intervals of 5 for N_{lower} and 0.5 kJ/mol for ΔE . The distribution of relative energies is better than we expected, with 35% of structures either lower than any others or within 0.5 kJ/mol of the global minimum; 53% are within 1 kJ/mol. 56% of the observed crystals are separated by fewer than five hypothetical structures from the global minimum and 74% have $N_{\text{lower}} \leq 10$. These distributions could be used to guide future prediction studies. For example, if we wanted to construct a list of possibilities for the crystal structure of a new molecule, with 90% confidence that the real structure will occur in this list, all structures within a cutoff of 4 kJ/mol above the global minimum should be included as possibilities.

The distributions tail off fairly quickly at higher N_{lower} or relative energy and the results are encouraging for the crystal structure prediction of small, rigid molecules. The rate at which these distributions fall away is a combination of two factors. The modeling of energies is fairly crude; lattice energies ignore temperature and

only approximate the energies at $T = 0$ K. Furthermore, the simple form of model potential that we have used has known limitations, so the errors in lattice energies are probably as great as the relative energies that we are calculating for many of the molecules. The second factor is that metastable polymorphs do exist and it is possible that more stable polymorphs are experimentally realizable for some of the molecules in our set. Hence, the relative energies that we calculate for some of the higher ranked known crystals could be a realistic measure of their metastability. To separate the effects of modeling deficiencies and our incomplete knowledge of the true number and stability of crystal forms, we could perform extensive polymorph screens on all 50 of the molecules. This is a mammoth task for this number of molecules. Alternatively, by systematically improving the theoretical basis of our energy ranking, we should get to a point where we can confidently estimate the proportion of crystals in our sample that are metastable.

4.4 Assessing the Importance of Alternate Conformations. For all but one of the molecules where alternate conformations exist, the conformation in the known crystal is the most stable in the gas phase (Table 4). Furthermore, for several of the molecules, the

Table 4. Relative Energies of Alternate Molecular Conformations

molecule	conformational difference from conformation in known crystal	molecular energy relative to conformation found in known crystal (kJ/mol)
<i>m</i> -hydroxybenzaldehyde	180° rotation of hydroxyl	+0.33
	180° rotation of aldehyde	+0.37
nicotinic acid	rotation of hydroxyl and aldehyde	-3.27
nicotinamide	180° rotation of COOH	+1.01
paracetamol	180° rotation of amide	+3.40
<i>m</i> -nitrophenol	180° rotation of hydroxyl	+1.42
<i>o</i> -nitrobenzaldehyde	180° rotation of hydroxyl	+2.02
isoxazol-3-ol	180° rotation of aldehyde	+9.52
1,2-dihydropyridazine-3,6-dione	180° rotation of hydroxyl	+17
pyrazinamide	180° rotation of hydroxyl	+21.0
quinoline-2-carboxamide	180° rotation of amide	+39.5
	180° rotation of amide	+45

conformational energy difference is too great to be balanced by improved crystal packing. Where the mo-

so the N_{lower} and ΔE based on the known conformation are unaffected.

The ranking of the known crystal was only affected for *m*-hydroxybenzaldehyde. For this molecule, one of the molecular conformations, with the hydroxyl and aldehyde substituents rotated 180 degrees from their orientation in the observed conformation, is predicted to be significantly more stable than the observed conformer. On lattice energy, the lowest energy crystal predicted for this conformation is 1.6 kJ/mol less stable than global minimum with the observed conformation, but is more stable on total (lattice + conformational) energy. In fact, 18 predicted crystals with this molecular conformation are more stable on total energy than the known form (refcode XAYCIJ). Of the two conformations that are less stable in the gas phase, one packs poorly, with worse lattice energies than the known crystal, while the other (with just the aldehyde rotated with respect to the known conformation) packs with better lattice energies, which approximately balance the higher molecular energy. On total energy, two crystals of this conformation are lower in energy than the known form. In all, by taking account of these additional conformations, there are an additional 20 calculated structures lower in energy than the observed crystal and the relative energy, ΔE , is increased by 1.63 kJ/mol. This ranking and relative energy were used in Figure 3a,b.

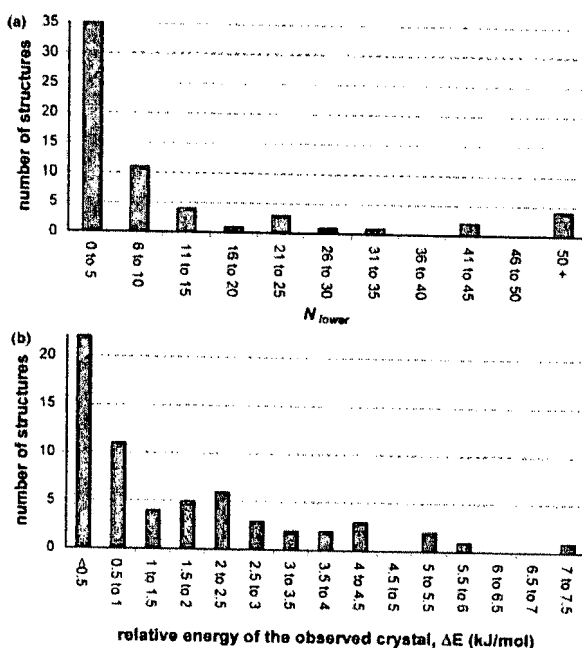


Figure 3. Distributions of the known crystals in our predicted lists.

lecular energy difference is only a few kJ/mol, these alternate conformations can give competitive low-energy crystals, so we performed the prediction for any conformations more stable than, or within 10 kJ/mol of the conformation in the known crystal. This meant extra searches for six of our molecules, each in one extra conformation, except *m*-hydroxybenzaldehyde, where three extra conformations had to be searched. For all of these molecules, the atomic charges were fitted separately to the DFT calculated charge distribution for each conformation.

For five of these molecules (nicotinic acid, nicotinamide, *m*-nitrophenol, *o*-nitrobenzaldehyde, and paracetamol), the global minimum lattice energy in the search with the alternate conformation is less stable than with the observed conformation. Hence, the alternate conformation is disfavored on both molecular and packing energies. On the sum of lattice energy and gas-phase monomer energy, the observed crystal is more stable than any of those found for the alternate conformation,

5. Discussion

5.1 Which Crystals Are Predictable? In addition to the overall statistics on the performance of lattice energy minimization for crystal structure prediction, we hoped that our results would distinguish between types of molecules that are more or less easily predicted than others.

The search procedure used here has been very successful at locating the experimentally observed crystal structures, in all but a few cases—XULDUD01, BZAMID, and PYRZIN01. It is difficult to say what the reasons are for this, although we can speculate that the potential energy well is narrow and difficult to access via the simulated annealing search. That two of these are aromatic amides suggests that such molecules may be problematic for the search routine, although the other two polymorphs of pyrazinamide and the crystal structure of nicotinamide were located without problems.

Besides these infrequent difficulties with the searching of phase space, the success of crystal structure prediction by lattice energy minimization depends on

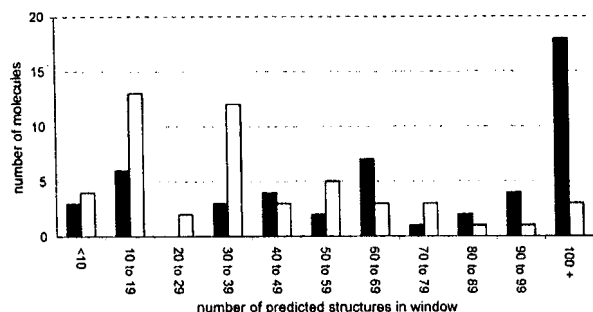


Figure 4. Density of structures in the lowest 5 kJ/mol (filled bars) and lowest 5% in lattice energy (unfilled bars).

the number of predicted low-energy structures and the computation of the relative energies of the predicted crystal structures. We tried correlating several molecular descriptors with the position of crystals in the predicted lists. No easily quantifiable descriptor, such as molecular mass or dipole moment, shows any correlation with how well that molecule's known crystal is predicted. It seems reasonable to suggest that particularly bulky molecules with very asymmetric shapes should have less possible ways to closely pack, so should be more easily predictable. This is not an easily quantified molecular property, but can be judged by eye. We deliberately included four similarly shaped molecules in our set, chosen for their obvious bulkiness. The four crystals (refcodes HOBOP, HEBBEV, BOQUT, and DOGTIC) all rank quite well ($N_{\text{lower}} = 4, 0, 6$ and 0 , respectively). Although less so, 3a,6a-dihydro-isoxazolo-(5,4)isoxazole (refcode DIHXL) also has a distinctive bent shape and its $N_{\text{lower}} = 2$.

The density of structures in the lowest energy part of the list is a measure of the difficulty in predicting the correct structure. A large number of structures in a small energy range means small energy differences and, so, requires much more accurate energy calculations to predict the correct order of stability. We counted the number of structures within 5 kJ/mol of the global minimum for all of our molecules and the distribution (filled bars in Figure 4) shows that about a third of molecules have more than 100 distinct structures in this small energy window. The median of this distribution is about 67. Considering these densely packed lists, the low overall values of N_{lower} are a testament to the usefulness of the empirical atom-atom model potential.

We noticed that the smaller molecules with lower lattice energies generally have more molecules in the 5 kJ/mol energy window, so we also present a distribution that is less dependent on the size of the molecule and magnitude of the lattice energy—the number of structures within 5% of the global minimum (white bars in Figure 4). More than half of the molecules have between 10 and 40 structures in this relative energy range and the median of the distribution is 33. Of the five bulky molecules mentioned earlier, all but norbornene (refcode HOBOP) have fewer than 20 structures within 5% of the global minimum. The distinctive shape of these molecules may be a reason for their having few low-energy close packed possibilities, helping their overall predictability.

5.2 Consequences of Hydrogen Bonding on Predicting Crystal Structures. Apart from global mo-

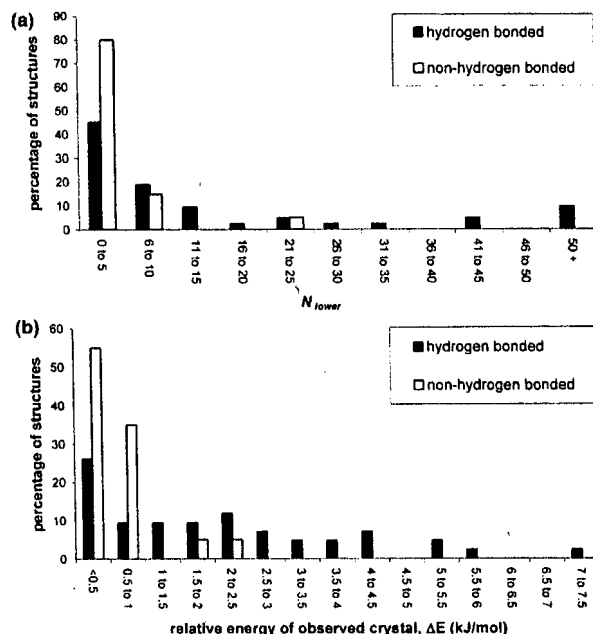


Figure 5. Distributions of (a) N_{lower} and (b) ΔE for hydrogen bonding (filled bars) and non-hydrogen bonding (unfilled bars) molecules.

lecular characteristics, local attributes may be important in determining the predictability of a molecule's crystal structure. Probably the most important such attribute is the presence or absence of hydrogen-bond donors and acceptors. Molecules with the ability to hydrogen bond behave very differently from those whose crystals have no such strong, directional interactions. To examine differences between hydrogen-bonding and non-hydrogen-bonding molecules, the distributions of N_{lower} and ΔE were split into these two categories. For this classification, the presence or absence of weak C-H...O interactions was ignored. Because of the different numbers of each type of molecule in our test set, the distributions are presented as percentages instead of the absolute number of structures in each bin (Figure 5).

Differences between the two classes of molecules are striking. For 80% of non-hydrogen-bonded crystals, there are five or fewer theoretical structures lower in energy than the observed crystal structure and there are never more than 25 lower in energy. In contrast, less than half of hydrogen-bonded crystals have N_{lower} between 0 and 5 and all of the poorly ranked crystals are heavily hydrogen bonded. Over half of the non-hydrogen-bonded crystals are found as either global minima or within 0.5 kJ/mol of the global minimum and 90% are within 1 kJ/mol (Figure 5b). Again, the distribution is much flatter for the hydrogen-bonding molecules; we must consider predicted structures up to 4.5 kJ/mol above the global minimum to include 90% of the observed structures.

The differences in results for the two classes of molecules are likely to be due partly to the increased difficulty in modeling the energies of hydrogen-bonded crystals. Such strong interactions involve very close contacts with hydrogen atoms, so the calculations are especially sensitive to slight changes in molecular

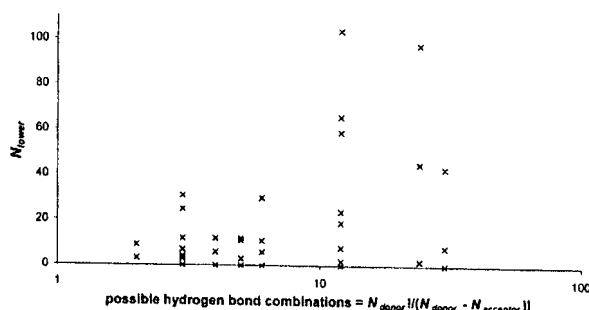


Figure 6. Ranking of the observed hydrogen bonding crystals versus the number of hydrogen bond acceptor–donor combinations.

geometry. The dominant contribution of electrostatics to these interactions also means that the simple atomic point charge model used here limits the quality of calculated energies far more for crystals with hydrogen bonding. Such a simple representation of the charge distribution cannot describe important features such as lone pairs, which dictate the directionality of hydrogen bonds and relative energies of different hydrogen-bond motifs. Thus, the errors in relative lattice energies are greater for molecules with hydrogen-bonding capability. As a result, molecules with a plurality of competing hydrogen-bond motifs are a greater challenge for CSP. For each molecule, a measure of the number of competing hydrogen bond options can be roughly quantified as the number of possible hydrogen-bond acceptors/donors pairwise combinations ($N_{\text{acceptor}}!/(N_{\text{acceptor}} - N_{\text{donor}})!$). While this measure ignores differences in extended motifs, our results strongly suggest that molecules with more hydrogen-bonding possibilities have a higher likelihood of a poor lattice energy ranking (Figure 6).

While much of the difficulty with hydrogen-bonding molecules results from limitations of the model for the interaction energies, the distributions in Figures 5 and 6 could reveal a reality of crystallization. It seems sensible that strong, directional interactions lead to higher barriers between valleys on the potential energy surface. Such higher energy barriers facilitate the trapping of a crystal in a local energy minimum, possibly leading to the growth of metastable polymorphs. This is a likely contributor to our results, but we must reduce the errors in our modeling methods to confidently describe real differences between classes of molecules—higher quality energy calculations are desirable and are the subject of current investigations.

6. Conclusions

Lattice energy minimization, the most widely used method for the prediction of crystal structures, gives promising results for rigid organic molecules. This study of a diverse set of 50 molecules shows the success rate that can be achieved when using a carefully parametrized, but simple form of model potential—about half of all molecules can be found among the five lowest energy predicted structures, or within 1 kJ/mol of the global minimum in lattice energy. Due to differences in the details of modeling methods, these statistics would have been difficult to determine from a survey of the literature.¹²

We might expect that certain molecular characteristics affect the predictability of a crystal and there is some evidence that more bulky molecules have less possibilities for low-energy packing, so are easier to predict than those with simple shapes. However, the greatest difference in types of molecules is the presence or absence of hydrogen-bonding sites. The known crystals of almost all non-hydrogen-bonding molecules are at, or within 1 kJ/mol of, the global minimum. These relative energies are certainly well within the expected errors due to the model potential and omission of thermal effects in lattice energy calculations. There is a much greater variability of ranking for molecules with hydrogen bonding, with up to approximately 100 computed structures lower in energy than the known crystal. While part of this variability may be due to deficiencies in the modeling, the results do point toward a greater propensity for the formation of metastable polymorphs of hydrogen-bonded molecules. Investigations of the effect of improving the model potential and thermodynamic basis of the calculations are required to determine the true proportion of metastable crystals in our test set, which we hope is reasonably representative of all small organic molecules in the Cambridge Structural Database.

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Supporting Information Available: Experimental and lattice energy minimized unit cells (Table S1); experimental and lattice energy minimized unit cells (Table S2); and structure references. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Pharmaceutical Cocrystals: An Emerging Approach to Physical Property Enhancement

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Andrew V. Trask

Abstract

Pharmaceutical cocrystals are crystalline molecular complexes containing therapeutic molecules. They represent an emerging class of pharmaceutical materials offering the prospect of optimized physical properties. This article highlights important opportunities and challenges associated with the design and synthesis of pharmaceutical cocrystals. Cocrystallization is first placed into context with the more established approaches to physical property optimization of polymorph, hydrate, and salt selection. A directed, intermolecular-interaction-based approach to cocrystal design is described. The enhancement of specific physical properties, such as dissolution rate and physical stability, is illustrated by summarizing several recent reports. Synthetic approaches to cocrystallization are considered; in particular, the selectivity and screening-related opportunities afforded by solid-state grinding and solvent-drop grinding methods are discussed. Finally, an outlook on future developments summarizes the growth potential in this field, especially with regard to targeted, informatics-driven cocrystal screening approaches.

Keywords: biomedical, crystal growth, crystalline.

Introduction

On average, about a decade of research and development is expended in the discovery and commercialization of a new pharmaceutical product. Initial R&D efforts center on the identification of a suitable molecular structure, physical form, and formulation. Whereas the molecular structure of the active pharmaceutical ingredient (API) of a drug substance is selected to optimize therapeutic properties, selecting the physical form of an API represents a strategic opportunity for optimizing such physical properties as solubility, dissolution rate, hygroscopicity, physical stability, and chemical stability.¹

Most APIs are dosed as solids, and most solid APIs exist in the crystalline form.

Frequently, however, the API does not crystallize on its own or it crystallizes into one or more crystal forms that possess undesirable physical properties. In either case, an alternative crystal form is typically sought. Various options include single-component and multiple-component modifications of an API, including polymorphs, salts, solvates, and hydrates. In addition to these established crystalline API modifications, pharmaceutical cocrystals, or crystalline molecular complexes involving an API, have recently attracted interest as an alternative approach.

This article outlines how pharmaceutical cocrystals offer an alternative approach to physical property optimization

during crystal form selection. Important design strategies for making cocrystals are described, along with some recent examples of using cocrystals to enhance specific physical properties. Cocrystal screening and synthesis are also covered, particularly using solid-state grinding and solvent-drop selective cocrystal synthesis.

Solid-State Modifications of APIs Polymorphs

A polymorph is "a solid crystalline phase of a given compound resulting from the possibility of at least two different arrangements of the molecules of that compound in the solid state."² Different polymorphs of a given compound each possess a unique set of physicochemical properties, and many, if not most, compounds exhibit polymorphism to some extent.^{1,3,4} Some compounds exist in more than ten crystal form modifications.⁵ At present, it is not generally possible to computationally predict the number of observable polymorphs of even the simplest molecules,⁶ and as a result, the use of high-throughput screening methods to search for new polymorphic forms has become an important tool in form screening.⁵

Hydrates and Solvates

Frequently during crystallization, solvent may be taken up and incorporated as part of the crystal structure. Most solvents, however, are biologically toxic; as a result, most solvate-containing crystals are avoided in the development of the solid form of an API.

An important exception is the subclass of API hydrates, which are well known in pharmaceutical products.^{7,8} It has been estimated that one-third of pharmaceutical molecules are capable of forming hydrates.^{9,10} As a result of process-induced stresses, such as changes in temperature, pressure, or relative humidity, hydrates often convert into anhydrous crystal forms. This conversion from hydrate to anhydrate can result in significant changes in physical properties and can present major issues, for example, during storage, where hydrate conversion can compromise dosage form appearance and integrity.

Pharmaceutical Salts

Salt formation is a common approach to modifying the properties of an API.¹¹⁻¹³ Salt formation is an acid-base reaction between the API and an acidic or basic substance. It is an attractive strategy, because most pharmaceutical compounds possess either acidic or basic functionality, and the widespread use of salt formation is evidenced by the large number of marketed crystalline salts of APIs.¹⁴

Pharmaceutical Cocrystals

A more recent approach to pharmaceutical physical property optimization is pharmaceutical cocrystal formation. A cocrystal may be thought of as a crystalline complex of two or more neutral molecular constituents bound together in the crystal lattice through noncovalent interactions, often including hydrogen bonding.

The application of cocrystallization to the pharmaceutical industry provides inherent benefits as compared with salt formation in at least two ways. The first is that, at least in theory, all types of molecules can form cocrystals, including weakly ionizable and non-ionizable APIs, which are traditionally considered to present a higher risk in terms of physical property optimization because they have either limited or no capacity for salt formation.

A second benefit is that, whereas for toxicological reasons only 12 or so acidic or basic counterions are explored in a typical API salt screen,^{12,15} there are many potential counter-molecules that may be used in cocrystal synthesis. (A counter-molecule may be defined as the species cocrystallized with the API.) The U.S. Food and Drug Administration manages several lists of substances that have precedence as food ingredients (e.g., the FDA's GRAS list, a list of substances "generally recognized as safe"), with the total amount of substances numbering in the thousands. Although the increased scope of cocrystals is a benefit in that it suggests a greater likelihood of achieving a desirable physical property profile for an API physical form, it also presents a challenge in terms of screening efforts, even with high-throughput screening.

To maximize efficiency in screening for cocrystals, therefore, improved rational cocrystal design and more efficient cocrystal screening protocols are needed.

Synthon Approach to Cocrystal Design

The formation of cocrystals has been studied for some time in academic research, and various important studies aimed at understanding cocrystal design have been published.

In early studies, Etter and co-workers proposed several "hydrogen-bond rules," including the observations that (1) all good proton donors and acceptors are used in hydrogen bonding, and (2) the best donor typically pairs with the best acceptor in a given crystal structure.¹⁶ The combined use of the hydrogen-bond rules with a geometric analysis (known as graph-set analysis¹⁷) assisted Etter and co-workers in implementing rational cocrystal design

in the synthesis of many new supramolecular structures.

Allen et al. demonstrated a quantification of the "robustness" of a certain class of intermolecular arrangements (commonly called motifs, or synthons) involving strong hydrogen-bonded bimolecular ring motifs. Their analysis involved examining trends within the Cambridge Structural Database (CSD), a searchable repository containing more than 300,000 small-molecule crystal structures.¹⁸ They assessed the robustness of a motif in terms of its "formation probability," that is, the observed frequency of motif formation among all structures containing the necessary functional group components. A higher formation probability suggested a greater utility in a cocrystal design scheme.

By relying on robust intermolecular interactions with demonstrated solid-state reproducibility, synthon-based cocrystal design has become increasingly important to the synthesis of new cocrystal materials. In the future, automated searches for formation probabilities pertaining to the molecular structure of an API of interest will be an important step toward rational pharmaceutical cocrystal design.

Pharmaceutical Cocrystals and Physical Property Enhancement

During the past few years, the focus on pharmaceutical cocrystals has increased significantly.¹⁹ In 2002, Oswald et al. demonstrated cocrystallization of the analgesic drug paracetamol (acetaminophen) with six different counter-molecules, each of which was capable of acting as a hydrogen-bond acceptor.²⁰ Shortly thereafter, Zaworotko and co-workers reported cocrystals of the APIs ibuprofen, flurbiprofen, and aspirin with several hydrogen-bond acceptors.²¹ These examples served as early proof that a series of cocrystals with common hydrogen-bonding features may be obtained with APIs. Aside from melting point data, however, these reports focused essentially on structural features without addressing the functional properties that these cocrystals might offer. Additionally, from an industrial standpoint, these were only model systems, in that the non-API components were, in most cases, not known to be safe for human ingestion.

In a subsequent paper, Zaworotko and co-workers reported on a series of cocrystals of the API carbamazepine, a drug used in the treatment of epilepsy, with a variety of different counter-molecules, including several that are biologically nontoxic, including acetic acid, nicotinamide (vitamin B₃), and the well-known sweetener saccharin.²² The report brought to light the

variety of possible counter-molecules that may form cocrystals with a single API.

Despite the increase in reports containing new pharmaceutical cocrystal structures, however, only a limited number of studies have directly addressed the realization of physical property modification. One report of a pharmaceutical cocrystal with enhanced dissolution properties involved cocrystals of several nontoxic C₄ (four-carbon) 1,4-dicarboxylic acids with itraconazole, an antifungal drug with very low aqueous solubility in its crystalline free base form.²³ The cocrystals reportedly resulted from a high-throughput crystal form screen of itraconazole, and the acids in the study were known to be biologically nontoxic at common pharmaceutical dosage levels.¹⁵ Single-crystal data were reported for one of the cocrystals, a 2:1 itraconazole:succinic acid cocrystal (Figure 1), where it was observed that the diacid spanned two itraconazole molecules via OH...N hydrogen bonds. All of the cocrystals demonstrated an enhanced dissolution profile as compared with itraconazole free base, and in some cases the dissolution profiles of the cocrystals approached that of amorphous itraconazole, which itself had been developed for the specific aim of enhancing the dissolution rate of the API.

The same study also emphasized the value of performing high-throughput cocrystal screening in addition to implementing rational design methodology. In the itraconazole:succinic acid cocrystal, the diacid formed a hydrogen bond with the five-membered triazole ring rather than with the most basic site on the drug molecule, the nitrogen of the six-membered piperazine (pK_a = 3.7). This example appeared to violate the best-donor–best-acceptor hydrogen-bond rule in preference for what may have been a geometric consideration: attempts to cocrystallize itraconazole with dicarboxylic acid chain lengths other than C₄, including malonic (C₃), glutaric (C₅), and adipic (C₆) acids, were reportedly unsuccessful. Until it becomes possible to confidently predict which counter-molecules will form cocrystals with a given API, high-throughput screening will continue to be of tremendous value to this research field.

A second demonstration of dissolution rate enhancement via cocrystallization involved three pharmaceutical cocrystals of the API fluoxetine, the active ingredient in the antidepressant drug Prozac.²⁴ This case was of particular interest from the standpoint of cocrystal design: the researchers formed cocrystals by combining a carboxylic acid with the hydrochloric acid (HCl) salt of fluoxetine, generating three novel cocrystals of salts. For example,

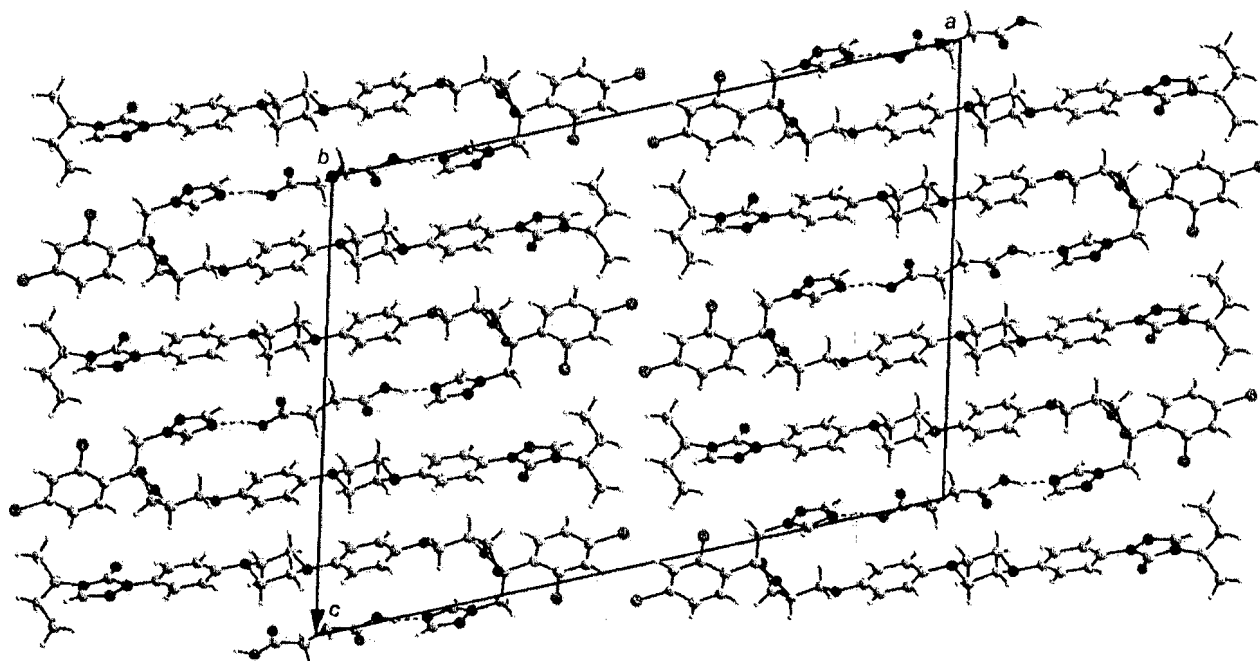


Figure 1. Crystal packing diagram and corresponding unit cell of the 2:1 itraconazole:succinic acid cocrystal.²³ Carbon atoms are large and gray, hydrogen atoms are small and white, nitrogen atoms are blue, oxygen atoms are red, and chlorine atoms are green.

fluoxetine:HCl salt was cocrystallized with succinic acid to form a succinic acid cocrystal of the fluoxetine:HCl salt, with a stoichiometry of 2:2:1 fluoxetine:chloride:succinic acid (see Structure 1). Significant differences in the measured dissolution rates of each of the three cocrystals were observed, such that individual cocrystals were found to exhibit rates above, below, and comparable with that of the crystalline HCl salt. In general, multiple-component cocrystals (and cocrystals of salts) with more than two components are evidence of the wide supramolecular diversity that may be achieved via cocrystal design.

The issue of physical stability enhancement via cocrystallization was

addressed using caffeine as a model API. Caffeine is known to exhibit solid-state physical instability as a function of relative humidity (RH); its stable anhydrous polymorph undergoes conversion to a crystalline hydrate upon exposure to high RH, and the hydrate loses water below a critical RH and reverts to the anhydrate. This form of physical instability limits the processing and storage conditions of an API in development. Caffeine also has a limited salt-forming capacity attributable to its weak basicity (its conjugate acid has a reported pK_a of 3.6), meaning that it is capable of forming salts only with strong acids. Only one pharmaceutically acceptable salt of caffeine had been reported in the CSD, a caffeine HCl salt that existed as a dihydrate.

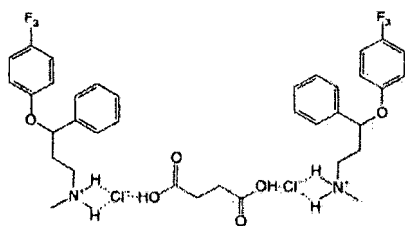
A cocrystallization study was initiated to obtain a series of cocrystals of caffeine that could be measured with regard to RH stability.²⁵ A strategy was devised whereby caffeine cocrystallization was attempted with several pharmaceutically acceptable dicarboxylic acids of various chain lengths. The strategy relied upon a caffeine-acid hydrogen-bond interaction that satisfied the hydrogen-bond rules, forming a motif that exhibited a good degree of robustness in the CSD.

Six caffeine:dicarboxylic acid cocrystals were reported, and the results of storing

cocrystal materials at several RH conditions were described. One cocrystal (the 2:1 caffeine:oxalic acid cocrystal, Figure 2), was physically stable at all RH conditions and all time points across the study. This cocrystal material was also fully stable upon slurrying in water. The stability of the caffeine:oxalic acid cocrystal is particularly remarkable given that both caffeine and oxalic acid, as pure materials, are known to convert to crystalline hydrates. The reason for this stability is currently being studied.

Supramolecular Synthesis via Solid-State Grinding

Solid-state grinding is the act of mixing, pressing, and crushing materials manually



Structure 1. Hydrogen-bond arrangement in the crystal structure of succinic acid cocrystal of fluoxetine:HCl salt; taken from crystal structure data.²⁴

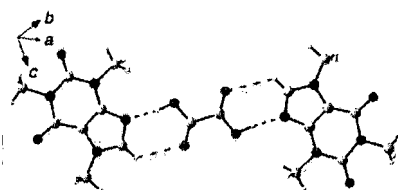


Figure 2. Hydrogen bonding in a 2:1 caffeine:oxalic acid cocrystal. Carbon atoms are large and gray, hydrogen atoms are small and white, nitrogen atoms are blue, and oxygen atoms are red.

with a mortar and pestle or mechanically in a mill. A common means of particle size reduction, solid-state grinding may also be performed on a mixture of materials to induce covalent or supramolecular reactivity. In the context of pharmaceutical cocrystals, solid-state grinding has emerged recently as a viable synthetic alternative to solution-based crystallization methods. In certain cases, pharmaceutical cocrystal synthesis by solid-state grinding offers enhanced selectivity as compared with that of solution crystallization. Moreover, the simplicity of the technique in revealing the cocrystallization potential between two molecular species suggests application in cocrystal screening efforts.

Caira and co-workers provided an early demonstration of the application of solid-state grinding to pharmaceutical cocrystal synthesis in a study of six cocrystals of the sulfa drug sulfadimidine with various carboxylic acids, including anthranilic acid (AA) and salicylic acid (SA).²⁶ Additionally, a remarkable preference was demonstrated for one particular cocrystal, the sulfadimidine:AA cocrystal. In a grinding competition experiment, a sulfadimidine:SA cocrystal, for which the crystal structure had been previously determined, was ground in the presence of AA. The result was a displacement of SA by AA as the cocrystal partner of sulfadimidine. Because of the common hydrogen-bonding pattern in both cocrystals, the authors based their explanation for the preference on the relative strengths of hydrogen bonding in the ingoing homomeric acid crystals. In extending these results to pharmaceutical processing considerations, it can be imagined that a grinding competition experiment such as the one just described might be used to assess the stability of a given pharmaceutical cocrystal material in the presence of excipients (i.e., substances other than the pharmacologically active drug in the final drug product) that may be encountered in the course of a formulation process.

With regard to the caffeine cocrystals described in the previous section, whereas single crystals were obtained by solution growth, it was reported that most cocrystals could also be prepared by grinding together the reactants in a ball mill. This finding was not unexpected: solid-state grinding has been repeatedly relied upon as a viable synthetic method for organic cocrystals. Solid-state grinding was often used by Etter and co-workers as a means of preparing cocrystal materials for the investigation of hydrogen-bond preferences. Furthermore, in a number of instances they reported that certain cocrystal modifications could be formed only by the

grinding method. These and other examples of this phenomenon were recently summarized.²⁷

The ability of solid-state grinding to reveal alternative cocrystal modifications would be particularly useful in pharmaceuticals, where unforeseen polymorphic transformations can bring disastrous consequences, including the withdrawal of a pharmaceutical product from the market.²⁸ For example, in a model system of cocrystals with caffeine and several monocarboxylic acids, solid-state grinding generated crystal forms which were initially inaccessible from solution. In experiments involving caffeine and trifluoroacetic acid, cocrystal material synthesis was initially found to be possible only via grinding. Two 1:1 polymorphs were identified and could be prepared separately depending upon the quantity of starting material in the grinding jars. The structure of each was solved from powder x-ray diffraction (PXRD) data. Subsequently, by using seeds obtained by grinding, cocrystal material of each polymorph was prepared by solution growth methods. This seeding method was used to obtain a single crystal for one of the structures, which confirmed the initial PXRD structure solution of that polymorph.²⁹

Enhanced Supramolecular Selectivity via Solvent-Drop Grinding

A modification to the solid-state grinding experiment has enabled enhanced supramolecular selectivity in certain cocrystal systems. Termed "solvent-drop" grinding, this method allows for stoichiometric and polymorphic selectivity in two model cocrystal systems.

Solvent-drop grinding involves the grinding of two materials together, as with solid-state grinding, but with the addition of a minor quantity of solvent (typically a few tenths of one equivalent of solvent per mole of starting material). The added solvent acts in what may be described as a catalytic role, in that the quantities employed are small and the solvent is not a component of the final cocrystal product.

The usefulness of solvent-drop grinding was first demonstrated in the context of cocrystallization rate enhancement in a system involving several cocrystals of nitrogenous bases with a cyclohexanetricarboxylic acid derivative, all of which were initially prepared by solution growth. It was found that some cocrystals could be readily prepared by solid-state grinding, whereas others exhibited only minor cocrystal content after grinding together starting materials for a significant time. For those that did not proceed to completion

upon solid-state grinding, it was found that solvent-drop grinding could be used to prepare an essentially phase-pure cocrystal material after significantly reduced periods of time.³⁰

Solvent-drop grinding was then found to enable selective polymorphic synthesis between two 1:1 caffeine:glutaric acid cocrystals (Forms I and II).³¹ The two polymorphs, which shared an identical sheet-like hydrogen-bonding arrangement and differed primarily in terms of the stacking of sheets, were first found to precipitate concomitantly from solution. In an effort to prepare each polymorphic modification separately, grinding was explored as a method of cocrystal synthesis. It was found that solid-state grinding of caffeine and glutaric acid produced predominantly Form I and that solvent-drop grinding with polar solvents (cyclohexane, hexane, and heptane) also produced Form I in the absence of Form II. Alternatively, phase-pure Form II could be prepared by the grinding of starting materials in the presence of more polar solvents (acetonitrile, chloroform, and water). A possible factor that may have had a role in this observed selectivity was the observation of a potential nonpolar cleavage plane in the Form I polymorph. Examples have also been reported of stoichiometric selectivity via solvent-drop grinding.²⁵

In addition to the ability of solvent-drop grinding to provide for polymorphic and stoichiometric selectivity in cocrystallization, the technique has also been demonstrated as a way of interconverting crystal forms of polymorphic organic materials, such as succinic acid and anthranilic acid.³² In the case of succinic acid, grinding of the stable polymorph in the presence of nonpolar solvents was found to result in significant quantities of the metastable polymorph, hitherto known to crystallize only at high temperatures. AA, a trimorphic system, underwent interconversions between the three different polymorphs depending upon the solvent that was added in minor quantities to the grinding experiment. A schematic depicting the interconversions among AA polymorphs is provided as Figure 3.

Solvent-drop grinding has also found application with regard to crystalline salt synthesis with pharmaceuticals.³³ Salt screening is an important aspect of physical property optimization, as well as intellectual property protection, for many API candidates. Much effort, increasingly using high-throughput robotics, is expended in revealing all potential salts (and polymorphs of salts) to ensure that the salt selection is made from the widest knowledge of potential candidates.⁵

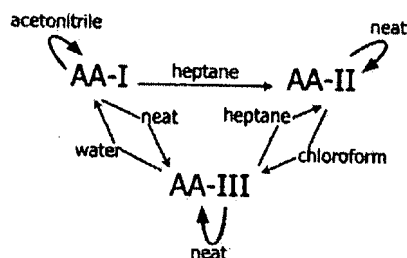


Figure 3. Interconversions among anthranilic acid (AA) polymorphs via solvent-drop grinding.

Conclusions and Outlook

Physical property improvement via cocrystallization will be of increasing importance in the area of pharmaceutical solid form selection in the near future. Several hurdles remain, however, before this technique can become fully implemented in the industry.

The wide number and variety of possible counter-molecules that may be considered in a cocrystal screen with an API is a significant benefit of this approach, but offers challenges in terms of screening efforts. There is a tremendous number of potential counter-molecule combinations to be explored in a given cocrystal screen, especially if ternary systems are to be considered (e.g., three-component cocrystals and cocrystals of salts). Current crystal form screening methodology, to a certain extent, applies a predetermined set of crystallization variables to any system under study. This unguided approach could generate an insurmountable number of different cocrystal synthetic possibilities.

In screening for cocrystals, it is therefore necessary to develop a guided screening methodology. This approach may include an initial stage during which potential counter-molecules for an API are automatically pre-screened and ranked using informatics tools such as the CSD, described in the earlier section Synthon Approach to Cocrystal Design. Counter-molecules that are capable of forming more robust hydrogen-bond motifs with the API may be ranked higher in terms of likelihood of cocrystal formation. In subsequent experimental efforts, higher-ranking cocrystal counter-molecules might justify increased experimental screening resources with the API of interest.

In consideration of the typically small quantity of material available during the development stages of an API, it also appears necessary to improve experimental screening methodology to facilitate cocrystal screening efforts. Solution-based

techniques, which are most common in current polymorph and salt screens, require a small amount of sample per experiment, but entail a large number of experiments to cover variables such as solvent system choice, concentration, and heating or cooling profiles, among others. Techniques such as solid-state grinding, as well as the developing approach of solvent-drop grinding, appear to offer a highly efficient alternative for offering evidence of whether two materials will cocrystallize. Other techniques, such as crystallization from the melt using techniques such as thermal microscopy and differential scanning calorimetry, may also offer the opportunity to screen for cocrystals with minimal expenditure of API material and reduced experimental effort.

This two-step cocrystal screening approach, involving an informatics-based ranking of counter-molecules followed by targeted, efficient cocrystal screening methodology, remains to be fully demonstrated. Nonetheless, as examples mount that indicate the ability of cocrystals to overcome the physical property shortcomings of API candidates, a guided approach to pharmaceutical cocrystal screening should become increasingly important for the successful implementation of cocrystallization in the pharmaceutical industry.

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121

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17

122

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The pharmaceutical formulator has need for specialized areas of science in order to acquire scientific information about the drug substance which is necessary to develop an optimum dosage form. We are in an era in the pharmaceutical industry where we can no longer rely on past experience to formulate. We must have a thorough understanding of the physical-chemical properties as well as the pharmacokinetic and biopharmaceutical behavior of each drug substance being de-

Preformulation work is usually initiated after a compound has shown sufficient activity to merit further testing in humans. When this decision is made, the various disciplines begin to generate data essential for properly evaluating the performance of the drug substance. A stability-indicating analytical assay method is very important. Since this often takes considerable time, it is sometimes necessary to rely on thin-layer chromatographic procedures to determine if a drug molecule is degrading. Accelerated testing procedures are utilized to promote breakdown of the compound being tested. Attempts are made to isolate and characterize the breakdown products in order to identify the mechanism of breakdown.

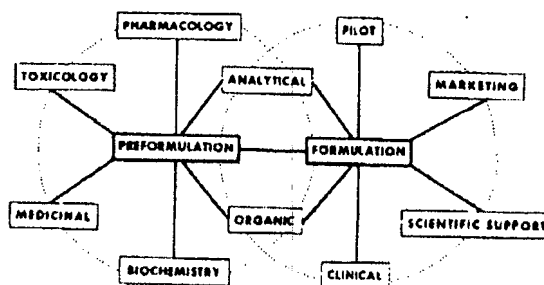


Fig 76-1. The wheels of product development.

This information provides a lead to the development pharmacist in his efforts to formulate the product.

During a preformulation study it is necessary to maintain some degree of flexibility. Problem areas must be identified early. For example, selection of a suitable salt form is critical. Toxicity studies are usually scheduled early. Consequently, if the salt form under consideration has some deficiencies they should be pointed out so that alternate salts may be prepared and evaluated prior to beginning toxicity studies.

When preformulation studies are initiated, the chemical is usually in short supply. Twenty-five grams of chemical is an ample supply, but many preliminary evaluations have been done with less. The initial supply usually originates as excess from batches prepared by the medicinal chemists. They usually have preliminary data such as melting point, spectral data, and structure of the compound. The direction taken for the evaluation is determined by the structure and the intended dosage forms to be developed. For example, one would not waste time determining the stability of a solution of a compound if there was no interest in a liquid dosage form. Many areas must be critically evaluated for each compound, and it is essential that problem areas be identified early, otherwise delays could occur if a problem surfaced during the development phase for the compound. Some consequences of poor preformulation work are: (1) possible use of unsatisfactory salt form; (2) poor stability of the active ingredient; (3) testing compound of marginal activity; (4) increased development costs; (5) increased development time. When preformulation studies are completed, the data are compiled and transferred to the development pharmacist, who, in turn, utilizes this information to plan his development work on the finished dosage forms.

Physical Properties

Description

Since chemical is in short supply at the outset of most preliminary evaluations, it is extremely important to note the general appearance, color, and odor of the compound. These characteristics provide a basis for comparison with future lots. During the preparation of scale-up lots the chemist usually refines or alters the original chemical synthetic route. This sometimes results in a change in some of the physical properties. When this takes place, comparisons can be made with earlier lots and decisions made regarding solvents for recrystallization.

Taste usually warrants some consideration, especially if the drug is intended for oral use in pediatric dosage forms. In such cases consideration should be given to the preparation of alternate salt forms or possible evaluation of excipients that mask the undesirable taste.

Microscopic Examination

Each lot of chemical, regardless of size, is examined microscopically and a photomicrograph taken. The microscopic examination gives a gross indication of particle size and characteristic crystal properties. These photomicrographs are useful in determining the consistency of particle size and crystal habit from batch to batch, especially during the early periods of chemical synthesis. If a synthesis step is changed, they also give an indication of any effect the change may have on crystal habit. One must keep in mind that the photomicrograph only gives a qualitative indication of particle size distribution; it is always necessary to do a particle size analysis for a more accurate picture of the distribution of particles in any particular batch of chemical.

Particle Size

The uses of pharmaceutical products in a finely divided form are diverse. From knowledge of their particle size, such drugs as griseofulvin, nitrofurantoin, spironolactone, procaine penicillin, and phenobarbital have been formulated so as to optimize activity. Other drugs, formulated in suspension or emulsion systems, in inhalation aerosols, and in oral dosage forms, may contain finely divided material as an essential component. One of the basic physical properties common to all these finely divided substances is the particle size distribution, i.e., the frequency of occurrence of particles of every size. What is of practical interest is usually not the characteristics of single particles but rather the mean characteristics of a large number of particles. It must be emphasized, however, that knowledge of size characteristics is of no value unless adequate correlation has been established with functional properties of specific interest in the drug formulation. Many investigations demonstrating the significance of particle size are reported in the literature. It has been shown that dissolution rate, absorption rate, content uniformity, color, taste, texture, and stability are dependent to varying degrees on particle size and distribution. In preformulation work it is important that the significance of particle size in relation to formulation be established early. Preliminary physical observations can sometimes detect subtle differences in color. If this can be attributed to differences in particle size distribution it is important to define this distribution and recommend that more attention be given to particle size in preparing future batches of chemical. This effect is also evident when preparing suspensions of poorly soluble materials. One may observe batch-to-batch differences in the color of a suspension which can be related to differences in particle size. Sometimes, when small particles tend to agglomerate, a subtle change in color or texture may be evident.

Sedimentation and flocculation rates in suspensions are in part governed by particle size. In concentrated deflocculated suspensions the larger particles exhibit hindered settling and the smaller particles settle more rapidly. In flocculated suspensions, the particles are linked together into flocs which settle according to the size of the floc and porosity of the aggregated mass. Flocculated suspensions are preferred since they have less tendency to cake and are more rapidly dispersible. Thus it is apparent that the ultimate height, H_u , of sediment as a suspension settles depends on particle size. The ratio H_u/H_0 or the degree of suspendibility as affected by particle size is valuable information for the formulator in order to prepare a satisfactory dosage form.

The rate of dissolution of small particles is usually faster than that of larger ones because rate of dissolution is dependent on the specific surface area in contact with the liquid medium. This is usually described by the modified Noyes-Whitney equation for dissolution rate dA/dt .

$$\frac{dA}{dt} = KS(C_s - C)$$

where A is the amount of drug in solution, K is the intrinsic dissolution rate constant, S is the surface area, C_s the concentration of a saturated solution of the drug, and C is the drug concentration at time t . The surface area of an object, regardless of shape, varies inversely with its diameter and confirms the above effect of particle size on dissolution rate. Solubility has also been observed to be dependent on particle size. Dittert et al¹ reported data for an experimental drug, 4-acetamidophenyl 2,2,2-trichloroethyl carbonate, which demonstrated that the dissolution rate and in turn bioavailability were affected by particle size. Although the ultimate amount of drug in solution may not be significant with respect to the dose administered, the formulator should be aware of this potential. With poorly soluble drugs it is extremely im-

124

portant that these factors be taken into account during the design of the dosage form.

Flow properties of drugs can be influenced by particle size, and particle size reduction to extremely small sizes (less than 10 μm) may be inadvisable for some drug substances. Entrapped air adsorbed on the surface of the particles and/or surface electrical charges sometimes impart undesirable properties to the drug. For example, adsorbed air at the drug-particle surface may prevent wetting of the drug by surrounding fluid, and electrically induced agglomeration of fine particles may decrease exposure of the drug surface to surrounding dissolution medium. Such effects act as dissolution rate-limiting steps since they minimize maximum drug surface-liquid contact.

Crystal growth is also a function of particle size. Finer particles tend to dissolve and subsequently recrystallize and adhere to larger particles. This phenomenon is referred to as *Ostwald ripening*. Protective colloid systems can be utilized to suppress this nucleation. Preformulators can generate information concerning the effectiveness of different colloids that is extremely important to the formulator when he is given the task of preparing a suspension dosage form.

Particle size reduction may be deleterious for some drug substances. Increasing surface area by milling or other methods may lead to rapid degradation of a compound. Drug substances may also undergo polymorphic transformation during the milling process. The preformulator must always be cognizant of these potential problems and whenever the decision is made to reduce particle size, the conditions must be controlled and the stability profile evaluated. If a problem does arise, it is the responsibility of the preformulator to note it and attempt to resolve it prior to turning the drug substance over to the formulating pharmacist.

Gastrointestinal absorption of a poorly soluble drug may be affected by the particle size distribution. If the dissolution rate of the drug is less than the diffusion rate to the site of absorption and the absorption rate itself, then the particle size of the drug is of great importance. Smaller particles should increase dissolution rate and thus bring about more rapid gastrointestinal absorption. One of the first observations of this phenomenon was made with sulfadiazine. Blood-level determinations showed that the drug in suspension containing particles 1 to 3 μm in size was absorbed more rapidly and more efficiently than from a suspension containing particles 7 times larger. Maximum blood levels were about 40% higher and occurred 2 hours earlier. Increased bioavailability with particle size reduction has also been observed with griseofulvin. The extent of absorption of an oral dose increased 2.5 times when the surface area was increased approximately six-fold. Micronized griseofulvin permits a 50% decrease in dosage to obtain a satisfactory clinical response.

On the other hand it was found that with nitrofurantoin there was an optimal average particle size that minimized side effects without affecting therapeutic response. In fact a commercial product containing large particles is available. For chloramphenicol, particle size has virtually no effect on total absorption but it significantly affects the rate of appearance of peak blood-levels of the drug. After administration of 50- μm particles, as well as 200- μm particles, peak levels occurred in 1 hour; with 400- μm particles peak levels occurred in 2 hours; with 800- μm particles peak levels occurred in 3 hours. All four preparations had the same physiological availability, which implies that the absorption of chloramphenicol occurs uniformly over a major portion of the intestinal tract.

Reduction of particle size may also create adverse responses. For example, fine particles of the prodrug trichloroethyl carbonate were more toxic in mice than regular and coarse particles.² Increasing the surface area for water-soluble drugs and possibly for weakly basic drugs appears to be of little

value. Absorption of weak bases is usually rate-limited by stomach emptying time rather than by dissolution. As previously mentioned, particle size is of importance only when the absorption process is rate-limited by the dissolution rate in gastrointestinal fluids.

We have been discussing the effect of particle size of the drug substance and its relationship to formulation. The particle size of the inert ingredients merits some attention. When one is concerned with particle size all ingredients utilized in preparing the dosage form should be evaluated and some recommendation regarding their control should be made prior to full-scale development of a dosage form. It is highly recommended that particle size and its distribution be determined, optimized, monitored, and controlled when applicable, particularly during early preformulation studies when the decision is made with regard to a suitable dosage form. The more common methods of determining particle size of powders used in the pharmaceutical industry include sieving, microscopy, sedimentation, and stream scanning.

Sieving or Screening—Sieving or screening is probably one of the oldest methods of sizing particles and is still commonly used to determine the size distribution of powders in the size range of 325 mesh (44 μm) and greater. These data usually serve as a rough guideline in evaluating raw materials with regard to the need for milling. The basic disadvantages of screen analysis are the large sample size required and the tendency for blinding of the screens due to static charge or mechanical clogging. The advantages include simplicity, low cost, and little skill requirement.

Microscopy—Microscopy is the most universally accepted and direct method of determining particle-size distribution of powders in the subsieve range. The method is tedious and time-consuming. The preparation of the slide for counting particles is important because the sample must represent the particle size distribution of the bulk sample. Extreme care must be taken in obtaining a truly representative sample from the bulk chemical. The cone and quartering technique usually gives a satisfactory sample. The sample should be properly suspended, dispersed, and thoroughly mixed in a liquid which has a different refractive index from the particles being counted. A representative sample is mounted on a slide having a calibrated grid. For counting, random fields are selected on the slide and the particles are sized and counted. Between 500 and 1000 particles should be counted to make statistical treatment of the data meaningful.

Sedimentation—Sedimentation techniques utilize the dependence of velocity of fall of particles on their size. Application is made of the Stokes equation (see page 314) which describes a relationship between the rate at which a particle settles in a fluid medium to the size of that particle. Although the equation is based on spherical-shaped particles, it is widely used to determine the weight-size distribution of irregularly-shaped particles. Data obtained by this procedure are usually reliable; however, the result may not agree with those obtained by other methods because of the limitations of the shape factor.

The *Andreasen Pipette Method* is most commonly used for sedimentation studies. Exact volumes are withdrawn at prescribed times and at a specified liquid depth. The liquid is evaporated and the residue of powder is weighed. The data are utilized in the Stokes equation and a weight-size distribution is calculated. Precautions must be observed with this method. Proper dispersion, consistent sampling, temperature control of the suspending medium, and concentration should be achieved in order to obtain consistent results.

Stream Scanning—Stream scanning is a technique in which a fluid suspension passes through a sensing zone where the individual particles are electronically sized, counted, and tabulated. The great advantage of this technique is that data can be generated in relatively short periods of time with rea-

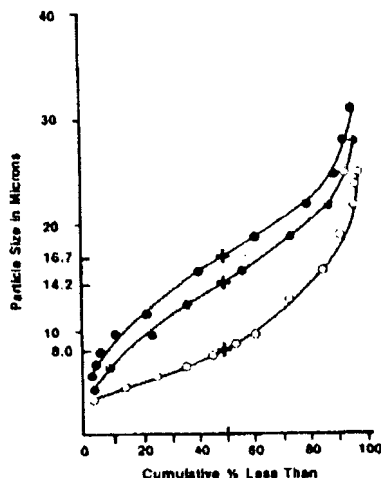


Fig 76-2. Particle size distribution of NBS glass beads expressed in terms of ○ = number of particles; ● = weight of particles; ⊙ = surface area of particles.

reasonable accuracy. Literally thousands of particles can be counted in seconds and used in determining the size distribution curve. The data are in a number of particles per class interval and can be expressed mathematically as the arithmetic mean diameter and graphed accordingly. Fig 76-2 illustrates a plot of typical data obtained for NBS Standard Reference Material No 1003.

The *Coulter Counter* and the *HIAC Counter* are widely used in the field of particle size analysis in the pharmaceutical industry. They can be used to follow crystal growth in suspensions very effectively. Fig 76-3 shows the change in particle size with time for an aqueous suspension of Form I of an experimental drug. It appears that the growth of the particles decreases significantly after six hours. The photomicrograph shown in Fig 76-4 depicts the significant increase in particle size after six hours. Further treatment of the data as shown in Fig 76-5 enables one to establish rates of growth for suspended particles. Simply reading off the intercepts at the 1%, 2% or 3% oversize and plotting this increase in diameter with time enables one to calculate the rate of growth of particles in a suspension. This is shown in Fig 76-6.

Partitioning Effect

If an excess of liquid or solid is added to a mixture of two immiscible liquids, it will distribute itself between the two phases so that each becomes saturated. If the substance is added to the immiscible solvents in an amount insufficient to saturate the solutions it will still distribute between the two layers in a definite concentration ratio. If C_1 and C_2 are the equilibrium concentrations of the substance in solvent 1 and solvent 2 the equilibrium expression becomes

$$\frac{C_1}{C_2} = k$$

The equilibrium constant k is known as the distribution ratio or partition coefficient. Biologically, in order for a pharmacological response to occur it is necessary that the drug molecule cross a biological membrane. The membrane, consisting of protein and lipid material, acts as a lipophilic barrier to most drugs. The resistance of this barrier to drug transfer is related to the lipophilic nature of the molecule involved. (See Chapter 37, on *Drug Absorption, Action, and Disposition*.)

Understanding the partitioning effect and the dissociation constant enables one to estimate the site of absorption of a

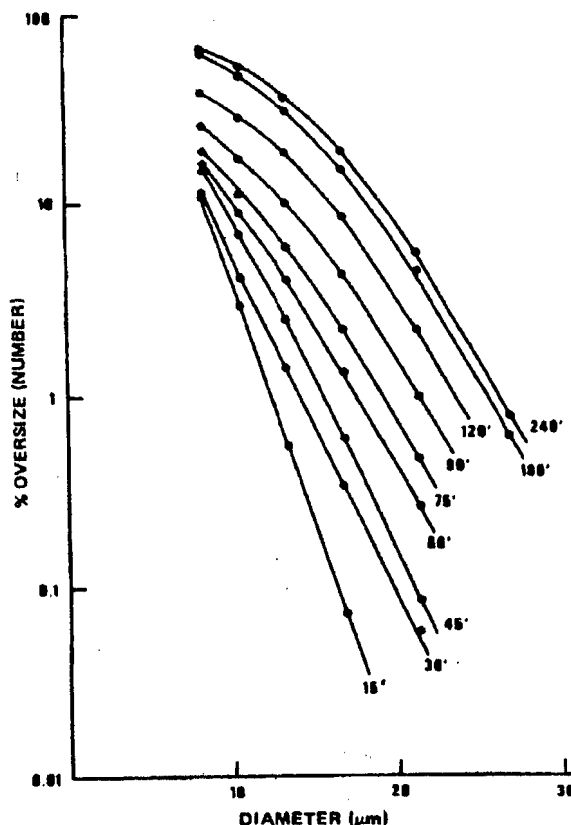


Fig 76-3. Change in particle size with time for an aqueous suspension of Form I of experimental drug.

new chemical entity. If one assumes the stomach to have a pH range of 1.0 to 3.0 and the small intestines to have a pH range from 5 to 8, in most cases acidic drugs (pK_a 3) will be more rapidly absorbed in the stomach while more basic drugs (pK_a 8) will be absorbed more rapidly in the intestinal tract. There are, however, exceptions. Some compounds have low partition coefficients and/or are highly ionized over the entire physiological pH range, but still show good bioavailability.

Polymorphism.

A polymorph is a solid crystalline phase of a given compound resulting from the possibility of at least two different arrangements of the molecules of the compound in the solid state. The molecule itself may be of different shape in the two polymorphs, but that is not necessary and indeed, certain changes in shape involve formation of different molecules and hence do not constitute polymorphism. Geometric isomers or tautomers, even though interconvertible and reversibly so, cannot be called polymorphs although they may behave in a confusingly similar manner.

A safe criterion for classification of a system as polymorphic is the following: Two polymorphs will be different in crystal structure but identical in the liquid or vapor states. Dynamic isomers will melt at different temperatures, as do polymorphs, but will give melts of different composition. In time each of these melts changes to an equilibrium mixture of the two isomers with temperature-dependent compositions. Some reported cases of polymorphism are undoubtedly dynamic isomerism, since the two behave quite similarly.

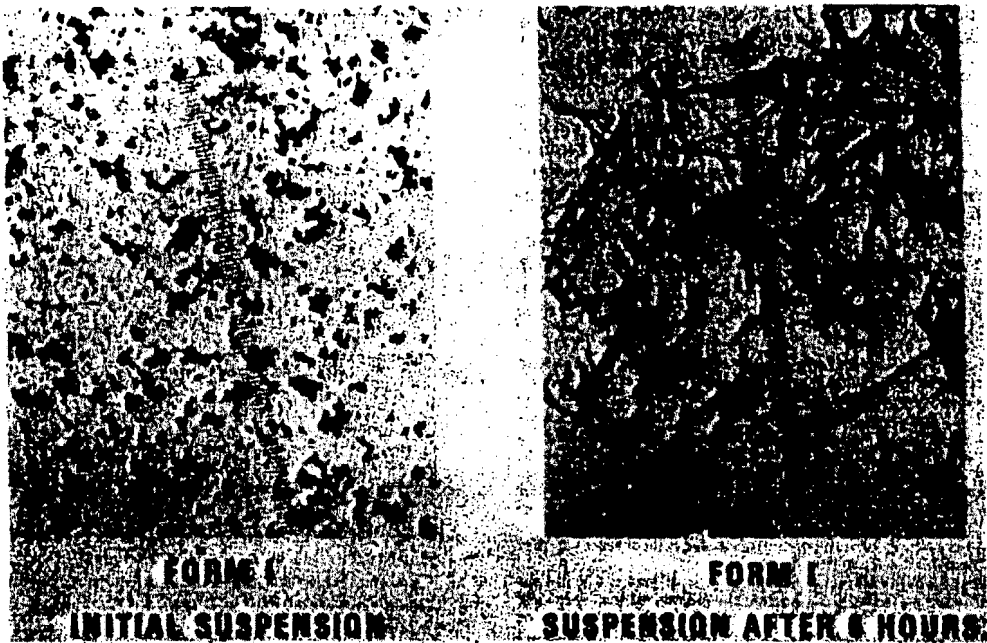


Fig 76-4. Photomicrographs showing change in crystal size for a suspension of Form I of experimental drug.

Polymorphism is the ability of any element or compound to crystallize as more than one distinct crystalline species, eg, carbon as a cubic diamond or hexagonal graphite. Different polymorphs of a given compound are, in general, as different

in structure and properties as the crystals of two different compounds. Solubility, melting point, density, hardness, crystal shape, optical and electrical properties, vapor pressure, stability, etc. all vary with the polymorphic form. In general, it should be possible to obtain different crystal forms of a drug substance and thus modify the performance properties for that compound. To do so requires a knowledge of the behavior of polymorphs. There are numerous reviews on the subject of polymorphism. In addition, numerous indications of the importance of polymorphism in pharmaceuticals are reported in the literature. The work of Kuhnert-Brandstatter *et al* with steroids, barbiturates, and antihistamines probably represents the most intensive study of polymorphism and drugs. In England, Mesley *et al*, using infrared spectroscopy described the polymorphism of steroids, barbiturates, and

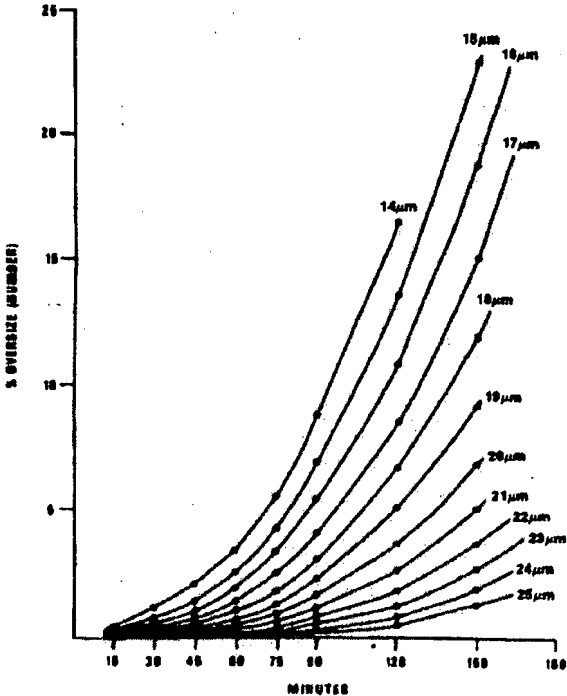


Fig 76-6. Change in cumulative count with time for an aqueous suspension of Form I of experimental drug.

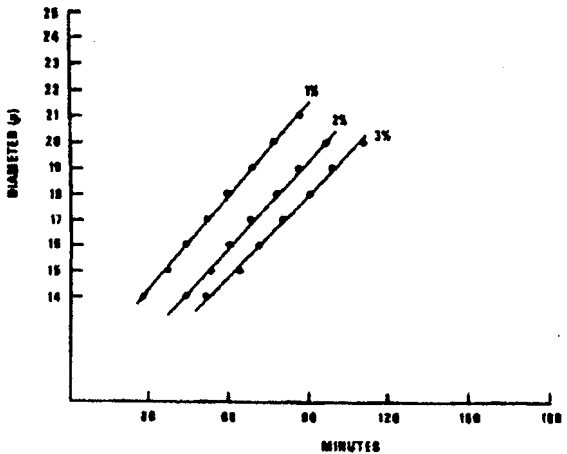


Fig 76-8. Rate of growth of Form I of experimental drug in aqueous suspension.

sulfonamides. Preformulation studies usually include rigorous studies to determine the presence of polymorphs in new drug substances being prepared for preliminary investigation in test animals. Some of the parameters routinely investigated are the number of polymorphs that exist, relative degree of stability of the various polymorphs, presence of a glassy state, stabilization of metastable forms, temperature stability ranges for each polymorph, solubilities, method of preparation of each form, effect of micronization or tableting, and interaction with formulation ingredients.

The initial task of the preformulator is to determine whether or not the drug substance being evaluated exists in more than one crystalline form. The following procedures are usually followed to cause crystallization of a metastable form:³

(a) Melt completely a small amount of the compound on a slide and observe the solidification between crossed polars. If, after spontaneous freezing, a transformation occurs spontaneously or can be induced by seeding or scratching, the compound probably exists in at least two polymorphic forms. It is essential to prevent nucleation of the stable form by inducing supercooling. Supercooling can be induced by using a small sample size, by holding the melt for approximately 30 seconds about 10° above the melting point; by carefully setting aside the compound without physical shock before observing it; and by rapid cooling of the compound.

(b) Heat a sample of the compound on a hot stage and observe whether a solid-solid transformation occurs during heating.

(c) Sublime a small amount of the compound and attempt to induce a transformation between the sublimate and the original sample by mixing the two in a drop of saturated solution of one of them. If the two are polymorphs, the more stable one will be more insoluble and will grow at the expense of the more soluble metastable form. This process will continue until the metastable form is completely transformed to the stable form. If the samples are not polymorphs, one may dissolve but the other will not grow. If the two are identical forms nothing will occur.

(d) Maintain an excess of the compound in a small amount of solvent held near the melting point of the compound. Isolate the suspended solid. Care should be taken to maintain the temperature during this step. Test the isolated material with an original sample using the procedure outlined in (c).

(e) Recrystallize the compound from solution by shock-cooling, and observe a portion of the precipitated material suspended in a drop of the mother liquor. The drop may then be seeded with the original compound to check for solution phase transformation. If the precipitate is a different polymorph, a solution phase transformation should take place.

Once it has been established that polymorphism occurs there are procedures which enable the preformulator to prepare the various forms in larger quantities for further evaluation and suitability for incorporation into dosage forms.

Once a compound has been shown to exist in more than one crystalline form, a number of techniques are available to identify the different polymorphic phases present. Each of these techniques could be successful in identifying the phase, but a combination of methods provides a means for isolation and identification of each crystalline modification. In order to confirm the presence of more than one crystalline form of a compound it is advisable to identify the modifications present by more than one method. Using only one method for confirming the presence of polymorphs may sometimes be misleading.

Microscopy—Optical crystallography is used in the identification of polymorphs. Biles in his review of crystallography discusses this application. Crystals exist in isotropic and anisotropic forms. When isotropic crystals are present the velocity of light is the same in all directions while anisotropic crystals have two or three different light velocities or refractive indices. This method requires the services of a trained crystallographer.

Hot-Stage Methods—The polarizing microscope fitted with a hot or cold stage is very useful for investigating polymorphs. An experienced microscopist can quickly tell whether polymorphs exist; the degree of stability of the metastable forms; transition temperatures and melting points; rates of transition under various thermal and physical conditions and whether to pursue polymorphism as a route to an improved dosage form. Kodlers and McCrone discuss these methods in detail.

X-Ray Powder Diffraction—Crystalline materials in powder form give characteristic X-ray diffraction patterns made up of peaks in certain positions and varying intensities. Each powder pattern of the crystal

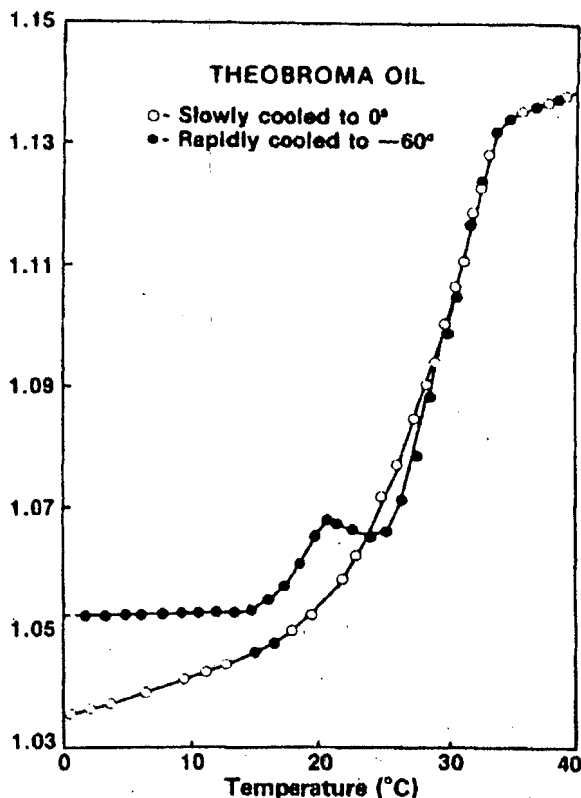


Fig 76-7. Dilatometric curves, theobroma oil, slowly and rapidly cooled.

lattice is characteristic for a given polymorph. This method has the advantage over other identification techniques in that the sample is examined as presented. Some care should be exercised in reducing and maintaining particle size control. A very small sample size is needed and the method is nondestructive. This method has been used by several investigators in identifying polymorphs in pharmaceuticals.

Infrared Spectroscopy—This procedure is useful in identification of polymorphs. Solid samples must be used since polymorphs of a compound have identical spectra in solution. The technique can be used for both qualitative and quantitative identification.

Thermal Methods—Differential scanning calorimetry and differential thermal analysis have been used extensively to identify polymorphs. In both methods, the heat loss or gain resulting from physical or chemical changes occurring in a sample is recorded as a function of temperature as the substance is heated at a uniform rate. Enthalpic changes, both endothermic and exothermic, are caused by phase transitions. For example, fusion, sublimation, solid-solid transition, and water loss generally produce endothermic effects while crystallization produces exothermic effects. Thermal analysis enables one to calculate the thermodynamic parameters for the systems being evaluated. Guillory obtained the heats of fusion for sulfathiazole and methylprednisolone. Ravin *et al.* utilized differential scanning calorimetry to follow the rate of conversion of sulfathiazole polymorphs.

Dilatometry—Dilatometry measures the change in volume caused by thermal or chemical effects. Ravin and Higuchi⁴ utilized dilatometry to follow the melting behavior of theobroma oil by measuring the specific volume of both rapidly and slowly cooled theobroma oil as a function of increasing temperature. The presence of the metastable form was shown by a contraction in the temperature range of 20° to 24°. This is illustrated in Fig 76-7. Dilatometry is extremely accurate; however, it is extremely tedious and time-consuming. It is not widely used.

Proton magnetic resonance, nuclear magnetic resonance and electron microscopy are sometimes used to study polymorphism.

Polymorphs can be classified into one of two types: (1) **Enantiotropic**—one polymorphic form can be reversibly

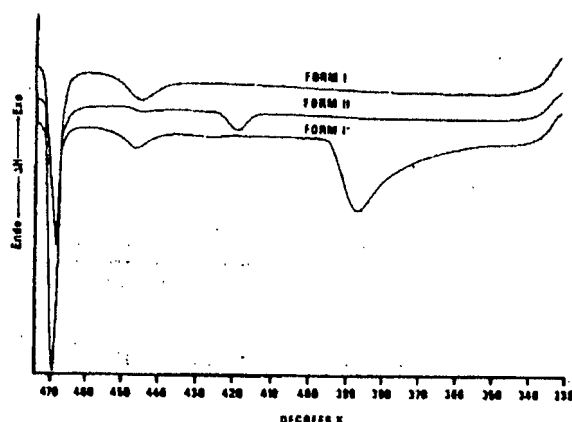


Fig 76-8. Thermograms for Forms I, I' and II of SK&F 30097.

changed into another one by varying the temperature or pressure, eg, sulfur. (2) Monotropic—one polymorphic form is unstable at all temperatures and pressures, eg, glyceryl stearates. At a specified temperature and pressure only one polymorphic form will be thermodynamically stable. However, other metastable forms may exist under the same conditions. These metastable forms will convert to the stable lattice structures with time. The first indication of the significance of a polymorphic transformation in a pharmaceutical system was noted with novobiocin. The amorphous form of novobiocin was found to be well absorbed; however, when formulated into a suspension a reversion of the metastable form to the more stable crystalline form occurred resulting in poor absorption.

After it has been determined that a drug substance does exist in more than one crystalline form, the conditions under which each can be produced should be established. In this manner, proper crystallizing conditions can be maintained from batch to batch to ensure a uniform and acceptable raw material. Recrystallization solvent, rate of crystallization, and other factors may cause one crystal form to dominate. During the preliminary investigation to establish these conditions it is necessary to monitor the forms prepared. For example, during the preliminary work with an indole derivative, differential scanning calorimetry, X-ray analysis, and infrared analysis were utilized to establish that polymorphs were present and that they could be prepared satisfactorily. Figs 76-8, 76-9, and 76-10 show the respective data for this conclusion. When polymorphs are shown to be present, experiments should be designed to determine whether or not the properties differ sufficiently to alter their pharmaceutical or biological behavior.

Dissolution tests can be utilized initially to show differences in apparent equilibrium solubilities provided a discriminating solvent system is utilized. Fig 76-11 illustrates dissolution data for two polymorphs of an indole derivative which had similar dissolution in the medium used; however, when a more discriminating dissolution medium was used it was possible to show differences in their dissolution characteristics. This is illustrated in Fig 76-12. From the data presented for the indole derivative it was concluded that there would be no appreciable difference in the availability of the two forms if they were to be administered orally in a solid dosage form. Subsequent testing in animals confirmed this. The Nernst equation relates the rate of concentration increase to the solubility of a dissolving solid and is commonly written as:

$$\frac{dc}{dt} = \frac{AD}{Vh} (C_s - C_t)$$

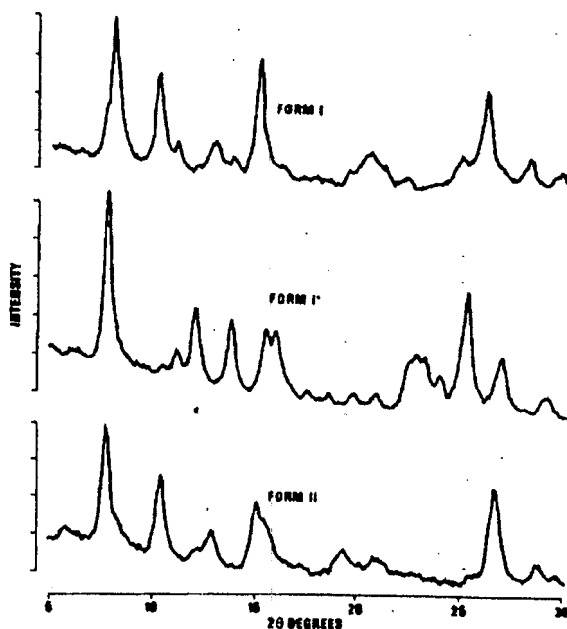


Fig 76-9. X-Ray diffractograms for Forms I, I' and II of SK&F 30097.

where A is the area of the dissolving interface of the solid, D is the diffusion coefficient of the solute in the solvent, V is the volume of the solvent, h is the thickness of the diffusion layer and C_s and C_t are concentration of the solute at saturation and at time t respectively. The equation reduces to:

$$\frac{dc}{dt} = \frac{AD}{Vh} C_s$$

for the experimental conditions where $C_s > C_t$. Since D is a property of the solute molecule and the solvent, it is independent of the solid state form. The experimental conditions can be selected such that A , V and h can be maintained constant in measuring the dissolution rates of different polymorphic forms. The dissolution rate is then directly pro-

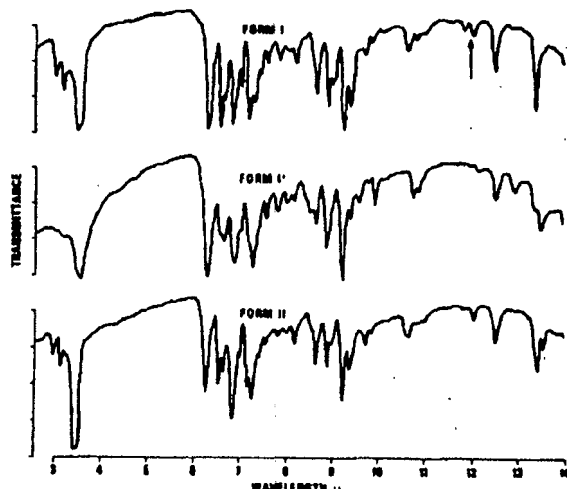


Fig 76-10. Infrared spectra of Forms I, I' and II of SK&F 30097.

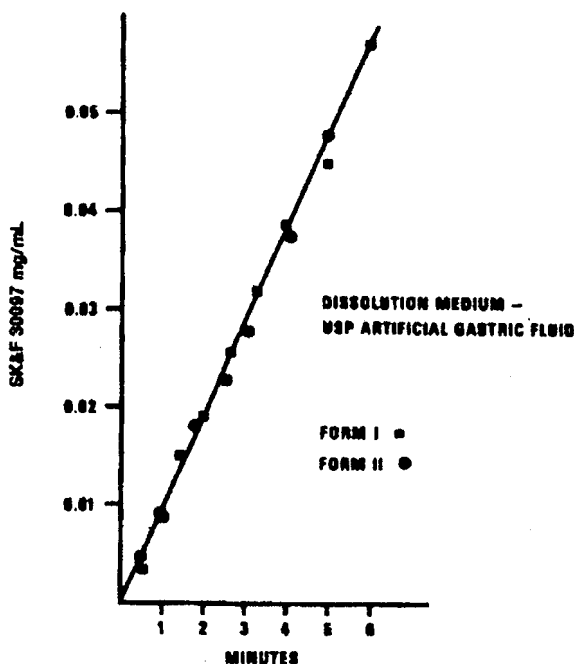


Fig 76-11. Dissolution behavior of Forms I and II of SK&F 30097 in artificial gastric fluid.

portional to C_s , the saturation solubility and the differences in the solubilities can be related to their free energies.

The solubility and dissolution behavior of several polymorphs of chloramphenicol palmitate have been determined. Figs 76-13 and 76-14 illustrate the data obtained at several temperatures. It is apparent from the dissolution behavior

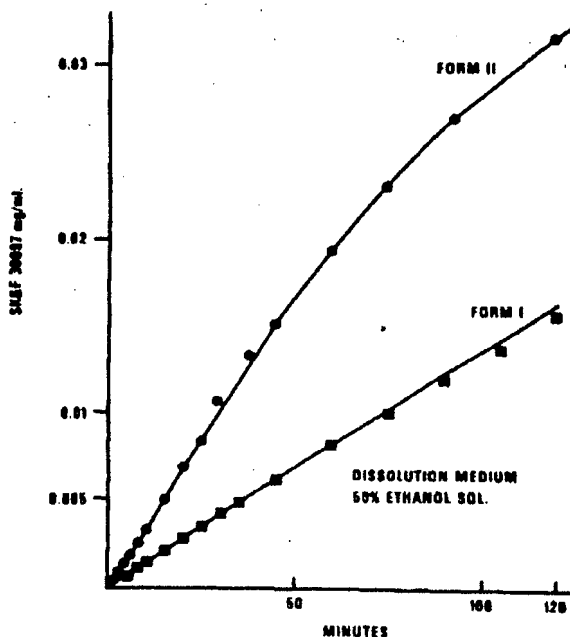


Fig 76-12. Dissolution behavior of Forms I and II of SK&F 30097 in 50% ethanol solution.

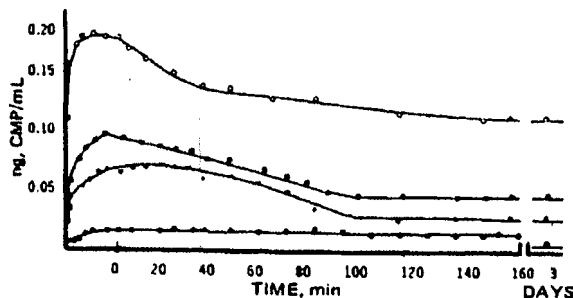


Fig 76-13. Dissolution curves for Polymorph C of chloramphenicol palmitate in 35% *t*-butyl alcohol and water at 30, 20, 15 and 6°. Key: 30°, O—O; 20°, ■—■; 15°, Δ—Δ; 6°, ●—●.

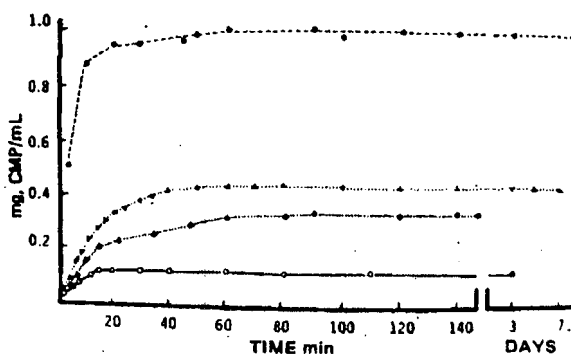


Fig 76-14. Dissolution curves for Polymorphs A and B of chloramphenicol palmitate in 35% *t*-butyl alcohol and water at 30 and 38°. Key: Polymorph A, 30°, O—O; Polymorph B, 30°, Δ—Δ; Polymorph A, 38°, ◆—◆; Polymorph B, 38°, ●—●.

that the maximum values obtained were good approximations of the solubility of the various forms. Therefore obtaining data at several temperatures would enable one to calculate the thermodynamic quantities involved in the transition from the metastable to the stable form. A plot of the solubility data as a function of temperature in a typical van't Hoff fashion is shown in Fig 76-15. The straight-line relationship enables one to calculate the heats of solution for the various forms and also by extrapolation to approximate the transition temperatures for the various forms. These values are shown in Table I.⁶

At constant temperature and pressure, the free energy differences between the polymorphs can be calculated by:

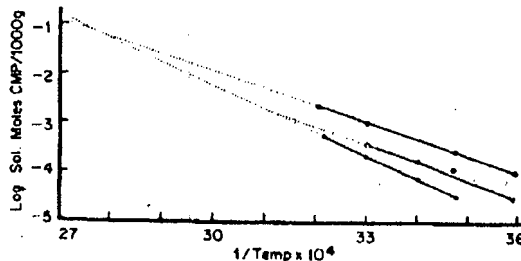


Fig 76-15. The van't Hoff type plot for Polymorphs A, B, and C of chloramphenicol palmitate. Key: Polymorphs A —; B ●—●; and C O—O.

Table I—Thermodynamic Values Calculated for Polymorphs A, B, and C of Chloramphenicol Palmitate

Poly-morph	Transi-tion Temp. (°C.) to Form A	Heat of Solution, kcal./mole	ΔG_f , cal./mole*	ΔS_{303} , e. s. u.	ΔS_{300} , e. s. u.*
A	—	21.8	—	—	—
B	88	15.4	-774	-18	-17
C	50	17.2	-465	-13	-14

* Calculated for the conversion to Polymorph A.

$$\Delta G_f = RT \ln \frac{C_s \text{ Polymorph A}}{C_s \text{ Polymorph B}}$$

This equation relates the solubility, C_s , of the polymorphic forms at a particular temperature, T , to the free energy differences, ΔG_f . Table I also contains the free energy differences calculated for the polymorphs. The enthalpy changes can also be determined for the various transitions by subtracting the heat of solution derived for the stable form from that of the metastable form. Also at any particular temperature, T , the entropy for the transition of polymorphs can be evaluated by the following relationship:

$$\Delta S_f = \frac{\Delta H_{B \rightarrow A} - \Delta G_f}{T}$$

The values computed for the transitions are also included in Table I. At the transition temperature the ΔG_f is equal to zero and the entropy can be calculated neglecting the free energy term in the above equation.

The thermodynamic relationships discussed are based on the assumption that Henry's Law is obeyed. Knowledge of these thermodynamic relationships enables the preformulator to select more rationally the more energetic polymorphic form of the drug being investigated for further pharmacological studies and also to have a preliminary assessment of its probable stability.

When a preformulation group inadequately investigates polymorphic drug forms, problems may develop during the development stage. Crystal growth in suspensions resulting in poor uniformity, poor appearance, poor bioavailability, transformation occurring during milling or granulation resulting in changes in the physical and biological characteristics, inadequate pharmacologic response, and poor chemical stability are typical problems that will become evident.

Solubility

In dealing with new drug substances, it is extremely important to know something about their solubility characteristics, especially in aqueous systems since they must possess some limited aqueous solubility to elicit a therapeutic response. When a drug substance has an aqueous solubility less than 1 mg/mL in the physiologic pH range (1-7), a potential bioavailability problem may exist and preformulation studies should be initiated to alleviate the problem. Equilibrium solubility of the drug substance should be determined in a solvent or solvent system which does not have any toxic effects on the test animal. This is done by placing an excess of drug in a vial with the solvent. The vial is agitated at constant temperature and the amount of drug determined periodically by analysis of the supernatant fluid. Equilibrium is not achieved until at least two successive samples have the same result. Experience with solubility determinations would indicate that equilibrium is usually attained by agitating overnight (approximately 24 hours). Solubility determinations

can be conducted at several temperatures since the resultant drug products will ultimately be subjected to a wide variation in temperature.

If the solubility of the drug substance is less than the required concentration necessary for the recommended dose, steps must be taken to improve its solubility. The approach taken will usually depend on the chemical nature of the drug substance and the type of drug product desired. If the drug substance is acidic or basic, its solubility can be influenced by pH. Through the application of the Law of Mass Action, the solubility of weakly acidic and basic drug substances can be predicted as a function of pH with a considerable degree of accuracy, utilizing the following equations for the weakly acidic and basic drugs.

$$\begin{array}{ll} \text{Weak Acid} & \text{Weak Base} \\ S_t = K_s \left(1 + \frac{K_a}{[H^+]} \right) & S_t = K_s \left(1 + \frac{[H^+]}{K_a} \right) \end{array}$$

There are many drug substances for which pH adjustment does not provide an appropriate means for effecting solution. Very weakly acidic or basic drugs may require a pH that may be outside the accepted tolerable physiological range or may cause stability problems with formulation ingredients. For example, an experimental indole had an equilibrium solubility at pH 1.2 of approximately 50 mg/mL. However, when the pH of this system was increased to approximately 2.0 the solubility decreased to less than 0.1 mg/mL. In cases like this one or with nonelectrolytes, it is necessary to utilize some other means of achieving better solubility.

Cosolvent systems have been utilized quite effectively to achieve solubility for poorly soluble drug substances under investigation. Propylene glycol, glycerin, sorbitol, and polyethylene glycols have enjoyed a wide range of success in this area. They have been very useful and generally acceptable for improving solubility. Additional solvents, such as glyceryl formal, glycofurool, ethyl carbonate, ethyl lactate, and dimethylacetamide have been cited in a review article by Spiegel and Nosworthy,⁸ however, it must be emphasized that with the possible exception of dimethylacetamide all of these solvents have not been utilized in oral products and their acceptability may be doubtful. The number of vehicles readily available to improve solubility is rather limited yet the frequency of their use is rather high. Solubilizing a new drug substance can improve its availability. For example, when a triazinoindole was administered in a 0.02% solution it showed an equivalent response in antiviral activity to a 2.5% suspension. Information generated early in the preformulation stage can result in a refinement of the dosage regimen and allow for a more accurate estimation of the effective dose.

Cosolvents usually serve a two-fold purpose in many pharmaceutical liquid products. Not only do they effect solution of the drug substance but also they improve the solubility of flavoring constituents added to the product. Ideally in determining the appropriate ratio of cosolvents to achieve the concentration one must achieve, it is recommended to effect solution at the concentration desired and then place the solution at 5° and allow it to equilibrate. If precipitation occurs under these conditions it may be necessary to alter the cosolvent ratio.

Use of surfactants of various types—nonionic, cationic, anionic—as solubilizing agents for medicinal substances is widespread (see Chapter 20, on *Colloidal Dispersions*, page 297, for illustrations of specific uses). The effect of Triton WR-1339 in solubilizing several steroids is shown in Fig 76-16.⁷ The effect of an anionic, a cationic, and a nonionic surfactant on the solubility of an antianginal compound being considered for clinical trials is shown in Fig 76-17. From such data investigators may be guided in selecting solubilizing

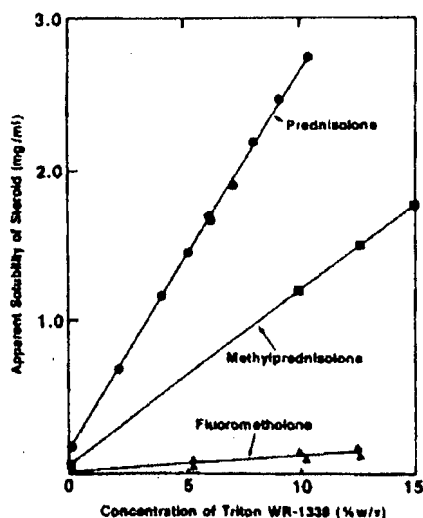


Fig 76-16. The effect of varying concentrations of Triton WR-1339 in water on the solubility of some anti-inflammatory steroids.

agents for use in preparations to be studied in humans, but it must be emphasized that the acceptability of a particular solubilizing agent is dependent also on other factors that determine its suitability for the intended use. For example, surfactants are known to interact with some preservatives and thereby decrease preservative action, for which reason the preformulator should always recommend some type of biological test to demonstrate that the activity of the drug substance being studied is not reduced when it is solubilized by a surfactant.

Complexation phenomena can sometimes be utilized to impart better solubility characteristics. However, the degree of association and the extent to which solubility can be increased is generally not adequate for use in pharmaceutical

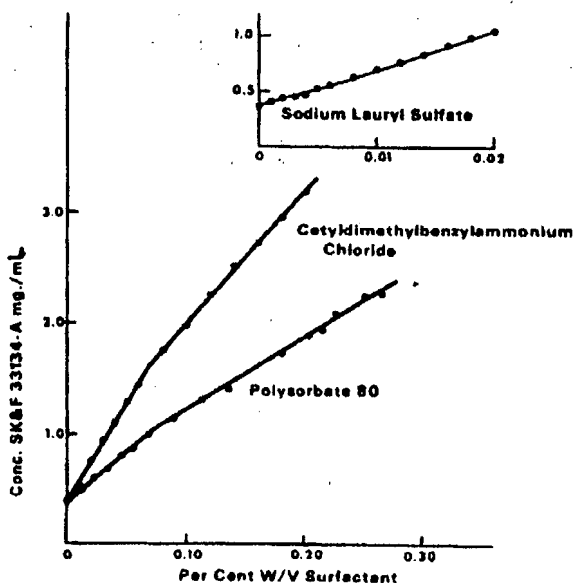


Fig 76-17. Effect of surfactant concentration on the solubility of SK&F 33134-A.

products. In addition, many complexing agents have physiological activity. The most noteworthy example of the utility of complexation to enhance solubility is the PVP-iodine complex. Hydrotropy can sometimes be utilized to enhance solubility. High concentrations of urea, salicylates, and xanthenes have been used successfully on several occasions. Again, the concept is available but the increase in solubility normally observed is not adequate for use in pharmaceutical products.

Salt Formation

Salt-forming agents are often chosen empirically by the pharmaceutical chemist primarily on the basis of the cost of raw materials, the ease of recrystallization, and the percentage yield. Unfortunately there is no reliable way of predicting the influence of a particular salt species on the behavior of the parent compound in dosage forms. Furthermore, even when many salts of the basic compound have been prepared there are no effective screening techniques which make the selection process of the salt an easier task for the pharmacist. The basic considerations which may have some influence on salt selection are physical and chemical stability, hygroscopicity, flowability, and solubility.

The number of salt forms available to the chemist is large. Table II lists the cations and anions present in FDA-approved

Table II—FDA-Approved Commercially Marketed Salts.

Anion	Percent ^a	Anion	Percent ^a
Acetate	1.26	Iodide	2.02
Benzenesulfonate	0.25	Isethionate ¹	0.88
Benzoate	0.51	Lactate	0.76
Bicarbonate	0.13	Lactobionate	0.13
Bitartrate	0.63	Malate	0.13
Bromide	4.68	Maleate	3.03
Calcium edetate	0.25	Mandelate	0.38
Camylate ^b	0.25	Mesylate	2.02
Carbonate	0.38	Methylbromide	0.76
Chloride	4.17	Methylnitrate	0.38
Citrate	3.03	Methylsulfate	0.88
Dihydrochloride	0.51	Mucate	0.13
Edetate	0.25	Napsylate	0.25
Edisylate ^c	0.38	Nitrate	0.64
Estolate ^d	0.13	Pamoate (Embonate)	1.01
Esylate ^e	0.13	Pantothenate	0.25
Fumarate	0.25	Phosphate/diphosphate	3.16
Glucoplate ^f	0.18	Polygalacturonate	0.13
Gluconate	0.51	Salicylate	0.88
Glutamate	0.25	Stearate	0.25
Glycylglycylsarcosinate ^g	0.13	Subacetate	0.38
Hexylresorcinate	0.13	Succinate	0.38
Hydrabamine ^h	0.25	Sulfate	7.46
Hydrobromide	1.90	Tannate	0.88
Hydrochloride	42.96	Tartrate	3.54
Hydroxynaphthoate	0.25	Teoclate ⁱ	0.13
		Triethiodide	0.13
Cation	Percent ^a	Cation	Percent ^a
Organic:		Metallic:	
Benzathine ^a	0.66	Aluminum	0.66
Chlorprocaine	0.33	Calcium	10.49
Choline	0.33	Lithium	1.64
Diethanolamine	0.98	Magnesium	1.31
Ethylenediamine	0.66	Potassium	10.82
Meglumine ¹	2.29	Sodium	61.97
Procaine	0.66	Zinc	2.95

^a Percent is based on total number of anionic or cationic salts in use through 1974. ^b Camphorsulfonate. ^c 1,2-Ethanedithiolate. ^d Lauryl sulfate. ^e Ethanesulfonate. ^f Glucoheptonate. ^g *p*-Glycylamidophenylarsionate. ^h *N,N'*-Di(dehydroabietyl)ethylenediamine. ⁱ 2-Hydroxyethanesulfonate. ^j 8-Chlorotheophyllinate. ^k *N,N'*-Dibenzylethylenediamine. ^l *N*-Methylglucamine.

133

commercially marketed salts of pharmaceutical agents.⁸ The monoprotic hydrochlorides have been the most frequent choice of the available anionic salt-forming radicals, while sodium has been the most predominant cation. During preformulation evaluation it is extremely important to establish that the particular salt form in question will have properties that will result in a minimum of problems during the development of the dosage forms. Since toxicity studies are usually initiated soon after a compound has been designated for further studies in man it is important that the salt form selected has been given a critical evaluation to determine whether or not its properties are suitable.

Since physical and chemical stability are vital to any pharmaceutical product it is imperative that the preformulator evaluate both parameters. A systematic determination of the thermal stability, solution stability (at several pH's) and light sensitivity of the drug substance provides essential input toward the selection of the most suitable derivative. Studies are usually initiated early to identify problems. Samples of the salts in question are usually placed under exaggerated conditions of heat and light in the presence and absence of moisture and subsequently analyzed to determine the amount of breakdown. In many instances stability-indicating analytical methods may not be available. In these cases it is necessary to resort to thin-layer chromatography to establish a qualitative assessment of stability. At the same time samples are placed under high-humidity conditions and weighed periodically to determine the degree of hygroscopicity of the compounds. Compounds that have a tendency to adsorb or absorb moisture may present flowability problems during encapsulation.

Solubility characteristics are also evaluated. When a particular salt form has very good solubility (greater than 10%) it is sometimes difficult to prepare a suitable granulation using an aqueous granulating fluid, especially for high doses. Granulations prepared by these methods will not dry satisfactorily or the granulation will not flow uniformly from the hopper, resulting in large weight variation during the compression stage. A critical evaluation of this type with different salt forms has been proven quite effective in enabling the preformulator to make the selection of the salt form of choice for further development.

Chemical Properties

The evaluation of the physical and chemical stability of a new drug substance is an important function of the preformulation group. The initial work should be designed to identify those factors that may result in an alteration of the drug substance under study. The physical pharmacist can initially anticipate the possible type of breakdown that a compound will be subjected to by examination of the chemical structure of the compound. For example, esters and amides are sensitive to hydrolytic degradation while acridanes and catecholamines are sensitive to oxidation degradation. With this preliminary knowledge he can more effectively design his studies to identify the problems early. At this point the primary concern is not the pathway or mechanism of degradation. A stability-indicating method of analysis is usually not available early in the preformulation phase. Techniques such as thin-layer chromatography, diffuse reflectance, and thermal analysis can be utilized to provide data to assess preliminary stability. Sometimes the preliminary evaluation is complicated by the presence of impurities. It is essential that the drug under study be pure before any stability tests are undertaken. The presence of impurities can lead to erroneous conclusions in the preformulation evaluation.

Drug Substance Stability—It is extremely important to determine the stability of the bulk chemical as early as possible. One would hardly expect to prepare stable dosage

forms with a chemical substance that was not stable in the pure state. Samples of the chemical are usually subjected to various conditions of light, heat, and moisture in the presence and absence of oxygen. The chemical is placed in sealed vials with and without moisture and stored at various elevated temperatures which may vary to some degree from laboratory to laboratory. Light sensitivity is measured by exposing the surface of the compound to light. Sunlamps are sometimes used to exaggerate light conditions. Hygroscopicity is evaluated by placing the chemical in open petri dishes at relative humidities from 30 to 100%. The samples are monitored regularly for physical changes, moisture pick-up and chemical degradation.

Most drug substances are either stable at all conditions, or stable under special conditions of handling, or unstable with special handling, or unstable. When drug substances are found to have some stability problems, then it may be important to define the pathway of degradation and initiate studies to stabilize the compound with appropriate additives.

At this point it may be advisable to consider some of the more prominent reactions accounting for instability of new drug substances. Obviously, some compounds will not undergo any appreciable decomposition if kept dry and away from air in a sealed container. We must always assume that the new drug substance is in some kind of formulation environment that may lead to instability problems.

Hydrolytic Degradation—Hydrolysis is probably the degradative process most frequently encountered in the formulation of new drugs. It is safe to assume that most new drugs will be exposed to water at some stage during processing or during storage; hence, hydrolysis may occur unless the conditions are optimum. Hydrolysis occurs with esters, amides, salts of weak acids and strong bases, and thioesters among others. A few drug compounds that undergo hydrolytic degradation are procaine, penicillin, aspirin, and chlorothiazide.

From a kinetic standpoint hydrolysis reactions are second-order reactions because the rate is proportional to the concentration of two reactants. However, in aqueous solutions since water is usually present in excess and at relatively constant concentration, the reactions are treated experimentally as monomolecular or first-order reactions. This simplification permits calculations of the extent of decomposition under precise experimental conditions by less complicated means. Extrapolation of the exaggerated rates to room temperature makes it possible to more expeditiously establish shelf-life stability of potential new drug products.

The rate of hydrolysis can be affected by temperature and by hydrogen or hydroxyl ion concentration when the hydrolytic process is dependent on pH. Fig 76-18 shows the

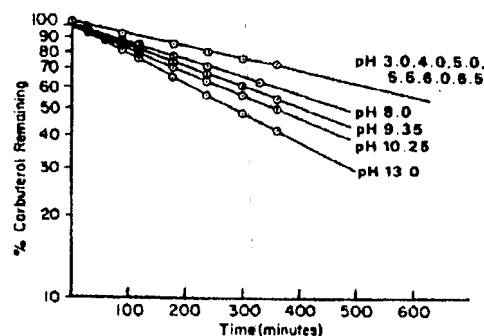


Fig 76-18. Effect of pH on carbutole degradation at 85° ($\mu = 0.5$).

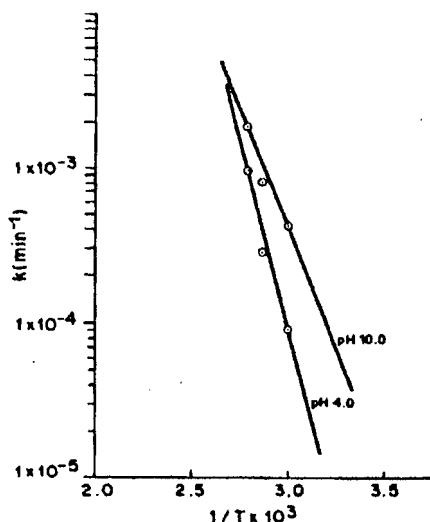


Fig 76-19. Typical Arrhenius-type plot depicting the temperature dependency of carbuterol hydrolysis at pH 4.0 and 10.0.

pseudo-first-order behavior as a function of pH for carbuterol in aqueous solution at constant ionic strength at 85°. The effect of temperature is illustrated in Fig 76-19 for carbuterol at pH 4.0 and 10.0 respectively.⁹ When we are concerned with solids, the amount of moisture present is minimal. When a physical pharmacist has a drug substance that undergoes hydrolytic degradation, he usually designs studies to establish the conditions of pH and buffer concentration where minimum decomposition occurs. There is sometimes a wide range of pH adjustment that a drug substance can tolerate. For example, idoxuridine was shown to have maximum stability over a pH range from 2 to 6. Fig 76-20 shows the pH-stability profile.¹⁰ Another drug substance, carbuterol, hydrolyzed by an intra-molecular process showed maximum stability over a wide pH range. Even though these compounds had a wide range of pH for optimum stability in aqueous solution they could not be formulated and provide products with satisfactory shelf lives without special cosolvent systems and/or

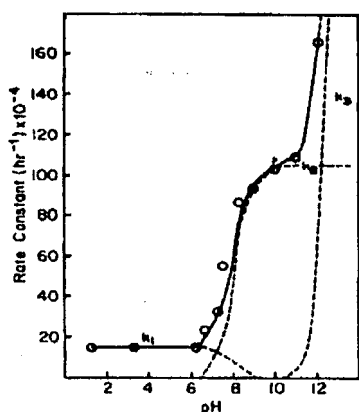


Fig 76-20. Plot showing pH-rate profile for hydrolysis of idoxuridine at 60°. Circles represent experimental results. Solid line corresponds to theoretical pH-rate profile. Broken line designates contribution of k_1 , k_2 , and k_3 at any pH value.

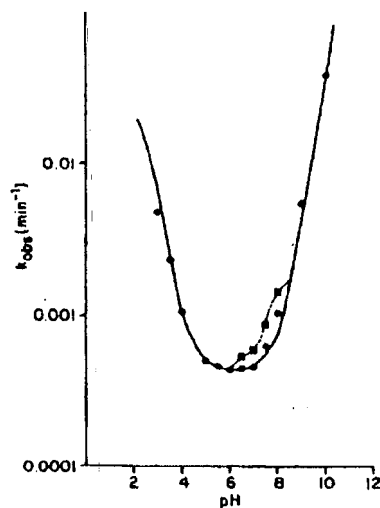


Fig 76-21. pH-Rate profile of cefazolin degradation in aqueous solution at 60° ($\mu = 0.5$). Solid line: theoretical profile; circles: experimental profile; squares: rates uncorrected for buffer effect.

special storage conditions. Cefazolin was shown to have a narrow pH range for maximum stability as indicated in Fig 76-21.¹¹ Buffering aqueous solutions to provide a pH for optimum stability can lead to stability problems. Stability is sometimes affected by buffer concentration, for example, carbuterol stability was shown to be affected by phosphate buffer concentration.

Another manner in which the physical pharmacist can overcome an instability due to hydrolysis is to recommend the preparation of an insoluble salt form or to prepare a solid dosage form. Insoluble chlorothiazide is stable in neutral aqueous suspensions but solutions of the sodium salt at relatively high pH's decompose rapidly. Frequently the replacement of water by some other solvent such as alcohol or the polyhydroxy solvents reduces the hydrolytic rate of degradation for some systems. Acetylsalicylic acid suspensions containing high concentrations of sorbitol improved stability. Ampicillin was also shown to be more stable when the concentration of alcohol was increased. Higuchi and his associates greatly reduced the hydrolytic rates of degradation for aromatic esters by forming molecular complexes.

It has also recently been shown that stability of some compounds may vary depending on whether or not they exist in the micellar or nonmicellar state. Recently Koetenbauder showed a difference in the chemical stability of penicillin that existed in the micellar state from that in the monomeric state.

Oxidation—Oxidative degradation is as important as hydrolysis in the preliminary stability evaluation of new drug substances. Studies should be initiated to establish the oxidative route, then steps should be taken to determine what additives can minimize the degradation. Oxidative degradation is common with many drug compounds. Ascorbic acid, epinephrine, vitamin A, chlorpromazine, isoproterenol, morphine, resorcinol, and unsaturated fats and oils are subject to oxidative degradation. The oxidation reaction depends on several factors, including temperature, oxygen concentration in the liquid, impurities present, and the concentration of the oxidizable component. The temperature effect in solutions is usually minimal; however, in the dry state it is more pronounced since other factors such as moisture dictate its stability behavior.

Initially it is important to establish that oxidation is taking place. Solutions of the drug substance in question are exposed to various exaggerated conditions of light and oxygen tension in amber and flint-glass containers. Samples are analyzed for degradation. When it has been established that the oxidative route is the principal pathway for degradation, appropriate additives are utilized to determine what effect they might have on the stability. pH sometimes is critical since a great number of oxidation-reduction processes depend on the concentration of hydrogen or hydroxyl ions. Light usually accelerates the degradation, thus the storage of products in dark containers does much to preserve stability. Photochemical changes many times involve the formation of other reactive compounds or free radicals which function to propagate the decomposition, once started. Auto-oxidation may occur in the absence of light when susceptible materials such as fats and oils are stored in the presence of air. The auto-oxidation of phenolic compounds is of special significance since compounds such as epinephrine and isoproterenol degrade in this manner. Heavy metal ions, eg, cupric and ferric, accelerate the oxidation of ascorbic acid and the phenothiazines. Frequently only trace quantities of these ions occurring as impurities may be sufficient to cause an increased rate of decomposition. This can be a consistent problem since many of the so-called inert ingredients may have heavy metal contaminants.

The oxygen concentration in solution is a factor in many cases and often depends upon the temperature of storage or the solvent employed. Oxygen is more soluble in water at lower temperatures so that oxygen-dependent reactions can sometimes proceed more rapidly at the lower temperatures. Ascorbic acid is more stable in 90% propylene glycol or in USP syrup than in water, presumably because of the lower oxygen concentration in these vehicles. Oxidative degradation is an extremely complex process since the overall rate is dependent upon several factors. Preparations sensitive to oxidation are sometimes stabilized by effectively removing the oxygen and by the addition of suitable additives. Nitrogen flushing has been utilized successfully for this purpose. A wide variety of reducing agents and compounds to sequester metals and inhibit chain reactions have been employed for stabilization, but relatively few are acceptable for parenteral products. Oftentimes it is necessary to combine ingredients and adjust pH to maximize stability. Detailed kinetic studies have been reported for the oxidative decomposition of prednisolone.

The physical pharmacist has a difficult task with oxidative degradation. He must design experiments initially that will encompass many variables. Preparing samples at several concentrations containing antioxidants plus sequestering agents at several pH levels and placing them in flint or amber containers with and without nitrogen is commonplace procedure. The subsequent evaluation of these limited data is critical. Light-sensitivity studies with several formulations of prochlorperazine resulted in the selection of a stable formula. In a study with idoxuridine it was shown that placing the aqueous solution in an amber container was sufficient to protect the product from oxidative degradation.

Drug Substance-Excipient Interaction—Drug substance-excipient studies are designed to determine a list of excipients that can be used routinely in the final dosage forms. Lactose, sucrose, calcium sulfate, dicalcium phosphate, starch, and magnesium stearate are some of the substances routinely tested in combinations. Some basic observations with the drug substance and/or its salt form can sometimes dictate what excipients can be used. For example, one would not consider using sucrose or lactose if the drug substance being considered is a primary amine. This system has the potential for interaction to form a colored compound readily detected by a color change.

Various means have been utilized for detecting potential

interactions and incompatibilities. Diffuse reflectance techniques have been used to detect interactions. This has been done by comparing the spectra obtained initially with those obtained after storage at exaggerated conditions. A shift in absorption has been interpreted as an interaction. Thin-layer chromatography has also been used. When excipients are present it is usually advisable to set a mixture of the excipients at the same conditions as the active mixtures. This will give a comparison of the thin layer of both systems. If any new degradation products are present the source can more easily be determined.

Mixtures containing at least two levels of drug concentration with excipients are sealed in vials containing 5% water in half of the samples. These vials are stored under exaggerated conditions of light and heat for various time periods. The resultant samples are observed physically and analyzed by an appropriate technique to get a qualitative determination. At this point in the stability evaluation, which is a preliminary screening process, it is not necessary to know exactly how much has degraded. It is an all-or-none type effect. The search is for the excipients that have no effect on the stability of the active ingredient.

When solution interactions are being investigated and no incompatibilities are evident, it is wise to recommend an *in vivo* experiment to evaluate availability. On occasion interaction may occur in solution that is not detectable with routine procedures. For example, clindamycin was found to interact with cyclamates and interfere with the absorption of the drug.

Other Changes—Optically active substances may lose their optical activity without a structural change. If the enantiomeric compounds possess different degrees of physiologic action, such changes may result in reduced therapeutic effects. Epinephrine has been shown to undergo racemization under various acidic and basic conditions. Although the potential for this to become evident during a preformulation evaluation is rare, one should always be aware of this possibility. Polymerization is also a remote possibility. Darkening of glucose solution is attributed to polymerization of the breakdown product, 5-hydroxymethylfurfural. Isomerization, which is the process involving the change of one structure into another having the same empirical formula but with different properties in one or more respects, can also occur. Again, the occurrence is rare. Deamination and decarboxylation can sometimes occur. This type of change would be easily detected since the resultant degradation products would have completely different properties.

Permeability

A preformulation evaluation should include studies to assess the passage of drug molecules across biological membranes. These membranes act as lipid barriers to most drugs and permit the absorption of lipid-soluble substances by passive diffusion. Lipid-insoluble substances can cross the barrier only with considerable difficulty. The pH-partition theory explains the interrelationship of the dissociation constant, lipid solubility, pH at the absorption site, and the absorption characteristics of drugs across membranes. The theory has evolved following a series of investigations in laboratory animals and man and is the basis of much of the current understanding of absorption of drugs.

Data obtained from basic physical-chemical studies described earlier may give the preformulation scientist an indication of possible absorption difficulties. Experimental techniques are available that can be utilized to give a more accurate assessment of absorption problems. An *in vitro* system that has been used extensively consists of an aqueous/organic solvent/aqueous system which has the advantage of being simple, allows for accurate pH control, membrane

thickness, and other variables. It can be described mathematically in precise terms. However, the interpretation and correlation of data are limited when applied to biological systems.

Another *in vitro* procedure, the everted sac technique, is a simple and reproducible method for determining the absorption characteristics of drugs. Isolated segments of rat small intestines are everted and filled with a solution of the drug being evaluated and the passage of drug through the membrane is determined. This technique has been used to measure the permeability of a number of drug substances.¹² It can also evaluate both passive and active transport of drugs. The fact that the preparation has been removed from the animal and its normal blood supply is a distinct disadvantage.

The *in situ* technique developed by Doluisio, et al¹³ for the study of membrane permeability appears to overcome the disadvantages of the everted sac technique. Since the intestine is not removed from its blood supply the results would be expected to be similar to those obtained in intact animals. A disadvantage of the technique is that the procedure does not account for the loss of fluid from the solution by absorption in the intestine. Nonabsorbable markers, ie, phenol red, can be added to the drug solution to solve this problem.

The techniques described can give the preformulation scientist an indication of possible absorption problems or suggest that little or no difficulty will be observed in the passage of a particular drug product through the biological membranes. This information, along with eventual studies in man, serves to establish possible *in vitro/in vivo* correlation for dissolution and bioavailability. These data are important in establishing quality control specifications for the products which will ensure consistent biological performance from subsequent lots.

Formulation Ingredients

Although preliminary screening of commonly used excipients with new drug substances has become routine in preformulation studies, there are occasions when problems arise because of the interaction with additives such as preservatives, stabilizers, dyes, and possibly flavors. A discussion of some problems that have arisen is in order to make formulators aware that they should be concerned about the potential for interaction whenever another ingredient is added to a formulation.

Preservatives—Each time a liquid or semisolid pharmaceutical dosage form is prepared it is necessary to include a preservative in the formulation. Such preservatives as sodium benzoate, sorbic acid and the methyl and propyl esters of *p*-hydroxybenzoic acid have been utilized in these systems for many years. There have been reports that the parabens have been inactivated when used in the presence of various surface-active agents and vegetable gums. This loss of activity might be due to the formation of complexes between the preservative and the surfactant. Kostenbauder utilized a dialysis technique to demonstrate an interaction between polysorbate 80 and the methyl and propyl esters of *p*-hydroxybenzoic acid. This observation becomes critical if the level of preservative added is borderline with respect to the preservative activity threshold. The desired preservative effect may not be achieved unless an excess of the preservative is added to compensate for that which is complexed. It has also been shown that molecular complexes form when the *p*-hydroxybenzoates are mixed with polyethylene glycol, methylcellulose, polyvinylpyrrolidone, and gelatin. The degree of binding was less than that observed with polysorbate 80. Sorbic acid also interacts with the polysorbates but does not interact with the polyethylene glycols. The quaternary ammonium compounds are also bound by polysorbate 80 to

reduce their preservative activity. Benzyl alcohol was also shown to be adsorbed by certain types of rubber stoppers. Subsequent work has shown that butyl rubber does not interact with benzyl alcohol.

Antioxidants—During the preformulation evaluation of compounds that are sensitive to oxidation it is often commonplace to add several levels of antioxidant concentrations to aqueous systems in order to determine the relative effectiveness of the antioxidants. Sodium bisulfite and ascorbic acid are two antioxidants that enjoy widespread usage in pharmaceutical systems. Sodium bisulfite yields a colorless water-soluble salt when it is oxidized. It is also a very reactive ion. It will add to double bonds, react with aldehydes and certain ketones and contributes in bisulfite cleavage reactions. Many of the reactions with bisulfite are irreversible and the resulting sulfonic acids frequently are biologically inactive. Epinephrine has been shown to interact with bisulfite to form a bisulfite addition product. Other sympathomimetic drugs, principally the ortho- or para-hydroxybenzyl alcohol derivatives, also react with bisulfite in a similar manner. The meta-hydroxy alcohol did not react. Sometimes these interactions are reversible as in the case with the adrenocorticosteroid molecules.

Ascorbic acid on the other hand is relatively nonreactive. However, when mixed with compounds having a primary amine nucleus there is the tendency for interaction to form a highly colored Schiff base. One must be aware of this possibility when selecting a suitable antioxidant.

Suspending Agents—Occasionally it will be necessary to consider use of a suspending agent to prepare some preliminary suspension preparations for stability evaluation prior to starting toxicity testing. The physical pharmacist should be aware of the potential for these additives to react with the drug substance being evaluated. Anionic water-soluble compounds such as sodium carboxymethylcellulose, alginic acid, carrageenin and other hydrocolloids, although generally considered inert, frequently interact in solution with drug compounds. Carboxymethylcellulose and carrageenin form complexes or possibly salts with many medicinal agents including procaine, chlorpromazine, benadryl, quinine, chlorpheniramine, neomycin, and kanamycin. In some instances the formation of the complex imparted better stability to the system. When this problem is suspected it is important to conduct appropriate tests to insure that an interaction does not take place in the system being evaluated.

Dyes—Although preformulation tests are usually conducted long before any consideration of coloring the intended dosage forms, they should not be overlooked. Dyes are chemical in nature and contain reactive sites capable of causing incompatibilities. Several studies have demonstrated that certified dyes do react with drug substances. Sugars such as dextrose, lactose and sucrose were found to increase the rate of fading of FD&C Blue #2. Insoluble complexes were also formed when quaternary ammonium compounds were formulated with FD&C Red #1 and Blue #1.

Summary

The preformulation evaluation of new drug substances has become an integral part of the development process. A thorough understanding of the physical-chemical properties of the new drug substance under study provides the development pharmacist with data that are essential in designing stable and efficacious dosage forms. Many of the problems discussed and the solutions offered in this chapter resulted from application of scientific training of present-day pharmaceutical scientists. Their diverse skills, creative aptitudes, and initiative provide the pharmaceutical industry with the essential ingredients to develop drug products that help

136

137

maintain the health-care process at its highest level of excellence.

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(54) **ISOINDOLINES, METHOD OF USE, AND
PHARMACEUTICAL COMPOSITIONS**

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(51) Int. Cl.⁷ **A61K 31/445; C07D 401/04**

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(58) Field of Search **514/323; 546/201**

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(57) **ABSTRACT**

Substituted 1-oxo-2-(2,6-dioxopiperidin-3-yl)isoindolines are useful in treating inflammation, inflammatory disease, autoimmune disease, and oncogenic or cancerous conditions in a mammal. Typical embodiments are 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline and 1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4-aminoisoindoline.

26 Claims, No Drawings

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139

ISOINDOLINES, METHOD OF USE, AND
PHARMACEUTICAL COMPOSITIONSCROSS-REFERENCE TO RELATED
APPLICATIONS

This is a divisional of Ser. No. 09/230,389, now abandoned, which is based on PCT/US97/13375 filed Jul. 24, 1997, which is a continuation of Ser. No. 08/690,258 filed Jul. 24, 1996, now U.S. Pat. No. 5,635,517, which is a continuation of Ser. No. 08/701,494 filed Aug. 22, 1996 now U.S. Pat. No. 5,798,368, and provisional application Ser. No. 60/048,278 filed May 30, 1997.

DETAILED DESCRIPTION

The present invention relates to substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolines, the method of reducing levels of tumor necrosis factor α in a mammal through the administration thereof, and pharmaceutical compositions of such derivatives.

BACKGROUND OF THE INVENTION

Tumor necrosis factor α , or TNF α , is a cytokine which is released primarily by mononuclear phagocytes in response to a number of immunostimulators. When administered to animals or humans, it causes inflammation, fever, cardiovascular effects, hemorrhage, coagulation, and acute phase responses similar to those seen during acute infections and shock states. Excessive or unregulated TNF α production thus has been implicated in a number of disease conditions. These include endotoxemia and/or toxic shock syndrome {Tracey et al., *Nature* 330, 662-664 (1987) and Hinshaw et al., *Circ. Shock* 30, 279-292 (1990)}; cachexia {Dezube et al., *Lancet*, 335 (8690), 662 (1990)} and Adult Respiratory Distress Syndrome where TNF α concentration in excess of 12,000 pg/mL have been detected in pulmonary aspirates from ARDS patients {Millar et al., *Lancet* 2(8665), 712-714 (1989)}. Systemic infusion of recombinant TNF α also resulted in changes typically seen in ARDS {Ferrai-Baliviera et al., *Arch. Surg.* 124(12), 1400-1405 (1989)}.

TNF α appears to be involved in bone resorption diseases, including arthritis. When activated, leukocytes will produce bone-resorption, an activity to which the data suggest TNF α contributes. {Bertolini et al., *Nature* 319, 516-518 (1986) and Johnson et al., *Endocrinology* 124(3), 1424-1427 (1989)}. TNF α also has been shown to stimulate bone resorption and inhibit bone formation in vitro and in vivo through stimulation of osteoclast formation and activation combined with inhibition of osteoblast function. Although TNF α may be involved in many bone resorption diseases, including arthritis, the most compelling link with disease is the association between production of TNF α by tumor or host tissues and malignancy associated hypercalcemia {*Calc. Tissue Int. (US)* 46(Suppl.), S3-10 (1990)}. In Graft versus Host Reaction, increased serum TNF α levels have been associated with major complication following acute allogeneic bone marrow transplants {Holler et al., *Blood*, 75(4), 1011-1016 (1990)}.

Cerebral malaria is a lethal hyperacute neurological syndrome associated with high blood levels of TNF α and the most severe complication occurring in malaria patients. Levels of serum TNF α correlated directly with the severity of disease and the prognosis in patients with acute malaria attacks {Grau et al., *N. Engl. J. Med.* 320(24), 1586-1591 (1989)}.

Macrophage-induced angiogenesis TNF α is known to be mediated by TNF α . Leibovich et al. {*Nature*, 329, 630-632 (1987)} showed TNF α induces in vivo capillary blood vessel formation in the rat cornea and the developing chick chorioallantoic membranes at very low doses and suggest TNF α is a candidate for inducing angiogenesis in inflammation, wound repair, and tumor growth. TNF α production also has been associated with cancerous conditions, particularly induced tumors {Ching et al., *Brit. J. Cancer*, (1955) 72, 339-343, and Koch, *Progress in Medicinal Chemistry*, 22, 166-242 (1985)}.

TNF α also plays a role in the area of chronic pulmonary inflammatory diseases. The deposition of silica particles leads to silicosis, a disease of progressive respiratory failure caused by a fibrotic reaction. Antibody to TNF α completely blocked the silica-induced lung fibrosis in mice {Pignet et al., *Nature*, 344:245-247 (1990)}. High levels of TNF α production (in the serum and in isolated macrophages) have been demonstrated in animal models of silica and asbestos induced fibrosis {Bissonnette et al., *Inflammation* 13(3), 329-339 (1989)}. Alveolar macrophages from pulmonary sarcoidosis patients have also been found to spontaneously release massive quantities of TNF α as compared with macrophages from normal donors {Baughman et al., *J. Lab. Clin. Med.* 115(1), 36-42 (1990)}.

TNF α is also implicated in the inflammatory response which follows reperfusion, called reperfusion injury, and is a major cause of tissue damage after loss of blood flow {Vedder et al., *PNAS* 87, 2643-2646 (1990)}. TNF α also alters the properties of endothelial cells and has various pro-coagulant activities, such as producing an increase in tissue factor pro-coagulant activity and suppression of the anticoagulant protein C pathway as well as down-regulating the expression of thrombomodulin {Sherry et al., *J. Cell Biol.* 107, 1269-1277 (1988)}. TNF α has pro-inflammatory activities which together with its early production (during the initial stage of an inflammatory event) make it a likely mediator of tissue injury in several important disorders including but not limited to, myocardial infarction, stroke and circulatory shock. Of specific importance may be TNF α -induced expression of adhesion molecules, such as intercellular adhesion molecule (ICAM) or endothelial leukocyte adhesion molecule (ELAM) on endothelial cells {Munro et al., *Am. J. Path.* 135(1), 121-132 (1989)}.

TNF α blockage with monoclonal anti-TNF α antibodies has been shown to be beneficial in rheumatoid arthritis {Elliot et al., *Int. J. Pharmac.* 1995 17(2), 141-145} and Crohn's disease {von Dullemen et al., *Gastroenterology*, 1995 109(1), 129-135}.

Moreover, it now is known that TNF α is a potent activator of retrovirus replication including activation of HIV-1. {Duh et al., *Proc. Nat. Acad. Sci.* 86, 5974-5978 (1989); Poll et al., *Proc. Nat. Acad. Sci.* 87, 782-785 (1990); Monto et al., *Blood* 79, 2670 (1990); Clouse et al., *J. Immunol.* 142, 431-438 (1989); Poll et al., *AIDS Res. Hum. Retrovirus*, 191-197 (1992)}. AIDS results from the infection of T lymphocytes with Human Immunodeficiency Virus (HIV). At least three types or strains of HIV have been identified, i.e., HIV-1, HIV-2 and HIV-3. As a consequence of HIV infection, T-cell mediated immunity is impaired and infected individuals manifest severe opportunistic infections and/or unusual neoplasms. HIV entry into the T lymphocyte requires T lymphocyte activation. Other viruses, such as HIV-1, HIV-2 infect T lymphocytes after T cell activation and such virus protein expression and/or replication is mediated or maintained by such T cell activation. Once an activated T lymphocyte is infected with HIV, the T lympho-

cyte must continue to be maintained in an activated state to permit HIV gene expression and/or HIV replication. Cytokines, specifically TNF α , are implicated in activated T-cell mediated HIV protein expression and/or virus replication by playing a role in maintaining T lymphocyte activation. Therefore, interference with cytokine activity such as by prevention or inhibition of cytokine production, notably TNF α , in an HIV-infected individual assists in limiting the maintenance of T lymphocyte caused by HIV infection.

Monocytes, macrophages, and related cells, such as kupffer and glial cells, also have been implicated in maintenance of the HIV infection. These cells, like T cells, are targets for viral replication and the level of viral replication is dependent upon the activation state of the cells. {Rosenberg et al., *The Immunopathogenesis of HIV Infection*, Advances in Immunology, 57 (1989)}. Cytokines, such as TNF α , have been shown to activate HIV replication in monocytes and/or macrophages {Poli et al., *Proc. Natl. Acad. Sci.*, 87, 782-784 (1990)}, therefore, prevention or inhibition of cytokine production or activity aids in limiting HIV progression for T cells. Additional studies have identified TNF α as a common factor in the activation of HIV in vitro and has provided a clear mechanism of action via a nuclear regulatory protein found in the cytoplasm of cells (Osborn, et al., *PNAS* 86 2336-2340). This evidence suggests that a reduction of TNF α synthesis may have an antiviral effect in HIV infections, by reducing the transcription and thus virus production.

AIDS viral replication of latent HIV in T cell and macrophage lines can be induced by TNF α {Folks et al., *PNAS* 86, 2365-2368 (1989)}. A molecular mechanism for the virus inducing activity is suggested by TNF α 's ability to activate a gene regulatory protein (NF κ B) found in the cytoplasm of cells, which promotes HIV replication through binding to a viral regulatory gene sequence (LTR) {Osborn et al., *PNAS* 86, 2336-2340 (1989)}. TNF α in AIDS associated cachexia is suggested by elevated serum TNF α and high levels of spontaneous TNF α production in peripheral blood monocytes from patients {Wright et al., *J. Immunol.* 141(1), 99-104 (1988)}. TNF α has been implicated in various roles with other viral infections, such as the cytomegalia virus (CMV), influenza virus, adenovirus, and the herpes family of viruses for similar reasons as those noted.

The nuclear factor κ B (NF κ B) is a pleiotropic transcriptional activator (Lenardo, et al., *Cell* 1989, 58, 227-29). NF κ B has been implicated as a transcriptional activator in a variety of disease and inflammatory states and is thought to regulate cytokine levels including but not limited to TNF α and also to be an activator of HIV transcription (Dbalibo, et al., *J. Biol. Chem.* 1993, 17762-66; Duh et al., *Proc. Natl. Acad. Sci.* 1989, 86, 5974-78; Bachelierie et al., *Nature* 1991, 350, 709-12; Boswas et al., *J. Acquired Immune Deficiency Syndrome* 1993, 6, 778-786; Suzuki et al., *Biochem. And Biophys. Res. Comm.* 1993, 193, 277-83; Suzuki et al., *Biochem. And Biophys. Res Comm.* 1992, 189, 1709-15; Suzuki et al., *Biochem. Mol. Bio. Int.* 1993, 31(4), 693-700; Shakhov et al., *Proc. Natl. Acad. Sci. USA* 1990, 171, 35-47; and Staal et al., *Proc. Natl. Acad. Sci. USA* 1990, 87, 9943-47). Thus, inhibition of NF κ B binding can regulate transcription of cytokine gene(s) and through this modulation and other mechanisms be useful in the inhibition of a multitude of disease states. The compounds described herein can inhibit the action of NF κ B in the nucleus and thus are useful in the treatment of a variety of diseases including but not limited to rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, other arthritic conditions, septic

shock, sepsis, endotoxic shock, graft versus host disease, wasting, Crohn's disease, ulcerative colitis, multiple sclerosis, systemic lupus erythematosus, ENL in leprosy, HIV, AIDS, and opportunistic infections in AIDS. TNF α and NF κ B levels are influenced by a reciprocal feedback loop. As noted above, the compounds of the present invention affect the levels of both TNF α and NF κ B.

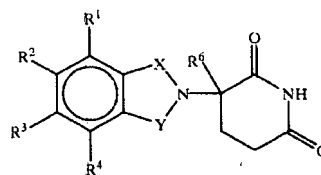
Many cellular functions are mediated by levels of adenosine 3',5'-cyclic monophosphate (cAMP). Such cellular functions can contribute to inflammatory conditions and diseases including asthma, inflammation, and other conditions (Lowe and Cheng, *Drugs of the Future*, 17(9), 799-807, 1992). It has been shown that the elevation of cAMP in inflammatory leukocytes inhibits their activation and the subsequent release of inflammatory mediators, including TNF α and NF κ B. Increased levels of cAMP also leads to the relaxation of airway smooth muscle.

Decreasing TNF α levels and/or increasing cAMP levels thus constitutes a valuable therapeutic strategy for the treatment of many inflammatory, infectious, immunological, and malignant diseases. These include but are not restricted to septic shock, sepsis, endotoxic shock, hemodynamic shock and sepsis syndrome, post ischemic reperfusion injury, malaria, mycobacterial infection, meningitis, psoriasis, congestive heart failure, fibrotic disease, cachexia, graft rejection, oncogenic or cancerous conditions, asthma, autoimmune disease, opportunistic infections in AIDS, rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, other arthritic conditions, Crohn's disease, ulcerative colitis, multiple sclerosis, systemic lupus erythematosus, ENL in leprosy, radiation damage, oncogenic conditions, and hyperoxic alveolar injury. Prior efforts directed to the suppression of the effects of TNF α have ranged from the utilization of steroids such as dexamethasone and prednisolone to the use of both polyclonal and monoclonal antibodies {Beutler et al., *Science* 234, 470-474 (1985); WO 92/11383}.

DETAILED DESCRIPTION

The present invention is based on the discovery that certain classes of non-polypeptide compounds more fully described herein decrease the levels of TNF α .

In particular, the invention pertains to (i) compounds of the formula:



in which:

one of X and Y is C=O and the other of X and Y is C=O or CH₂;

(i) each of R¹, R², R³, and R⁴, independently of the others, is halo, alkyl of 1 to 4 carbon atoms, or alkoxy of 1 to 4 carbon atoms or (ii) one of R¹, R², R³, and R⁴ is —NHR⁵ and the remaining of R¹, R², R³, and R⁴ are hydrogen;

R⁵ is hydrogen or alkyl of 1 to 8 carbon atoms;

R⁶ is hydrogen, alkyl of 1 to 8 carbon atoms, benzyl, or halo;

provided that R⁶ is other than hydrogen if X and Y are C=O and (i) each of R¹, R², R³, and R⁴ is fluoro or (ii) one of R¹, R², R³, or R⁴ is amino; and

742

5

(b) the acid addition salts of said compounds which contain a nitrogen atom capable of being protonated.

A preferred group of compounds are those of Formula I in which each of R^1 , R^2 , R^3 , and R^4 , independently of the others, is halo, alkyl of 1 to 4 carbon atoms, or alkoxy of 1 to 4 carbon atoms, and R^5 is hydrogen, methyl, ethyl, or propyl. A second preferred group of compounds are those of Formula I in which one of R^1 , R^2 , R^3 , and R^4 is $-NH_2$, the remaining of R^1 , R^2 , R^3 , and R^4 are hydrogen, and R^5 is hydrogen, methyl, ethyl, or propyl.

Unless otherwise defined, the term alkyl denotes a univalent saturated branched or straight hydrocarbon chain containing from 1 to 8 carbon atoms. Representative of such alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, and tert-butyl. Alkoxy refers to an alkyl group bound to the remainder of the molecule through an etheral oxygen atom. Representative of such alkoxy groups are methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, sec-butoxy, and tert-butoxy. Preferably R^1 , R^2 , R^3 , and R^4 are chloro, fluoro, methyl or methoxy.

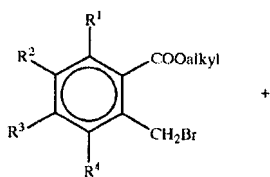
The compounds of Formula I are used, under the supervision of qualified professionals, to inhibit the undesirable effects of TNF α . The compounds can be administered orally, rectally, or parenterally, alone or in combination with other therapeutic agents including antibiotics, steroids, etc., to a mammal in need of treatment.

The compounds of the present invention also can be used topically in the treatment or prophylaxis of topical disease states mediated or exacerbated by excessive TNF α production, respectively, such as viral infections, such as those caused by the herpes viruses, or viral conjunctivitis, psoriasis, atopic dermatitis, etc.

The compounds also can be used in the veterinary treatment of mammals other than humans in need of prevention or inhibition of TNF α production. TNF α mediated diseases for treatment, therapeutically or prophylactically, in animals include disease states such as those noted above, but in particular viral infections. Examples include feline immunodeficiency virus, equine infectious anaemia virus, caprine arthritis virus, visna virus, and maedi virus, as well as other lentiviruses.

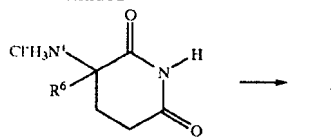
Compounds in which one of R^1 , R^2 , R^3 , R^4 is amino and R^5 and R^6 , as well as the remainder of R^1 , R^2 , R^3 , R^4 , are hydrogen, as for example, 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline or 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline are known. See, e.g. Jönsson, *Acta Pharma. Succica*, 9, 521-542 (1972).

The compounds can be prepared using methods which are known in general. In particular, the compounds can be prepared through the reaction of 2,6-dioxopiperidin-3-ammonium chloride, and a lower alkyl ester of 2-bromomethylbenzoic acid in the presence of an acid acceptor such as dimethylaminopyridine or triethylamine.



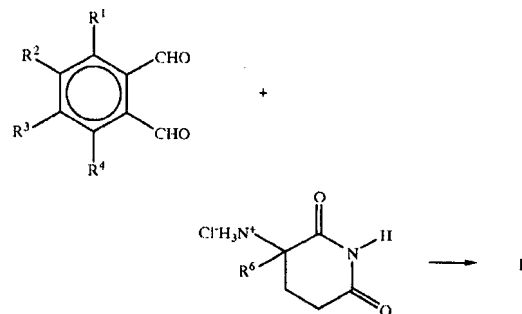
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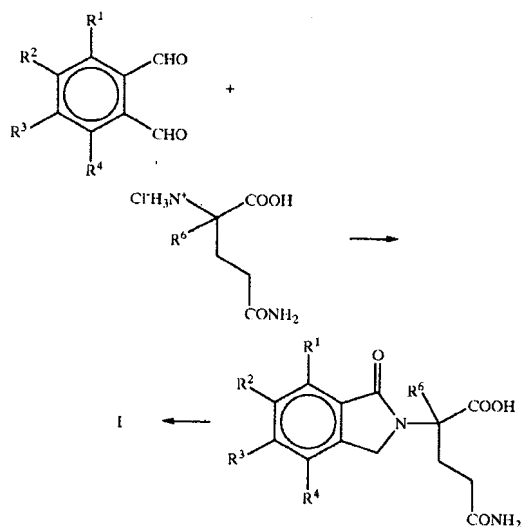


The substituted benzoate intermediates are known or can be obtained through conventional processes. For example, a lower alkyl ester of an ortho-toluic acid is brominated with N-bromosuccinimide under the influence of light to yield the lower alkyl 2-bromomethylbenzoate.

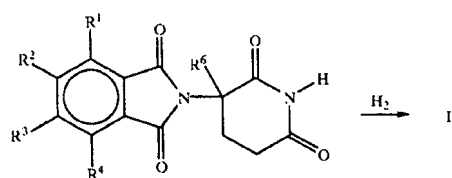
Alternatively, a dialdehyde is allowed to react with 2,6-dioxopiperidin-3-ammonium chloride:



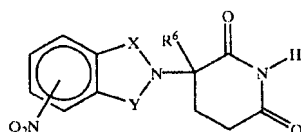
In a further method, a dialdehyde is allowed to react with glutamine and the resulting 2-(1-oxoisoindolin-2-yl)glutaric acid then cyclized to yield a 1-oxo-2-(2,6-dioxopiperidin-3-yl)-isoindoline of Formula I:



Finally, an appropriately substituted phthalidimide intermediate is selectively reduced:



Amino compounds can be prepared through catalytic hydrogenation of the corresponding nitro compound:



The nitro intermediates of Formula IA are known or can be obtained through conventional processes. For example, a nitrophthalic anhydride is allowed to react with α -aminoglutarimide hydrochloride {alternatively named as 2,6-dioxopiperidin-3-ylammonium chloride} in the presence of sodium acetate and glacial acetic acid to yield an intermediate of Formula IA in which X and Y are both C=O.

In a second route, a lower alkyl ester of nitro-ortho-toluic acid is brominated with N-bromosuccinimide under the influence of light to yield a lower alkyl 2-(bromomethyl) nitrobenzoate. This is allowed to react with 2,6-dioxopiperidin-3-ylammonium chloride in, for example, dimethylformamide in the presence of triethylamine to yield an intermediate of Formula II in which one of X is C=O and the other is CH₂.

Alternatively, if one of R₁, R₂, R₃, and R₄ is protected amino, the protecting group can be cleaved to yield the corresponding compound in which one of R₁, R₂, R₃, and R₄ is amino. Protecting groups utilized herein denote groups which generally are not found in the final therapeutic compounds but which are intentionally introduced at some stage of the synthesis in order to protect groups which otherwise might be altered in the course of chemical manipulations. Such protecting groups are removed at a later stage of the synthesis and compounds bearing such protecting groups thus are of importance primarily as chemical intermediates (although some derivatives also exhibit biological activity). Accordingly the precise structure of the protecting group is not critical. Numerous reactions for the formation and removal of such protecting groups are described in a number of standard works including, for example, "Protective Groups in Organic Chemistry", Plenum Press, London and N.Y., 1973; Greene, Th. W. "Protective Groups in Organic Synthesis", Wiley, New York, 1981; "The Peptides", Vol. 1, Schröder and Lubke, Academic Press, London and New York, 1965; "Methoden der organischen Chemie", Houben-Weyl, 4th Edition. Vol.15/1, Georg Thieme Verlag, Stuttgart 1974, the disclosures of which are incorporated herein by reference. An amino group can be protected as an amide utilizing an acyl group which is selectively removable under mild conditions, especially benzyloxycarbonyl, formyl, or a lower alkanoyl group which is branched in 1-or α position to the carbonyl group, particularly tertiary alkanoyl such as pivaloyl, a lower alkanoyl group which is substituted in the position α to the carbonyl group, as for example trifluoroacetyl.

The compounds of the present invention possess a center of chirality and can exist as optical isomers. Both the racemates of these isomers and the individual isomers themselves, as well as diastereomers when there are two chiral centers, are within the scope of the present invention. The racemates can be used as such or can be separated into their individual isomers mechanically as by chromatography using a chiral adsorbent. Alternatively, the individual isomers can be prepared in chiral form or separated chemically from a mixture by forming salts with a chiral acid, such as the individual enantiomers of 10-camphorsulfonic acid, camphoric acid, α -bromocamphoric acid, methoxyacetic acid, tartaric acid, diacetyltartaric acid, malic acid, pyrrolidone-5-carboxylic acid, and the like, and then freeing one or both of the resolved bases, optionally repeating the process, so as to obtain either or both substantially free of the other; i.e., in a form having an optical purity of >95%.

The present invention also pertains to the physiologically acceptable non-toxic acid addition salts of the compounds of Formula I. Such salts include those derived from organic and inorganic acids such as, without limitation, hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, methanesulphonic acid, acetic acid, tartaric acid, lactic acid, succinic acid, citric acid, maleic acid, malic acid, sorbic acid, aconitic acid, salicylic acid, phthalic acid, embonic acid, enanthic acid, and the like.

Oral dosage forms include tablets, capsules, dragees, and similar shaped, compressed pharmaceutical forms containing from 1 to 100 mg of drug per unit dosage. Isotonic saline solutions containing from 20 to 100 mg/mL can be used for parenteral administration which includes intramuscular, intrathecal, intravenous and intra-arterial routes of administration. Rectal administration can be effected through the use of suppositories formulated from conventional carriers such as cocoa butter.

Pharmaceutical compositions thus comprise one or more compounds of the present invention associated with at least one pharmaceutically acceptable carrier, diluent or excipient. In preparing such compositions the active ingredients are usually mixed with or diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule or sachet. When the excipient serves as a diluent, it may be a solid, semi-solid, or liquid material which acts as a vehicle, carrier, or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, elixirs, suspensions, emulsions, solutions, syrups, soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders. Examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, and methyl cellulose, the formulations can additionally include lubricating agents such as talc, magnesium stearate and mineral oil, wetting agents, emulsifying and suspending agents, preserving agents such as methyl- and propylhydroxybenzoates, sweetening agents or flavoring agents.

The following examples will serve to further typify the nature of this invention but should not be construed as a limitation in the scope thereof, which scope is defined solely by the appended claims.

EXAMPLE 1

1,3-Dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline

A mixture of 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-nitroisoindoline {alternatively named as N-(2,6-

1244

dioxopiperidin-3-yl)-4-nitrophthalimide} (1 g, 3.3 mmol) and 10% Pd/C (0.13 g) in 1,4-dioxane (200 mL) was hydrogenated at 50 psi for 6.5 hours. The catalyst was filtered through Celite and the filtrate concentrated in vacuo. The residue was crystallized from ethyl acetate (20 mL) to give 0.62 g (69%) of 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline {alternatively named as N-(2,6-dioxopiperidin-3-yl)-4-aminophthalimide} as an orange solid. Recrystallization from dioxane/ethyl acetate gave 0.32 g of yellow solid: mp 318.5–320.5° C.; HPLC (nova Pak C18, 15/85 acetonitrile/0.1% H₃PO₄) 3.97 min (98.22%); ¹H NMR (DMSO-d₆) δ 11.08(s, 1H), 7.53–7.50 (d, J=8.3 Hz, 1H), 6.94(s, 1H), 6.84–6.81(d, J=8.3 Hz, 1H), 6.55(s, 2H), 5.05–4.98(m, 1H), 2.87–1.99(m, 4H); ¹³C NMR (DMSO-d₆) δ 172.79, 170.16, 167.65, 167.14, 155.23, 134.21, 125.22, 116.92, 116.17, 107.05, 48.58, 30.97, 22.22; Anal. Calcd for C₁₃H₁₁N₃O₄: C, 57.14; H, 4.06; N, 15.38. Found: C, 56.52; H, 4.17; N, 14.60.

In a similar fashion from 1-oxo-2-(2,6-dioxopiperidin-3-yl)-5-nitroisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-nitroisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-nitroisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-7-nitroisoindoline, and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-nitroisoindoline, there is respectively obtained 1-oxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-7-aminoisoindoline, and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline, respectively, upon hydrogenation.

EXAMPLE 2

1,3-Dioxo-2-(2,6-dioxopiperidin-3-yl)-5-nitroisoindoline

A mixture of 4-nitrophthalic anhydride (1.7 g, 8.5 mmol), α-aminoglutarimide hydrochloride (1.4 g, 8.5 mmol) and sodium acetate (0.7 g, 8.6 mmol) in glacial acetic acid (30 mL) was heated under reflux for 17 hours. The mixture was concentrated in vacuo and the residue was stirred with methylene chloride (40 mL) and water (30 mL). The aqueous layer was separated, extracted with methylene chloride (2×40 mL). The combined methylene chloride solutions were dried over magnesium sulfate and concentrated in vacuo to give 1.4 g (54%) of 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-nitroisoindoline as a light brown solid. An analytical sample was obtained by recrystallization from methanol: mp 228.5–229.5° C.; ¹H NMR (DMOSO-d₆) δ 11.18(s, 1H), 8.69–8.65(d, J=1.9 and 8.0 Hz, 1H), 8.56(d, J=1.9 Hz, 1H), 8.21(d, J=8.2 Hz, 1H), 5.28(d, J=5.3 and 12.8 Hz, 1H), 2.93–2.07(m, 4H); ¹³C NMR (DMSO-d₆) δ 172.66, 169.47, 165.50, 165.23, 151.69, 135.70, 132.50, 130.05, 124.97, 118.34, 49.46, 30.85, 21.79; Anal. Calcd for C₁₃H₉N₃O₆: C, 51.49; H, 2.99; N, 13.86. Found: C, 51.59; H, 3.07; N, 13.73.

1-Oxo-2-(2,6-dioxopiperidin-3-yl)-5-nitroisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-nitroisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-nitroisoindoline, and 1-oxo-2-(2,6-dioxopiperidine-3-yl)-7-nitroisoindoline can be obtained by allowing 2,6-dioxopiperidin-3-ammonium chloride to react with methyl 2-bromomethyl-5-nitrobenzoate, methyl 2-bromomethyl-4-nitrobenzoate, methyl 2-bromomethyl-6-nitrobenzoate, and methyl 2-bromomethyl-7-nitrobenzoate, respectively, in dimethylformamide in the presence of triethylamine. The methyl 2-(bromomethyl)nitrobenzoates in turn are obtained from the corresponding methyl esters of nitro-orthotoluic acids by

conventional bromination with N-bromosuccinimide under the influence of light.

EXAMPLE 3

1-Oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline

A mixture of 16.25 g of 2,6-dioxopiperidin-3-ammonium chloride, and 30.1 g of methyl 2-bromomethyl-3,4,5,6-tetrafluorobenzoate, and 12.5 g of triethylamine in 100 mL of dimethylformamide is stirred at room temperature for 15 hours. The mixture is then concentrated in vacuo and the residue mixed with methylene chloride and water. The aqueous layer is separated and back-extracted with methylene chloride. The combined methylene chloride solutions are dried over magnesium sulfate and concentrated in vacuo to give 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline.

In a similar fashion 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrachloroisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetramethylisoindoline, and 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetramethoxyisoindoline are obtained by substituting equivalent amounts of 2-bromomethyl-3,4,5,6-tetrachlorobenzoate, 2-bromomethyl-3,4,5,6-tetramethylbenzoate, and 2-bromomethyl-3,4,5,6-tetramethoxybenzoate, respectively, for 2-bromomethyl-3,4,5,6-tetrafluorobenzoate.

EXAMPLE 4

N-Benzyloxycarbonyl-α-methyl-glutamic Acid

To a stirred solution of α-methyl-D,L-glutamic acid (10 g, 62 mmol) in 2 N sodium hydroxide (62 mL) at 0–5° C. was added benzyl chloroformate (12.7 g, 74.4 mmol) over 30 min. After the addition was complete the reaction mixture was stirred at room temperature for 3 hours. During this time the pH was maintained at 11 by addition of 2N sodium hydroxide (33 mL). The reaction mixture was then extracted with ether (60 mL). The aqueous layer was cooled in an ice bath and then acidified with 4N hydrochloric acid (34 mL) to pH=1. The resulting mixture was extracted with ethyl acetate (3×100 mL). The combined ethyl acetate extracts were washed with brine (60 mL) and dried (MgSO₄). The solvent was removed in vacuo to give 15.2 g (83%) of N-benzyloxycarbonyl-α-methylglutamic acid as an oil: ¹H NMR (CDCl₃) δ 8.73(m, 5H), 5.77(b, 1H), 5.09(s, 2H), 2.45–2.27(m, 4H), 2.0(s, 3H).

In a similar fashion from (α-ethyl-D,L-glutamic acid and α-propyl-D,L-glutamic acid, there is obtained N-benzyloxycarbonyl-α-ethylglutamic acid and N-benzyloxy-carbonyl-α-propylglutamic acid, respectively.

EXAMPLE 5

N-Benzyloxycarbonyl-α-methyl-glutamic Anhydride

A stirred mixture of N-benzyloxycarbonyl-α-methylglutamic acid (15 g, 51 mmol) and acetic anhydride (65 mL) was heated at reflux under nitrogen for 30 min. The reaction mixture was cooled to room temperature and then concentrated in vacuo to afford N-benzylcarbonyl-α-methylglutamic anhydride as an oil (15.7 g) which can be used in next reaction without further purification: ¹H NMR (CDCl₃) δ 7.44–7.26 (m, 5H), 5.32–5.30 (m, 2H), 5.11 (s, 1H), 2.69–2.61 (m, 2H), 2.40–2.30 (m, 2H), 1.68 (s, 3H).

In a similar fashion from N-benzyloxycarbonyl-α-ethylglutamic acid and N-benzyloxycarbonyl-α-

11

propylglutamic acid, there is obtained N-benzylcarbonyl- α -ethylglutamic anhydride and N-benzylcarbonyl- α -propylglutamic anhydride, respectively.

EXAMPLE 6

N-Benzylloxycarbonyl- α -methylisoglutamine

A stirred solution of N-benzylcarbonyl- α -methylglutamic anhydride (14.2 g, 51.5 mmol) in methylene chloride (100 mL) was cooled in an ice bath. Gaseous ammonia was bubbled into the cooled solution for 2 hours. The reaction mixture was stirred at room temperature for 17 hours and then extracted with water (2x50 mL). The combined aqueous extracts were cooled in an ice bath and acidified with 4N hydrochloric acid (32 mL) to pH 1. The resulting mixture was extracted with ethyl acetate (3x80 mL). The combined ethyl acetate extracts were washed with brine (60 mL) and then dried (MgSO₄). The solvent was removed in vacuo to give 11.5 g of N-benzylloxycarbonyl- α -amino- α -methylisoglutamine: ¹H NMR (CDCl₃/DMSO) δ 7.35 (m, 5H) 7.01 (s, 1H), 6.87 (s, 1H), 6.29 (s, 1H), 5.04 (s, 2H) 2.24–1.88 (m, 4H), 1.53 (s, 3H).

In a similar fashion from N-benzylcarbonyl- α -ethylglutamic anhydride and N-benzylcarbonyl- α -propylglutamic anhydride there is obtained N-benzylloxycarbonyl- α -amino- α -ethylisoglutamine and N-benzylloxycarbonyl- α -amino- α -propylisoglutamine, respectively.

EXAMPLE 7

N-Benzylloxycarbonyl- α -amino- α -methylglutarimide

A stirred mixture of N-benzylloxycarbonyl- α -methylisoglutamine (4.60 g, 15.6 mmol), 1,1'-carbonyldiimidazole (2.80 g, 17.1 mmol), and 4-dimethylaminopyridine (0.05 g) in tetrahydrofuran (50 mL) was heated to reflux under nitrogen for 17 hours. The reaction mixture was then concentrated in vacuo to an oil. The oil was slurried in water (50 mL) for 1 hour. The resulting suspension was filtered and the solid washed with water and air dried to afford 3.8 g of the crude product as a white solid. The crude product was purified by flash chromatography (methylene chloride:ethyl acetate 8:2) to afford 2.3 g (50%) of N-benzylloxycarbonyl- α -amino- α -methylglutarimide as a white solid: mp 150.5–152.5° C.; ¹H NMR (CDCl₃) δ 8.21 (s, 1H), 7.34 (s, 5H), 5.59 (s, 1H), 5.08 (s, 2H), 2.74–2.57 (m, 3H), 2.28–2.25 (m, 1H), 1.54 (s, 3H); ¹³C NMR (CDCl₃) δ 174.06, 171.56, 154.68, 135.88, 128.06, 127.69, 127.65, 66.15, 54.79, 29.14, 28.70, 21.98; HPLC: Waters Nova-Pak C18 column, 4 micron, 3.9x150 mm, 1 mL/min 240 nm, 20/80 CH₃CN/0.1% H₃PO₄(aq), 7.56 min (100%); Anal. Calcd For C₁₄H₁₆N₂O₆: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.88; H, 5.72; N, 10.07.

In a similar fashion from N-benzylloxycarbonyl- α -amino- α -ethylisoglutamine and N-benzylloxycarbonyl- α -amino- α -propylisoglutamine there is obtained N-benzylloxycarbonyl- α -amino- α -ethylglutarimide and N-benzylloxycarbonyl- α -amino- α -propylglutarimide, respectively.

EXAMPLE 8

 α -Amino- α -methylglutarimide hydrochloride

N-Benzylloxycarbonyl- α -amino- α -methylglutarimide (2.3 g, 8.3 mmol) was dissolved in ethanol (200 mL) with

12

gentle heat and the resulting solution allowed to cool to room temperature. To this solution was added 4N hydrochloric acid (3 mL) followed by 10% Pd/C (0.4 g). The mixture was hydrogenated in a Parr apparatus under 50 psi of hydrogen for 3 hours. To the mixture was added water (50 mL) to dissolve the product. This mixture was filtered through a Celite pad which was washed with water (50 mL). The filtrate was concentrated in vacuo to afford a solid residue. The solid was slurried in ethanol (20 mL) for 30 min. The slurry was filtered to afford 1.38 g (93%) of α -amino- α -methylglutarimide hydrochloride as a white solid: ¹H NMR (DMSO-d₆) δ 11.25 (s, 1H), 8.92 (s, 3H), 2.84–2.51 (m, 2H), 2.35–2.09 (m, 2H), 1.53 (s, 3H); HPLC, Waters Nova-Pak C₁₈ column, 4 micron, 1 mL/min, 240 nm, 20/80 CH₃CN/0.1% H₃PO₄(aq), 1.03 min (94.6%).

In a similar fashion from N-benzylloxycarbonyl- α -amino- α -ethylglutarimide and N-benzylloxycarbonyl- α -amino- α -propylglutarimide there is obtained α -amino- α -ethylglutarimide hydrochloride and α -amino- α -propylglutarimide hydrochloride, respectively.

EXAMPLE 9

3-(3-Nitrophthalimido)-3-methylpiperidine-2,6-dione

A stirred mixture of α -amino- α -methylglutarimide hydrochloride (1.2 g, 6.7 mmol), 3-nitrophthalic anhydride (1.3 g, 6.7 mmol), and sodium acetate (0.6 g, 7.4 mmol) in acetic acid (30 mL) was heated to reflux under nitrogen for 6 hours. The mixture then was cooled and concentrated in vacuo. The resulting solid was slurried in water (30 mL) and methylene chloride (30 mL) for 30 min. The suspension was filtered, the solid was washed with methylene chloride, and dried in vacuo (60° C., <1 mm) to afford 1.44 g (68%) of 3-(3-nitrophthalimido)-3-methylpiperidine-2,6-dione as a off-white solid: mp 265–266.5° C.; ¹HMR (DMSO-d₆) δ 11.05 (s, 1H), 8.31 (dd, J=1.1 and 7.9 Hz, 1H), 8.16–8.03 (m, 2H), 2.67–2.49 (m, 3H), 2.08–2.02 (m, 1H), 1.88 (s, 3H); ¹³C NMR (DMSO-d₆) δ 172.20, 171.71, 165.89, 163.30, 144.19, 136.43, 133.04, 128.49, 126.77, 122.25, 59.22, 28.87, 28.49, 21.04; HPLC, Water Nova-Pak/C₁₈ column, 4 micron, 1 mL/min, 240 nm, 20/80 CH₃CN/0.1% H₃PO₄(aq), 7.38 min(98%). Anal. Calcd For C₁₄H₁₃N₃O₆: C, 53.00; H, 3.49; N, 13.24. Found: C, 52.77; H, 3.29; N, 13.00.

In a similar fashion from α -amino- α -ethylglutarimide hydrochloride and α -amino- α -propylglutarimide hydrochloride there is obtained 3-(3-nitrophthalimido)-3-ethylpiperidine-2,6-dione and 3-(3-nitrophthalimido)-3-propylpiperidine-2,6-dione, respectively.

EXAMPLE 10

3-(3-Aminophthalimido)-3-methylpiperidine-2,6-dione

3-(3-Nitrophthalimido)-3-methylpiperidine-2,6-dione (0.5 g, 1.57 mmol) was dissolved in acetone (250 mL) with gentle heat and then cooled to room temperature. To this solution was added 10% Pd/C (0.1 g) under nitrogen. The mixture was hydrogenated in a Parr apparatus at 50 psi of hydrogen for 4 hours. The mixture then was filtered through Celite and the pad washed with acetone (50 mL). The filtrate was concentrated in vacuo to yield a yellow solid. The solid was slurried in ethyl acetate (10 mL) for 30 minutes. The slurry then was filtered and dried (60° C., <1 mm) to afford 0.37 g (82%) of 3-(3-aminophthalimido)-3-methylpiperidine-2,6-dione as a yellow solid: mp 268–269°

13

C.; ¹H NMR (DMSO-d₆) δ10.98 (s, 1H), 7.44 (dd, J=7.1 and 7.3 Hz, 1H), 6.99 (d, J=8.4 Hz, 1H), 6.94 (d, J=6.9 Hz, 1H), 6.52 (s, 2H), 2.71–2.47 (m, 3H), 2.08–1.99 (m, 1H), 1.87 (s, 3H); ¹³C NMR (DMSO-d₆) δ172.48, 172.18, 169.51, 168.06, 146.55, 135.38, 131.80, 121.51, 110.56, 108.30, 58.29, 29.25, 28.63, 21.00; HPLC, Water Nova-Pak/C₁₈ column, 4 micron, 1 mL/min, 240 nm, 20/80 CH₃CN/0.1% H₃PO₄(aq), 5.62 min (99.18%). Anal. Calcd For C₁₄H₁₃N₃O₄: C, 58.53; H, 4.56; N, 14.63. Found: C, 58.60; H, 4.41; N, 14.36.

In a similar fashion from 3-(3-nitrophthalimido)-3-ethylpiperidine-2,6-dione and 3-(3-nitrophthalimido)-3-propylpiperidine-2,6-dione there is obtained 3-(3-aminophthalimido)-3-ethylpiperidine-2,6-dione and 3-(3-aminophthalimido)-3-propylpiperidine-2,6-dione, respectively.

EXAMPLE 11

Methyl 2-bromomethyl-3-nitrobenzoate

A stirred mixture of methyl 2-methyl-3-nitrobenzoate (17.6 g, 87.1 mmol) and N-bromosuccinimide (18.9 g, 105 mmol) in carbon tetrachloride (243 mL) was heated under gentle reflux with a 100 W light bulb situated 2 cm away shining on the reaction mixture overnight. After 18 hours, the reaction mixture was cooled to room temperature and filtered. The filtrate was washed with water (2×120 mL), brine (120 mL), and dried (MgSO₄). The solvent was removed in vacuo to give a yellow solid. The product was purified by flash chromatography (hexane:ethyl acetate 8:2) to give 22g (93%) of methyl 2-bromomethyl-3-nitrobenzoate as a yellow solid: mp 69–72° C.; ¹H NMR (CDCl₃) δ8.13–8.09 (dd, J=1.36 and 7.86 Hz, 1H), 7.98–7.93 (dd, J=1.32 and 8.13 Hz, 1H), 7.57–7.51 (t, J=7.97 Hz, 1H), 5.16 (s, 2H), 4.0 (s, 3H); ¹³C NMR (CDCl₃) δ65.84, 150.56, 134.68, 132.64, 132.36, 129.09, 53.05, 22.70; HPLC: Waters Nova-Pak C₁₈ column, 4 micron, 1 mL/min, 240 nm, 40/60 CH₃CN/0.1% H₃PO₄(aq), 8.2 min 99 %. Anal. Calcd for C₉H₈NO₄Br: C, 39.44; H, 2.94; N, 5.11, Br, 29.15. Found: C, 39.51; H, 2.79; N, 5.02; Br, 29.32.

EXAMPLE 12

3-(1-Oxo-4-nitroisindolin-1-yl)-3-methylpiperidine-2,6-dione

To a stirred mixture of α-amino-α-methylglutarimide hydrochloride (2.5 g, 14.0 mmol) and methyl 2-bromomethyl-3-nitrobenzoate (3.87 g, 14.0 mmol) in dimethylformamide (40 mL) was added triethylamine (3.14 g, 30.8 mmol). The resulting mixture was heated to reflux under nitrogen for 6 hours. The mixture was cooled and then concentrated in vacuo. The resulting solid was slurried in water (50 mL) and CH₂Cl₂ for 30 min. The slurry was filtered, the solid washed with methylene chloride, and dried in vacuo (60° C., <1 mm) to afford 2.68 g (63%) of 3-(1-oxo-4-nitroisindolin-1-yl)-3-methylpiperidine-2,6-dione as a off-white solid: mp 233–235° C.; ¹H NMR (DMSO-d₆) δ10.95 (s, 1H), 8.49–8.46 (d, J=8.15 Hz, 1H), 8.13–8.09 (d, J=7.43 Hz, 1H), 7.86–7.79 (t, J=7.83 Hz, 1H), 5.22–5.0 (dd, J=19.35 and 34.6 Hz, 2H), 2.77–2.49 (m, 3H), 2.0–1.94 (m, 1H), 1.74 (s, 3H); ¹³C NMR (DMSO-d₆) δ173.07, 172.27, 164.95, 143.15, 137.36, 135.19, 130.11, 129.32, 126.93, 57.57, 48.69, 28.9, 27.66, 20.6; HPLC, Waters Nova-Pak C₁₈ column, 4 micron, 1 mL/min, 240 nm, 20/80 CH₃CN/0.1% H₃PO₄(aq), 4.54 min 99.6%. Anal. Calcd for C₁₄H₁₃N₃O₅: C, 55.45; H, 4.32; N, 13.86. Found: C, 52.16; H, 4.59; N, 12.47.

14

By substituting equivalent amounts of α-amino-α-ethylglutarimide hydrochloride and α-amino-α-propylglutarimide hydrochloride for α-amino-α-methylglutarimide hydrochloride, there is obtained respectively 3-(1-oxo-4-nitroisindolin-1-yl)-3-ethylpiperidine-2,6-dione and 3-(1-oxo-4-nitroisindolin-1-yl)-3-propylpiperidine-2,6-dione.

EXAMPLE 13

3-(1-Oxo-4-aminoisindolin-1-yl)-3-methylpiperidine-2,6-dione

3-(1-Oxo-4-nitroisindolin-1-yl)-3-methylpiperidine-2,6-dione (1.0 g, 3.3 mmol) was dissolved in methanol (500 mL) with gentle heat and allowed to cool to room temperature. To this solution was added 10% Pd/C (0.3 g) under nitrogen. The mixture was hydrogenated in a Parr apparatus at 50 psi of hydrogen for 4 hours. The mixture was filtered through Celite and the Celite washed with methanol (50 mL). The filtrate was concentrated in vacuo to an off white solid. The solid was slurried in methylene chloride (20 mL) for 30 min. The slurry was then filtered and the solid dried (60° C., <1 mm) to afford 0.54 g (60%) of 3-(1-oxo-4-aminoisindolin-1-yl)-3-methylpiperidine-2,6-dione as a white solid: mp 268–270° C.; ¹H NMR (DMSO-d₆) δ10.85 (s, 1H), 7.19–7.13 (t, J=7.63 Hz, 1H), 6.83–6.76 (m, 2H), 5.44 (s, 2H), 4.41 (s, 2H), 2.71–2.49 (m, 3H), 1.9–1.8 (m, 1H), 1.67 (s, 3H); ¹³C NMR (DMSO-d₆) δ173.7, 172.49, 168.0, 143.5, 132.88, 128.78, 125.62, 116.12, 109.92, 56.98, 46.22, 29.04, 27.77, 20.82; HPLC, Waters Nova-Pak/C₁₈ column, 4 micron, 1 mL/min, 240 nm, 20/80 CH₃CN/0.1% H₃PO₄(aq), 1.5 min (99.6%); Anal. Calcd for C₁₄H₁₃N₃O₃: C, 61.53; H, 5.53; N, 15.38. Found: C, 58.99; H, 5.48; N, 14.29.

From 3-(1-oxo-4-nitroisindolin-1-yl)-3-ethylpiperidine-2,6-dione and 3-(1-oxo-4-nitroisindolin-1-yl)-3-propylpiperidine-2,6-dione there is similarly obtained 3-(1-oxo-4-aminoisindolin-1-yl)-3-ethylpiperidine-2,6-dione and 3-(1-oxo-4-aminoisindolin-1-yl)-3-propylpiperidine-2,6-dione, respectively.

EXAMPLE 14

S-4-Amino-2-(2,6-dioxopiperid-3-yl)isindoline-1,3-dione

A. 4-Nitro-N-ethoxycarbonylphthalimide

Ethyl chloroformate (1.89 g 19.7 mmol) was added dropwise over 10 min to a stirred solution of 3-nitrophthalimide (3.0 g, 15.6 mmol) and triethylamine (1.78 g, 17.6 mmol) in dimethylformamide (20 mL) at 0–5° C. under nitrogen. The reaction mixture was allowed to warm to room temperature and stirred for 4 hours. The mixture was then slowly added to an agitated mixture of ice and water (60 mL). The resulting slurry was filtered and the solid was crystallized from chloroform (15 mL) and pet ether (15 mL) to afford 3.1 g (75%) of the product as an off-white solid: mp 100–100.5° C.; ¹H NMR (CDCl₃) δ8.25(d, J=7.5 Hz, 1H), 8.20(d, J=8.0 Hz, 1H), 8.03(t, J=7.9 Hz, 1H), 4.49(q, J=7.1 Hz, 2H), 1.44(t, J=7.2 Hz, 3H); ¹³C NMR (CDCl₃) δ161.45, 158.40, 147.52, 145.65, 136.60, 132.93, 129.65, 128.01, 122.54, 64.64, 13.92; HPLC, Waters Nova-Pak/C₁₈, 3.9×150 mm, 4 micron, 1 mL/min, 240 nm, 30/70 CH₃CN/0.1% H₃PO₄(aq), 5.17 min(98.11%); Anal. Calcd for C₁₁H₉N₃O₆: C, 50.00; H, 3.05; N, 10.60. Found: C, 50.13; H, 2.96; N, 10.54.

B. t-Butyl N-(4-nitrophthaloyl)-L-glutamine

A stirred mixture of 4-nitro-N-ethoxycarbonylphthalimide (1.0 g, 3.8 mmol), L-glutamine t-butyl ester hydrochloride (0.90 g, 3.8 mmol) and triethy-

15

amine (0.54 g, 5.3 mmol) in tetrahydrofuran (30 mL) was heated to reflux for 24 hours. The tetrahydrofuran was removed in vacuo and the residue was dissolved in methylene chloride (50 mL). The methylene chloride solution was washed with water (2x15 mL), brine (15 mL) and then dried (sodium sulfate). The solvent was removed in vacuo and the residue was purified by flash chromatograph (7:3 methylene chloride:ethyl acetate) to give 0.9 g (63%) of a glassy material: ^1H NMR (CDCl_3) δ 8.15(d, J=7.9 Hz, 2H), 7.94(t, J=7.8 Hz, 1H), 5.57(b, 2H), 4.84(dd, J=5.1 and 9.7 Hz, 1H), 2.53–2.30(m, 4H), 1.43(s, 9H); HPLC, Waters Nova-Pak/C18, 3.9x150 mm, 4 micron, 1 mL/min, 240 nm, 30/70 $\text{CH}_3\text{CN}/0.1\%$ $\text{H}_3\text{PO}_4(\text{aq})$, 6.48 min(99.68%); Chiral Analysis, Daicel Chiral Pak AD, 0.4x25 cm, 1 mL/min, 240 nm, 5.32 min(99.39%); Anal. Calcd for $\text{C}_{17}\text{H}_{19}\text{N}_3\text{O}_7$: C, 54.11; H, 5.08; N, 11.14. Found: C, 54.21; H, 5.08; N, 10.85.

C. N-(4-Nitrophthaloyl)-L-glutamine

Hydrogen chloride gas was bubbled into a stirred 5° C. solution of t-butyl N-(4-nitrophthaloyl)-L-glutamine (5.7 g, 15.1 mmol) in methylene chloride (100 mL) for 25 min. The mixture was then stirred at room temperature for 16 hours. Ether (50 mL) was added and the resulting mixture was stirred for 30 min. The resulting slurry was filtered to yield 4.5 g of crude product as a solid, which was used directly in the next reaction: ^1H NMR ($\text{DMSO}-d_6$) δ 8.36(dd, J=0.8 and 8.0 Hz, 1H), 8.24(dd, J=0.8 and 7.5 Hz, 1H), 8.11(t, J=7.9 Hz, 1H), 7.19(b, 1H), 6.72(b, 1H), 4.80(dd, J=3.5 and 8.8 Hz, 1H), 2.30–2.10(m, 4H).

D. (S)-2-(2,6-dioxo(3-piperidyl))-4-nitroisindoline-1,3-dione

A stirred suspension of N-(4-nitrophthaloyl)-L-glutamine (4.3 g, 13.4 mmol) in anhydrous methylene chloride (170 mL) was cooled to –40° C. (IPA/dry ice bath). Thionyl chloride (1.03 mL, 14.5 mmol) was added dropwise to the mixture followed by pyridine (1.17 mL, 14.5 mmol). After 30 minutes triethylamine (2.06 mL, 14.8 mmol) was added and the mixture was stirred at –30 to –40° C. for 3 hours. The mixture was allowed to warm to room temperature, filtered and washed with methylene chloride to afford 2.3 g (57%) of the crude product. Recrystallization from acetone (300 mL) afforded 2 g of the product as a white solid: mp 259.0–284.0° C.(dec.); ^1H NMR ($\text{DMSO}-d_6$) δ 11.19(s, 1H), 8.34(d, J=7.8 Hz, 1H), 8.23(d, J=7.1 Hz, 1H), 8.12(t, J=7.8 Hz, 1H), 5.25–5.17(dd, J=5.2 and 12.7 Hz, 1H), 2.97–2.82(m, 1H), 2.64–2.44(m, 2H), 2.08–2.05(m, 1H); ^{13}C NMR ($\text{DMSO}-d_6$) δ 172.67, 169.46, 165.15, 162.50, 144.42, 136.78, 132.99, 128.84, 127.27, 122.53, 49.41, 30.84, 21.71; HPLC, Waters Nova-Pak/C18, 3.9x150 mm, 4 micron, 1 mL/min, 240 nm, 10/90 $\text{CH}_3\text{CN}/0.1\%$ $\text{H}_3\text{PO}_4(\text{aq})$ 4.27 min(99.63%); Anal. Calcd for $\text{C}_{13}\text{H}_9\text{N}_3\text{O}_6$: C, 51.49; H, 2.99; N, 13.86. Found: C, 51.67; H, 2.93; N, 13.57.

E. S-4-Amino-2-(2,6-dioxopiperid-3-yl)isindoline-1,3-dione

A mixture of (S)-3-(4'-nitrophthalimido)-piperidine-2,6-dione (0.76 g, 2.5 mmol) and 10% Pd/C (0.3 g) in acetone (200 mL) was hydrogenated in a Parr-Shaker apparatus at 50 psi of hydrogen for 24 hours. The mixture was filtered through celite and the filtrate was concentrated in vacuo. The solid residue was slurried in hot ethyl acetate for 30 min and filtered to yield 0.47 g (69%) of the product as a yellow solid: mp 309–310° C.; ^1H NMR ($\text{DMSO}-d_6$) δ 11.10 (s, 1H), 7.47(dd, J=7.2 and 8.3 Hz, 1H), 7.04–6.99(dd, J=6.9 and 8.3 Hz, 2H), 6.53(s, 2H), 5.09–5.02(dd, J=5.3 and 12.4 Hz, 1H), 2.96–2.82(m, 1H), 2.6–2.46(m, 2H), 2.09–1.99(m, 1H); ^{13}C NMR ($\text{DMSO}-d_6$) δ 172.80, 170.10, 168.57, 167.36, 146.71, 135.44, 131.98, 121.69, 110.98, 108.54, 48.48, 30.97, 22.15; HPLC, Waters Nova-Pak/C18, 3.9x150

16

mm, 4 micron, 1 mL/min, 240 nm, 15/85 $\text{CH}_3\text{CN}/0.1\%$ $\text{H}_3\text{PO}_4(\text{aq})$ 4.99 min(98.77%); Chiral analysis, Daicel Chiral Pak AD, 0.46x25 cm, 1 mL/min, 240 nm, 30/70 Hexane/IPA 9.55 min (1.32%), 12.55 min (97.66%); Anal. Calcd for $\text{C}_{13}\text{H}_{11}\text{N}_3\text{O}_4$: C, 57.14; H, 4.06; N, 15.38. Found: C, 57.15; H, 4.15; N, 14.99.

EXAMPLE 15

R-4-Amino-2-(2,6-dioxopiperid-3-yl)isindoline-1,3-dione

A. t-Butyl N-(4-nitrophthaloyl)-D-glutamine

A stirred mixture of 4-nitro-N-ethoxycarbonylphthalimide (5.9 g, 22.3 mmol), D-glutamine t-butyl ester (4.5 g, 22.3 mmol) and triethylamine (0.9 g, 8.9 mmol) in tetrahydrofuran (100 mL) was refluxed for 24 hours. The mixture was diluted with methylene chloride (100 mL) and washed with water (2x50 mL), brine (50 mL) and then dried. The solvent was removed in vacuo and the residue was purified by flash chromatography (2% CH_3OH in methylene chloride) to afford 6.26 g (750%) of the product as a glassy material: ^1H NMR (CDCl_3) δ 8.12(d, J=7.5 Hz, 2H), 7.94(dd, J=7.9 and 9.1 Hz, 1H), 5.50(b, 1H), 5.41(b, 1H), 4.85(dd, J=5.1 and 9.8 Hz, 1H), 2.61–2.50(m, 2H), 2.35–2.27(m, 2H), 1.44(s, 9H); ^{13}C NMR (CDCl_3) δ 173.77, 167.06, 165.25, 162.51, 145.07, 135.56, 133.78, 128.72, 127.27, 123.45, 83.23, 53.18, 32.27, 27.79, 24.42; HPLC, Waters Nova-Pak/C18, 3.9x150 mm, 4 micron, 1 mL/min, 240 nm, 25/75 $\text{CH}_3\text{CN}/0.1\%$ $\text{H}_3\text{PO}_4(\text{aq})$ 4.32 min(99.74%); Chiral analysis, Daicel Chiral Pak AD, 0.46x25 cm, 1 mL/min, 240 nm, 55/45 Hexane/IPA 5.88 min(99.68%); Anal. Calcd for $\text{C}_{17}\text{H}_{19}\text{N}_3\text{O}_7$: C, 54.11; H, 5.08; N, 11.14. Found: C, 54.25; H, 5.12; N, 10.85.

B. N-(4-Nitrophthaloyl)-D-glutamine

Hydrogen chloride gas was bubbled into a stirred 5° C. solution of t-butyl N-(4-nitrophthaloyl)-D-glutamine (5.9 g, 15.6 mmol) in methylene chloride (100 mL) for 1 hour then stirred at room temperature for another hour. Ether (100 mL) was added and stirred for another 30 minutes. The mixture was filtered, the solid was washed with ether (60 mL) and dried (40° C., <1 mm Hg) to afford 4.7 g (94%) of the product: ^1H NMR ($\text{DMSO}-d_6$) δ 8.33(d, J=7.8 Hz, 1H), 8.22(d, J=7.2 Hz, 1H), 8.11(t, J=7.8 Hz, 1H), 7.19(b, 1H), 6.72(b, 1H), 4.81(dd, J=4.6 and 9.7 Hz, 1H), 2.39–2.12(m, 4H); ^{13}C NMR ($\text{DMSO}-d_6$) δ 173.21, 169.99, 165.41, 162.73, 144.45, 136.68, 132.98, 128.80, 127.23, 122.52, 51.87, 31.31, 23.87.

C. (R)-2-(2,6-dioxo(3-piperidyl))-4-nitroisindoline-1,3-dione

A stirred suspension of N-(4'-nitrophthaloyl)-D-glutamine (4.3 g, 13.4 mmol) in anhydrous methylene chloride (170 mL) was cooled to –40° C. with isopropanol/dry ice bath. Thionyl chloride (1.7 g, 14.5 mmol) was added dropwise followed by pyridine (1.2 g, 14.5 mmol). After 30 min, triethylamine (1.5 g, 14.8 mmol) was added and the mixture was stirred at –30 to –40° C. for 3 hours. The mixture was filtered, the solid washed with methylene chloride (50 mL) and dried (60° C., <1 mm Hg) to give 2.93 g of the product. Another 0.6 g of the product was obtained from the methylene chloride filtrate. Both fractions were combined (3.53 g) and recrystallized from acetone (450 mL) to afford 2.89 g (71%) of the product as a white solid: mp 256.5–257.5° C.; ^1H NMR ($\text{DMSO}-d_6$) δ 11.18(s, 1H), 8.34 (dd, J=0.8 and 7.9 Hz, 1H), 8.23(dd, J=0.8 and 7.5 Hz, 1H), 8.12(t, J=7.8 Hz, 1H), 5.22(dd, J=5.3 and 12.8 Hz, 1H), 2.97–2.82(m, 1H), 2.64–2.47(m, 2H), 2.13–2.04(m, 1H); ^{13}C NMR ($\text{DMSO}-d_6$) δ 172.66, 169.44, 165.14, 162.48, 144.41, 136.76, 132.98, 128.83, 127.25, 122.52, 49.41,

30.83, 21.70; HPLC, Waters Nova-Pak/C18, 3.9x150 mm, 4 micron, 1 mL/min, 240 nm, 10/90 CH₃CN/0.1% H₃PO₄(aq) 3.35 min(100%); Anal. Calcd for C₁₃H₉N₃O₆: C, 51.49; H, 2.99; N, 13.86. Found: C, 51.55; H, 2.82; N, 13.48.

D. (R)-4-Amino-2-(2,6-dioxopiperid-3-yl)isoindoline-1,3-dione

A mixture of R-3-(4'-nitrophthalimido)-piperidine-2,6-dione (1.0 g, 3.3 mmol) and 10% Pd/C (0.2 g) in acetone (250 mL) was hydrogenated in a Parr-Shaker apparatus at 50 psi of hydrogen for 4 hours. The mixture was filtered through celite and the filtrate was concentrated in vacuo. The resulting yellow solid was slurried in hot ethyl acetate (20 mL) for 30 min to give after filtration and drying 0.53 g (59%) of the product as a yellow solid: mp 307.5-309.5° C.; ¹H NMR (DMSO-d₆) δ 11.06(s, 1H), 7.47(dd, J=7.0 and 8.4 Hz, 1H), 7.02(dd, J=4.6 and 8.4 Hz, 2H), 6.53(s, 2H), 5.07(dd, J=5.4 and 12.5 Hz, 1H), 2.95-2.84(m, 1H), 2.62-2.46(m, 2H), 2.09-1.99(m, 1H); ¹³C NMR (DMSO-d₆) δ 172.78, 170.08, 168.56, 167.35, 146.70, 135.43, 131.98, 121.68, 110.95, 108.53, 48.47, 30.96, 22.14; HPLC, Waters Nova-Pak/C18, 3.9x150 mm, 4 micron, 1 mL/min, 240 nm, 10/90 CH₃CN/0.1% H₃PO₄(aq) 3.67 min(99.68%); Chiral analysis, Daicel Chiral Pak AD, 0.46x25 cm, 1 mL/min, 240 nm, 30/70 Hexane/IPA 7.88 min (97.48%); Anal. Calcd for C₁₃H₁₁N₃O₄: C, 57.14; H, 4.06; N, 15.38. Found: C, 57.34; H, 3.91; N, 15.14.

EXAMPLE 16

3-(4-Amino-1-oxoisindolin-2-yl)piperidine-2,6-dione

A. Methyl 2-bromomethyl-3-nitrobenzoate

A stirred mixture of methyl 2-methyl-3-nitrobenzoate (14.0 g, 71.7 mmol) and N-bromosuccinimide (15.3 g, 86.1 mmol) in carbon tetrachloride (200 mL) was heated under gentle reflux for 15 hours while a 100 W bulb situated 2 cm away was shining on the flask. The mixture was filtered and the solid was washed with methylene chloride (50 mL). The filtrate was washed with water (2x100 mL), brine (100 mL) and dried. The solvent was removed in vacuo and the residue was purified by flash chromatography (hexane/ethyl acetate, 8/2) to afford 19 g (96%) of the product as a yellow solid: mp 70.0-71.5° C.; ¹H NMR (CDCl₃) δ 8.12-8.09(dd, J=1.3 and 7.8 Hz, 1H), 7.97-7.94(dd, J=1.3 and 8.2 Hz, 1H), 7.54(t, J=8.0 Hz, 1H), 5.15(s, 2H), 4.00(s, 3H); ¹³C NMR (CDCl₃) δ 165.85, 150.58, 134.68, 132.38, 129.08, 127.80, 53.06, 22.69; HPLC, Waters Nova-Pak/C18, 3.9x150 mm, 4 micron, 1 mL/min, 240 nm, 40/60 CH₃CN/0.1% H₃PO₄(aq) 7.27 min(98.92%); Anal. Calcd for C₉H₈NO₄Br: C, 39.44; H, 2.94; N, 5.11; Br, 29.15. Found: C, 39.46; H, 3.00; N, 5.00; Br, 29.11.

B. t-Butyl N-(1-oxo-4-nitroisindolin-2-yl)-L-glutamate

Triethylamine (2.9 g, 28.6 mmol) was added dropwise to a stirred mixture of methyl 2-bromomethyl-3-nitrobenzoate (3.5 g, 13.0 mmol) and L-glutamine t-butyl ester hydrochloride (3.1 g, 13.0 mmol) in tetrahydrofuran (90 mL). The mixture was heated to reflux for 24 hours. To the cooled mixture was added methylene chloride (150 mL) and the mixture was washed with water (2x40 mL), brine (40 mL) and dried. The solvent was removed in vacuo and the residue was purified by flash chromatography (3% CH₃OH in methylene chloride) to afford 2.84 g (60%) of crude product which was used directly in the next reaction: ¹H NMR (CDCl₃) δ 8.40(d, J=8.1 Hz, 1H), 8.15(d, J=7.5 Hz, 1H), 7.71(t, J=7.8 Hz, 1H), 5.83(s, 1H), 5.61(s, 1H), 5.12(d, J=19.4 Hz, 1H), 5.04-4.98(m, 1H), 4.92(d, J=19.4 Hz, 1H) 2.49-2.22(m, 4H), 1.46(s, 9H); HPLC, Waters Nova-Pak/C18, 3.9x150 mm, 4 micron, 1 mL/min, 240 nm, 25/75 CH₃CN/0.1% H₃PO₄(aq) 6.75 min(99.94%).

C. N-(1-Oxo-4-nitroisindolin-2-yl)-L-glutamine

Hydrogen chloride gas was bubbled into a stirred 5° C. solution of t-butyl N-(1-oxo-4-nitroisindolin-2-yl)-L-glutamate (3.6 g, 9.9 mmol) in methylene chloride (60 mL) for 1 hour. The mixture was then stirred at room temperature for another hour. Ether (40 mL) was added and the resulting mixture was stirred for 30 minutes. The slurry was filtered, washed with ether and dried to afford 3.3 g of the product: ¹H NMR (DMSO-d₆) δ 8.45(d, J=8.1 Hz, 1H), 8.15(d, J=7.5 Hz, 1H), 7.83(t, J=7.9 Hz, 1H), 7.24(s, 1H), 6.76(s, 1H), 4.93(s, 2H), 4.84-4.78(dd, J=4.8 and 10.4 Hz, 1H), 2.34-2.10(m, 4H); ¹³C NMR (DMSO-d₆) δ 173.03, 171.88, 165.96, 143.35, 137.49, 134.77, 130.10, 129.61, 126.95, 53.65, 48.13, 31.50, 24.69; Anal. Calcd for C₁₃H₁₃N₃O₆: C, 50.82; H, 4.26; N, 13.68. Found: C, 50.53; H, 4.37; N, 13.22.

D. (S)-3-(1-Oxo-4-nitroisindolin-2-yl)piperidine-2,6-dione

A stirred suspension mixture of N-(1-oxo-4-nitroisindolin-2-yl)-L-glutamate (3.2 g, 10.5 mmol) in anhydrous methylene chloride (150 mL) was cooled to -40° C. with isopropanol/dry ice bath. Thionyl chloride (0.82 mL, 11.3 mmol) was added dropwise to the cooled mixture followed by pyridine (0.9 g, 11.3 mmol). After 30 min, triethylamine (1.2 g, 11.5 mmol) was added and the mixture was stirred at -30 to -40° C. for 3 hours. The mixture was poured into ice water (200 mL) and the aqueous layer was extracted with methylene chloride (40 mL). The methylene chloride solution was washed with water (2x60 mL), brine (60 mL) and dried. The solvent was removed in vacuo and the solid residue was slurried with ethyl acetate (20 mL) to give 2.2 g (75%) of the product as a white solid: mp 285° C.; ¹H NMR (DMSO-d₆) δ 11.04(s, 1H), 8.49-8.45(dd, J=0.8 and 8.2 Hz, 1H), 8.21-8.17(dd, J=7.3 Hz, 1H), 7.84(t, J=7.6 Hz, 1H), 5.23-5.15(dd, J=4.9 and 13.0 Hz, 1H), 4.96(dd, J=19.3 and 32.4 Hz, 2H), 3.00-2.85(m, 1H), 2.64-2.49(m, 2H), 2.08-1.98(m, 1H); ¹³C NMR (DMSO-d₆) δ 172.79, 170.69, 165.93, 143.33, 137.40, 134.68, 130.15, 129.60, 127.02, 51.82, 48.43, 31.16, 22.23; HPLC, Waters Nova-Pak/C18, 3.9x150 mm, 4 micron, 1 mL/min, 240 nm, 20/80 CH₃CN/0.1% H₃PO₄(aq) 3.67 min(100%); Anal. Calcd for C₁₃H₁₁N₃O₅: C, 53.98; H, 3.83; N, 14.53. Found: C, 53.92; H, 3.70; N, 14.10.

E. (S)-3-(1-Oxo-4-aminoisindolin-2-yl)piperidine-2,6-dione

A mixture of (S)-1-(1-oxo-4-nitroisindolin-2-yl)piperidine-2,6-dione (1.0 g, 3.5 mmol) and 10% Pd/c (0.3 g) in methanol (600 mL) was hydrogenated in a Parr-Shaker apparatus at 50 psi of hydrogen for 5 hours. The mixture was filtered through Celite and the filtrate was concentrated in vacuo. The solid was slurried in hot ethyl acetate for 30 min, filtered and dried to afford 0.46 g (51%) of the product as a white solid: mp 235.5-239° C.; ¹H NMR (DMSO-d₆) δ 11.01(s, 1H), 7.19(t, J=7.6 Hz, 1H), 6.90(d, J=7.3 Hz, 1H), 6.78(d, J=7.8 Hz, 1H), 5.42(s, 2H), 5.12(dd, J=5.1 and 13.1 Hz, 1H), 4.17(dd, J=17.0 and 28.8 Hz, 2H), 2.92-2.85(m, 1H), 2.64-2.49(m, 1H), 2.34-2.27(m, 1H), 2.06-1.99(m, 1H); ¹³C NMR (DMSO-d₆) δ 172.85, 171.19, 168.84, 143.58, 132.22, 128.79, 125.56, 116.37, 110.39, 51.48, 45.49, 31.20, 22.74; HPLC, Waters Nova-Pak/C18, 3.9x150 mm, 4 micron, 1 mL/min, 240 nm, 10/90 CH₃CN/0.1% H₃PO₄(aq) 0.96 min(100%); Chiral analysis, Daicel Chiral Pak AD, 40/60 Hexane/IPA, 6.60 min(99.42%); Anal. Calcd for C₁₃H₁₃N₃O₃: C, 60.23; H, 5.05; N, 16.21. Found: C, 59.96; H, 4.98; N, 15.84.

EXAMPLE 17

3-(4-Amino-1-oxoisindolin-2-yl)-3-methylpiperidine-2,6-dione

A. N-Benzoyloxycarbonyl-3-amino-3-methylpiperidine-2,6-dione

149

A stirred mixture of N-benzyloxycarbonyl- α -methylisoglutamine (11.3 g, 38.5 mmol), 1,1'-carbonyldiimidazole (6.84 g, 42.2 mmol) and 4-dimethylaminopyridine (0.05 g) in tetrahydrofuran (125 mL) was heated to reflux under nitrogen for 19 hours. The reaction mixture was concentrated in vacuo to an oil. The oil was slurried in water (50 mL) for 1 hour then filtered, washed with water, air dried to afford 7.15 g of white solid. The crude product was purified by flash chromatography (2:8 ethyl acetate:methylene chloride) to afford 6.7 g (63%) of the product as a white solid: mp 151–152° C.; ¹H NMR (CDCl₃) δ 8.24 (s, 1H), 7.35 (s, 5H), 5.6 (s, 1H), 5.09 (s, 2H), 2.82–2.53 (m, 3H), 2.33–2.26 (m, 1H), 1.56 (s, 3H); ¹³C NMR (CDCl₃) δ 174.4, 172.4, 154.8, 136.9, 128.3, 127.8, 127.7, 65.3, 54.6, 29.2, 29.0, 22.18; HPLC: Waters Nova-Pak/C₁₈ column, 4 micron, 3.9–150 mm, 1 ml/min, 240 nm, 20/80 CH₃CN/H₃PO_{4(aq)}, 6.6 min. 100%. Anal. Calcd for C₁₄H₁₆N₂O₄. Theory: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.94; H, 5.76; N, 10.10.

B. 3-Amino-3-methylpiperidine-2,6-dione

N-benzyloxycarbonyl-3-amino-3-methylpiperidine-2,6-dione (3.0 g, 10.9 mmol) was dissolved in ethanol (270 mL) with gentle heat and then cooled to room temperature. To this solution was added 4 N HCl (7 mL) followed by 10% Pd/C (0.52 g). The mixture was hydrogenated under 50 psi of hydrogen for 3 hours. To the mixture was then added water (65 mL) to dissolve the product. The mixture was filtered through a celite pad and the celite pad washed with water (100 mL). The filtrate was concentrated in vacuo to a solid residue. This solid was slurried in ethanol (50 mL) for 30 min. The slurry was filtered to afford 3.65 g (94%) of the product as a white solid: ¹H NMR (DMSO-d₆) δ 11.25 (s, 1H), 8.9 (s, 3H), 2.87–2.57 (m, 2H), 2.35–2.08 (m, 2H), 1.54 (s, 3H); HPLC (Waters Nova-Pak/C₁₈ column, 4 micron, 1 ml/min, 240 nm, 15/85 CH₃CN/H₃PO_{4(aq)}, 1.07 min, 100%).

C. 3-Methyl-3-(4-nitro-1-oxoisindolin-2-yl)piperidine-2,6-dione

To a stirred mixture of α -amino- α -methyl-glutarimide hydrochloride (2.5 g, 14.0 mmol) and methyl 2-bromomethyl-3-nitro benzoate (3.87 g, 14 mmol in dimethylformamide (40 mL) was added triethylamine (3.14 g, 30.8 mmol) under nitrogen. The mixture was heated to reflux for 6 hours. The mixture was cooled and then concentrated in vacuo. The solid residue was slurried in water (50 mL) and methylene chloride for 30 min. The slurry was filtered and the solid washed with methylene chloride and dried (60° C., <1 mm). Recrystallization from methanol (80 mL) yielded 0.63 g (15%) of the product as an off white solid: mp 195–197° C.; ¹H NMR (DMSO-d₆) δ 10.95 (s, 1H), 8.49–8.46 (d, J=8.2 Hz, 1H), 8.13–8.09 (d, J=7.4 Hz, 1H), 7.86–7.79 (t, J=7.8 Hz, 1H), 5.22–5.0 (dd, J=19.4 and 34.6 Hz, 2H), 2.77–2.49 (m, 3H), 2.0–1.94 (m, 1H), 1.74 (s, 3H); ¹³C NMR (DMSO-d₆) δ 173.1, 172.3, 165.0, 143.2, 137.4, 135.2, 130.1, 129.3, 126.9, 57.6, 48.7, 28.9, 27.7, 20.6; HPLC (Waters Nova-Pak/C₁₈ column, 4 micron, 1 ml/min, 240 nm, 20/80 CH₃CN/H₃PO_{4(aq)}, 4.54 min, 99.6%); Anal. Calcd. For C₁₄H₁₃N₃O₅; C, 55.45; H, 4.32; N, 13.86. Found: C, 55.30; H, 4.48; N, 13.54.

D. 3-Methyl-3-(4-amino-1-oxoisindolin-2-yl)piperidine-2,6-dione

3-Methyl-3-(4-nitro-1-oxoisindolin-2-yl)piperidine-2,6-dione (1.0 g, 3.3 mmol) was dissolved in methanol (500 mL) with gentle heat and then cooled to room temperature. To this solution was added 10% Pd/C (0.3 g) under nitrogen. The mixture was hydrogenated in a Parr-Shaker apparatus at 50 psi of hydrogen for 4 hours. The mixture was filtered

through celite pad and the celite pad washed with methanol (50 mL). The filtrate was concentrated in vacuo to a off white solid. The solid was slurried in methylene chloride (20 mL) for 30 min. The slurry was filtered and the solid dried (60° C., <1 mm). The solid was to recrystallized from methanol (3 times, 100 mL/time) to yield 0.12 (13.3%) of the product as a white solid: mp 289–292° C.; ¹H NMR (DMSO-d₆) δ 10.85 (s, 1H), 7.19–7.13 (t, J=7.6 Hz, 1H), 6.83–6.76 (m, 2H), 5.44 (s, 2H), 4.41(s, 2H), 2.71–2.49 (m, 3H), 1.9–1.8 (m, 1H), 1.67 (s, 3H); ¹³C NMR (DMSO-d₆) δ 173.7, 172.5, 168.0, 143.5, 132.9, 128.8, 125.6, 116.1, 109.9, 57.0, 46.2, 29.0, 27.8, 20.8; HPLC (Waters Nova-Pak/C₁₈ column, 4 micron, 1 ml/min, 240 nm, 20/80 CH₃CN/H₃PO_{4(aq)}, 1.5 min, 99.6%); Anal. Calcd. For C₁₄H₁₅N₃O₃; C, 61.53; H, 5.53; N, 15.38. Found: C, 61.22; H, 5.63; N, 15.25.

EXAMPLE 18

Tablets, each containing 50 mg of 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisindoline, can be prepared in the following manner:

1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisindoline	50.0 g
lactose	50.7 g
wheat starch	7.5 g
polyethylene glycol 6000	5.0 g
talc	5.0 g
magnesium stearate	1.8 g
demineralized water	q.s.

The solid ingredients are first forced through a sieve of 0.6 mm mesh width. The active ingredient, lactose, talc, magnesium stearate and half of the starch then are mixed. The other half of the starch is suspended in 40 mL of water and this suspension is added to a boiling solution of the polyethylene glycol in 100 mL of water. The resulting paste is added to the pulverulent substances and the mixture is granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 6 mm diameter which are concave on both sides.

EXAMPLE 19

Tablets, each containing 100 mg of 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisindoline, can be prepared in the following manner:

1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisindoline	100.0 g
lactose	100.0 g
wheat starch	47.0 g
magnesium stearate	3.0 g

All the solid ingredients are first forced through a sieve of 0.6 mm mesh width. The active ingredient, lactose, magnesium stearate and half of the starch then are mixed. The other half of the starch is suspended in 40 mL of water and this suspension is added to 100 mL of boiling, water. The resulting paste is added to the pulverulent substances and the mixture is granulated, if necessary with the addition of water. The Granulate is dried overnight at 35° C., forced

through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 6 mm diameter which are concave on both sides.

EXAMPLE 20

Tablets for chewing, each containing 75 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline, can be prepared in the following manner:

1-oxo-2-(2,6-dioxo- piperidin-3-yl)-4-amino- isoindoline	75.0 g
mannitol	230.0 g
lactose	150.0 g
talc	21.0 g
glycine	12.5 g
stearic acid	10.0 g
saccharin	1.5 g
5% gelatin solution	q.s.

All the solid ingredients are first forced through a sieve of 0.25 mm mesh width. The mannitol and the lactose are mixed, granulated with the addition of gelatin solution, forced through a sieve of 2 mm mesh width dried at 50° C. and again forced through a sieve of 1.7 mm mesh width. 1-Oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline, the glycine and the saccharin are carefully mixed, the mannitol, the lactose granulate, the stearic acid and the talc are added and the whole is mixed thoroughly and compressed to form tablets of approximately 10 mm diameter which are concave on both sides and have a breaking groove on the upper side.

EXAMPLE 21

Tablets, each containing 10 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline, can be prepared in the following manner.

1-oxo-2-(2,6-dioxo- piperidin-3-yl)-5-amino- isoindoline	10.0 g
lactose	328.5 g
cornstarch	17.5 g
polyethylene glycol 6000	5.0 g
talc	25.0 g
magnesium stearate	4.0 g
demineralized water	q.s.

The solid ingredients are first forced through a sieve of 0.6 mm mesh width. Then the active imide ingredient, lactose, talc, magnesium stearate and half of the starch are intimately mixed. The other half of the starch is suspended in 65 mL of water and this suspension is added to a boiling solution of the polyethylene glycol in 260 mL of water. The resulting paste is added to the pulverulent substances, and the whole is mixed and granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 10 mm diameter which are concave on both sides and have a breaking notch on the upper side.

EXAMPLE 22

Gelatin dry-filled capsules, each containing 100 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline, can be prepared in the following manner:

1-oxo-2-(2,6-dioxo- piperidin-3-yl)-6-amino- isoindoline	100.0 g
microcrystalline cellulose	30.0 g
sodium lauryl sulfate	2.0 g
magnesium stearate	8.0 g

The sodium lauryl sulfate is sieved into the 1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline through a sieve of 0.2 mm mesh width and the two components are intimately mixed for 10 minutes. The microcrystalline cellulose is then added through a sieve of 0.9 mm mesh width and the whole is again intimately mixed for 10 minutes. Finally, the magnesium stearate is added through a sieve of 0.8 mm width and, after mixing for a further 3 minutes, the mixture is introduced in portions of 140 mg each into size 0 (elongated) gelatin dry-fill capsules.

EXAMPLE 23

A 0.2% injection or infusion solution can be prepared, for example, in the following manner:

1-oxo-2-(2,6-dioxo- piperidin-3-yl)-7-amino- isoindoline	5.0 g
sodium chloride	22.5 g
phosphate buffer pH 7.4	300.0 g
demineralized water	to 2500.0 mL

1-Oxo-2-(2,6-dioxopiperidin-3-yl)-7-aminoisoindoline is dissolved in 1000 mL of water and filtered through a microfilter. The buffer solution is added and the whole is made up to 2500 mL with water. To prepare dosage unit forms, portions of 1.0 or 2.5 mL each are introduced into glass ampoules (each containing respectively 2.0 or 5.0 mg of imide).

EXAMPLE 24

Tablets, each containing 50 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline, can be prepared in the following manner.

1-oxo-2-(2,6-dioxo- piperidin-3-yl)-4,5,6,7- tetrafluoroisoindoline	50.0 g
lactose	50.7 g
wheat starch	7.5 g
polyethylene glycol 6000	5.0 g
talc	5.0 g
magnesium stearate	1.8 g
demineralized water	q.s.

The solid ingredients are first forced through a sieve of 0.6 mm mesh width. The active ingredient, lactose, talc, magnesium stearate and half of the starch then are mixed. The other half of the starch is suspended in 40 mL of water and this suspension is added to a boiling solution of the polyethylene glycol in 100 mL of water. The resulting paste is added to the pulverulent substances and the mixture is granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 6 mm diameter which are concave on both sides.

23

EXAMPLE 25

Tablets, each containing 100 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrachloroisindoline, can be prepared in the following manner:

1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrachloroisindoline	100.0 g
lactose	100.0 g
wheat starch	47.0 g
magnesium stearate	3.0 g

All the solid ingredients are first forced through a sieve of 0.6 mm mesh width. The active ingredient, lactose, magnesium stearate and half of the starch then are mixed. The other half of the starch is suspended in 40 mL of water and this suspension is added to 100 mL of boiling water. The resulting paste is added to the pulverulent substances and the mixture is granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 6 mm diameter which are concave on both sides.

EXAMPLE 26

Tablets for chewing, each containing 75 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrafluoroisindoline, can be prepared in the following manner:

1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrafluoroisindoline	75.0 g
mannitol	230.0 g
lactose	150.0 g
talc	21.0 g
glycine	12.5 g
stearic acid	10.0 g
saccharin	1.5 g
5% gelatin solution	q.s.

All the solid ingredients are first forced through a sieve of 0.25 mm mesh width. The mannitol and the lactose are mixed, granulated with the addition of gelatin solution, forced through a sieve of 2 mm mesh width, dried at 50° C. and again forced through a sieve of 1.7 mm mesh width. 1-Oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrafluoroisindoline, the glycine and the saccharin are carefully mixed, the mannitol, the lactose granulate, the stearic acid and the talc are added and the whole is mixed thoroughly and compressed to form tablets of approximately 10 mm diameter which are concave on both sides and have a breaking groove on the upper side.

EXAMPLE 27

Tablets, each containing 10 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetramethylisindoline, can be prepared in the following manner:

1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetramethylisindoline	10.0 g
lactose	32.5 g

24

-continued

cornstarch	17.5 g
polyethylene glycol 6000	5.0 g
talc	25.0 g
magnesium stearate	4.0 g
demineralized water	q.s.

The solid ingredients are first forced through a sieve of 0.6 mm mesh width. Then the active imide ingredient, lactose talc, magnesium stearate and half of the starch are intimately mixed. The other half of the starch is suspended in 65 mL of water and this suspension is added to a boiling solution of the polyethylene glycol in 260 mL of water. The resulting paste is added to the pulverulent substances, and the whole is mixed and granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 10 mm diameter which are concave on both sides and have a breaking notch on the upper side.

EXAMPLE 28

Gelatin dry-filled capsules, each containing 100 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetramethoxyisindoline, can be prepared in the following manner:

1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetramethoxyisindoline	100.0 g
microcrystalline cellulose	30.0 g
sodium lauryl sulfate	2.0 g
magnesium stearate	8.0 g

The sodium lauryl sulfate is sieved into the 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetramethoxyisindoline through a sieve of 0.2 mm mesh width and the two components are intimately mixed for 10 minutes. The microcrystalline cellulose is then added through a sieve of 0.9 mm mesh width and the whole is again intimately mixed for 10 minutes. Finally, the magnesium stearate is added through a sieve of 0.8 mm width and, after mixing for a further 3 minutes, the mixture is introduced in portions of 140 mg each into size 0 (elongated) gelatin dry-fill capsules.

EXAMPLE 30

A 0.2% injection or infusion solution can be prepared, for example, in the following manner:

1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrafluoroisindoline	5.0 g
sodium chloride	22.5 g
phosphate buffer pH 7.4	300.0 g
demineralized water	to 2500.0 mL

1-Oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrafluoroisindoline is dissolved in 1000 mL of water and filtered through a microfilter. The buffer solution is added and the whole is made up to 2500 mL with water. To prepare dosage unit forms, portions of 1.0 or 2.5 mL each are introduced into glass ampoules (each containing respectively 2.0 or 5.0 mg of imide).

EXAMPLE 31

Tablets, each containing 50 mg of 1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4,5,6,7-tetrafluoroisindoline, can be prepared in the following manner:

1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4,5,6,7-tetrafluoroisindoline	50.0 g
lactose	50.7 g
wheat starch	7.5 g
polyethylene glycol 6000	5.0 g
talc	5.0 g
magnesium stearate	1.8 g
demineralized water	q.s.

The solid ingredients are first forced through a sieve of 0.6 mm mesh width. The active ingredient, lactose, talc, magnesium stearate and half of the starch then are mixed. The other half of the starch is suspended in 40 mL of water and this suspension is added to a boiling solution of the polyethylene glycol in 100 mL of water. The resulting paste is added to the pulverulent substances and the mixture is granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm, mesh width and compressed to form tablets of approximately 6 mm diameter which are concave on both sides.

EXAMPLE 32

Tablets, each containing 100 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisindoline, can be prepared in the following manner:

1-oxo-2-(2,6-dioxo-piperidin-3-yl)-4-aminoisindoline	100.0 g
lactose	100.0 g
wheat starch	47.0 g
magnesium stearate	3.0 g

All the solid ingredients are first forced through a sieve of 0.6 mm mesh width. The active ingredients lactose, magnesium stearate and half of the starch then are mixed. The other half of the starch is suspended in 40 mL of water and this suspension is added to 100 mL of boiling water. The resulting paste is added to the pulverulent substances and the mixture is granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 6 mm diameter which are concave on both sides.

EXAMPLE 33

Tablets for chewing, each containing 75 mg of 2-(2,6-dioxo-3-methylpiperidin-3-yl)-4-aminophthalimide, can be prepared in the following manner:

2-(2,6-dioxo-3-methylpiperidin-3-yl)-4-aminophthalimide	75.0 g
mannitol	230.0 g
lactose	150.0 g

-continued

talc	21.0 g
glycine	12.5 g
stearic acid	10.0 g
saccharin	1.5 g
5% gelatin solution	q.s.

All the solid ingredients are first forced through a sieve of 0.25 mm mesh width. The mannitol and the lactose are mixed, granulated with the addition of gelatin solution, forced through a sieve of 2 mm mesh width, dried at 50° C. and again forced through a sieve of 1.7 mm mesh width. 2-(2,6-Dioxo-3-methylpiperidin-3-yl)-4-aminophthalimide, the glycine and the saccharin are carefully mixed, the mannitol, the lactose granulate, the stearic acid and the talc are added and the whole is mixed thoroughly and compressed to form tablets of approximately 10 mm diameter which are concave on both sides and have a breaking groove on the upper side.

EXAMPLE 34

Tablets, each containing 10 mg of 2-(2,6-dioxoethylpiperidin-3-yl)-4-aminophthalimide, can be prepared in the following manner:

2-(2,6-dioxoethylpiperidin-3-yl)-4-aminophthalimide	100.0 g
lactose	328.5 g
cornstarch	17.5 g
polyethylene glycol 6000	5.0 g
talc	25.0 g
magnesium stearate	4.0 g
demineralized water	q.s.

The solid ingredients are first forced through a sieve of 0.6 mm mesh width. Then the active imide ingredient, lactose, talc, magnesium stearate and half of the starch are intimately mixed. The other half of the starch is suspended in 65 mL of water and this suspension is added to a boiling solution of the polyethylene glycol in 260 mL of water. The resulting paste is added to the pulverulent substances, and the whole is mixed and granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 10 mm diameter which are concave on both sides and have a breaking notch on the upper side.

EXAMPLE 35

Gelatin dry-filled capsules, each containing 100 mg of 1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4,5,6,7-tetrafluoroisindoline, can be prepared in the following manner:

1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4,5,6,7-tetrafluoroisindoline	100.0 g
microcrystalline cellulose	30.0 g
sodium lauryl sulfate	2.0 g
magnesium stearate	8.0 g

The sodium lauryl sulfate is sieved into the 1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4,5,6,7-

27

tetrafluoroisindoline through a sieve of 0.2 mm mesh width and the two components are intimately mixed for 10 minutes. The microcrystalline cellulose is then added through a sieve of 0.9 mm mesh width and the whole is again intimately mixed for 10 minutes. Finally, the magnesium stearate is added through a sieve of 0.8 mm width and, after mixing for a further 3 minutes, the mixture is introduced in portions of 140 mg each into size 0 (elongated) gelatin dry-fill capsules.

EXAMPLE 36

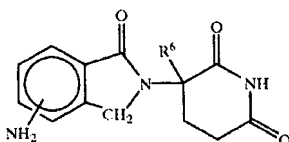
A 0.2% injection or infusion solution can be prepared, for example, in the following manner:

1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4,5,6,7-tetrafluoroisindoline	5.0 g
sodium chloride	22.5 g
phosphate buffer pH 7.4	300.0 g
demineralized water	to 2500.0 mL

1-Oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4,5,6,7-tetrafluoroisindoline is dissolved in 100 mL of water and filtered through a microfilter. The buffer solution is added and the whole is made up to 2500 mL with water. To prepare dosage unit forms, portions of 1.0 or 2.5 mL each are introduced into glass ampoules (each containing respectively 2.0 or 5.0 mg of imide).

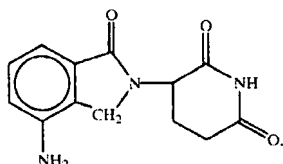
What is claimed is:

1. A method of treating inflammation, inflammatory disease or autoimmune disease in a mammal which comprises administering thereto an effective amount of a compound of the formula:



in which R⁶ is hydrogen or methyl.

2. The method according to claim 1 in which said compound has the formula:



3. The method according to claim 2 wherein said disease is rheumatoid arthritis.

4. The method according to claim 2 wherein said disease is osteoarthritis.

5. The method according to claim 2 wherein said disease is inflammatory bowel disease.

6. The method according to claim 5 wherein said inflammatory bowel disease is Crohn's disease.

7. The method according to claim 5 wherein said inflammatory bowel disease is ulcerative colitis.

8. The method according to claim 2 wherein said disease is an arthritic condition.

28

9. The method according to claim 2 wherein said disease is sepsis.

10. The method according to claim 2 wherein said disease is lupus.

11. The method according to claim 2 wherein said disease is erythema nodosum leprosum.

12. The method according to claim 2 wherein said compound is administered orally or parenterally.

13. The method according to claim 2 wherein said compound is administered in combination with a therapeutic agent.

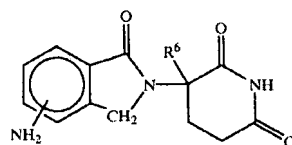
14. The method according to claim 2 in which said compound is administered in the form of a physiologically acceptable non-toxic acid addition salt.

15. The method according to claim 2 in which said compound has the R-configuration.

16. The method according to claim 2 in which said compound has the S-configuration.

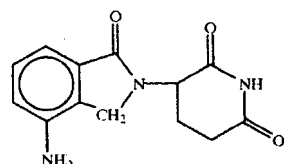
17. The method according to claim 2 in which said compound is administered in a single or multiple dosage regimen as a pharmaceutical composition containing from 1 to 100 mg. of said compound.

18. A method of treating an oncogenic or cancerous condition in a mammal which comprises administering thereto an effective amount of a compound of the formula:



in which R⁶ is hydrogen or methyl.

19. The method according to claim 18 in which said compound has the formula:



20. The method according to claim 19 wherein said compound is administered orally or parenterally.

21. The method according to claim 19 in which said compound is administered in combination with a therapeutic agent.

22. The method according to claim 21 wherein said therapeutic agent is a steroid, neoplastic agent or antibiotic.

23. The method according to claim 19 in which said compound is administered in the form of a physiologically acceptable non-toxic acid addition salt.

24. The method according to claim 19 in which said compound has the R-configuration.

25. The method according to claim 19 in which said compound has the S-configuration.

26. The method according to claim 19 in which said compound is administered in a single or multiple dosage regimen as a pharmaceutical composition containing from 1 to 100 mg. of said compound.

* * * * *

154

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,281,230 B1
DATED : August 28, 2001
INVENTOR(S) : Muller et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page.

Item [63], **Related U.S. Application Data**, revise as follows:

-- Division of application No. 09/230,389, which is a 371 of application No. PCT/US97/13375, filed on Jul. 24, 1997, now abandoned, which is a continuation-in-part of application Nos. 08/690,258, filed on Jul. 24, 1996, now Pat. No. 5,635,517, and 08/701,494, filed on Aug. 22, 1996, now Pat. No. 5,798,368. --.

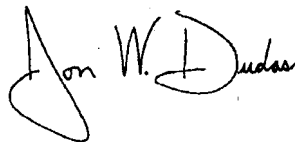
Column 1.

Lines 7-13, revise as follows:

-- This is a divisional of Ser. No. 09/230,389, now abandoned, which is a 371 of PCT/US97/13375 filed Jul. 24, 1997, which is a continuation-in-part of Ser. Nos. 08/690,258 filed Jul. 24, 1996, now U.S. Pat. No. 5,635,517, and 08/701,494 filed Aug. 22, 1996, now U.S. Pat. No. 5,798,368, and claims priority to provisional application Ser. No. 60/048,278 filed May 30, 1997. --.

Signed and Sealed this

Thirtieth Day of May, 2006



JON W. DUDAS
Director of the United States Patent and Trademark Office